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Julius-Kühn-Archiv

Edited by

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F.H. Arthur, C.G. Athanassiou, R. Bartosik, J. Campbell,
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Proceedings of the 12th International
Working Conference on Stored Product
Protection (IWCSPP)

in Berlin, Germany, October 7-11, 2018

Volume 1

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• Julius Kühn-Institut (JKI)

• Deutsche Phytomedizinische Gesellschaft e.V.

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Bundesministerium für Ernährung und Landwirtschaft (BMEL)

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Preface

Ladies and Gentlemen,

It is my pleasure welcoming all of you to the 12th International Working Conference for Stored Product Protection on behalf of the Federal Ministry of Food and Agriculture and the Federal Government. I am glad that Germany has the opportunity to host this important event for the first time. Facing challenges as the ever increasing world population, climate change and unrest in many places of the world, securing sufficient food supply is a crucial task in order to ensure food security.

The United Nation's Food and Agriculture Organization (FAO) estimates that approximately one third of the harvest gets lost before consumption. This is by far too much, and improvements are direly needed. Concerted research efforts and international cooperation are needed to find solutions in all fields of stored product protection from better conditions for on-farm storage to save and degradable food packaging. Therefore the purpose of the conference is to exchange new findings and ideas in order to improve stored product protection.

Sustainable intensification of crop production and improved stored product protection are two key approaches to improve the productivity of our agriculture. Both lead to better food supply and better protection of natural resources like bio-diversity, water, air and fertile soils. By contrast food losses are wasting resources and they are unethical. The broad and comprehensive range of topics at the conference underlines the diversity of challenges we are facing. We urgently need the exchange of thoughts and ideas amongst researchers, administration, civil society and entrepreneurs. I am convinced that the German Capital Berlin offers an excellent platform for your deliberations.

More than 60 nationalities take part in this conference. Thus, you resemble a large portion of this world's population and certainly take home some incitements for your important work and contacts for future collaboration. This conference could be realized because of the support and sponsorship of many organizations. Thank you very much to all of them. My special gratitude addresses the FAO and the WFP as well as the BMZ and the GIZ, who were strongly committed to this event. Last but not least I'd like to thank the President of Julius-Kühn-Institute and his staff for organizing the 12th IWCSPP in an excellent manner.

Julia Klöckner, Federal Minister of Food and Agriculture, Germany

Preface

Dear colleagues,

You hold in your hands the Program and book of abstracts of the IWCSPP 2018. To get this far, we needed some luck convincing the Permanent Committee of IWCSPP in 2014, and we learnt that many little challenges come along with organizing such a meeting. Now we hope, everything falls into place and you will have a good time.

This conference intends to cover all aspects of stored product protection. And we are convinced it comes at just the right time. There are new findings available on pest biology, chemotaxis, mycotoxins, there are new results regarding storage engineering, trapping, plant extracts and contact insecticides, on anoxia and fumigation, of course there are new regulations, and new results on physical and biological control. But we are also glad that extension of our research into the field is a topic in this meeting. And the global challenges we face by climate change, increasing unrest and the highest number of displaced people in decades will be discussed. Stored product protection has implications when we need to secure food for refugees, for people struck by severe droughts or other disasters. Are we prepared to take on these challenges? Should'nt we have more international research, more coordination, more funding, more cooperation across continents?

We are happy that you are here and that you participate in this meeting. Thanks to the many of you who sent their abstracts and prepared their presentations, thus adding value to this event.

We hope you will enjoy the few days of this conference and the chance to exchange thoughts. A number of organizations represent their tasks, many companies show their products and services. All thoughts and ideas are important like little mosaic stones that add up to give a complete picture of how stored product protection looks today or may look like in the future.

We hope you take home some happy memories of Berlin and Germany, about colleagues and new acquaintances, may be some new ideas or perspectives on stored product protection.

Your local IWCSPP organizers and Cornel Adler

Berlin, October 2018

Dear colleagues:

I welcome you to the 12th International Working Conference on Stored Product Protection (IWCSPP). This conference, which is held every four years, is the premier international conference for scientists and industry professionals working with stored agricultural commodities.

Since the first IWCSPP in 1974, the number of participants and the countries which they represent has increased along with the broad coverage of scientific topics related to storage of agricultural products including sessions on insect and pathogen biology, detection, and control, as well as engineering aspects of stored-product protection. The current conference also will have emphasis on protection of stored products in developing countries. The oral and poster presentations, along with specialized workshops, will inform participants on the latest advances in stored-product protection, while providing ample time to network with colleagues.

Berlin, the capital of Germany with a population of almost 4 million people, has much to offer historically (it was founded in the 13th century), culturally, and for outdoor activities. October is an ideal time to visit as the weather is very pleasant.

I welcome you to Berlin for what promises to be an exciting and productive conference for advancing the scientific fields involved in stored-product protection.

Sincerely,

James E. Throne, President
Permanent Committee of the International Working Conferences on Stored Product Protection

12th International Working Conference on Stored Product Protection

7 – 11 October 2018, Berlin, Germany

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IWCSPP, Past Conferences and Proceedings

1st International Working Conference on Stored-Product Entomology
Savannah, GA, USA, 1974 (articles available online)

2nd International Working Conference on Stored-Product Entomology
Ibadan, Nigeria, 1978 (articles available online)

3rd International Working Conference on Stored-Product Entomology
Manhattan, KS, USA, 1983 (articles available online)

4th International Working Conference on Stored-Product Protection
Tel Aviv, Israel, 1986 (articles available online)

5th International Working Conference on Stored-Product Protection
Bordeaux, France, 1990 (articles available online)

6th International Working Conference on Stored-Product Protection
Canberra, Australia, 1994 (articles available online)

7th International Working Conference on Stored-Product Protection
Beijing, China, 1998 (articles available online)

8th International Working Conference on Stored-Product Protection
York, UK, 2002

9th International Working Conference on Stored-Product Protection
Campinas, Brazil, 2006 (articles available online)

10th International Working Conference on Stored-Product Protection
Estoril, Portugal, 2010 (articles available online)

11th International Working Conference on Stored Product Protection
Chiang Mai, Thailand, 2014 (articles available online)

Link to former proceedings: http://bru.gmprc.ksu.edu/proj/iwcspp

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Session 1
Food Security and Challenges to Stored Product Protection

Food Safety and Global Challenges to Stored Product Protection – A WFP Perspective

Isabelle Mballa

Chief Food Safety & Quality, UN World Food Programme, Email: isabelle.mballa@wfp.org

The United Nations World Food Programme (WFP) is the leading humanitarian organization fighting hunger worldwide, delivering food assistance in emergencies, assisting 80 million people in around 80 countries each year. In 2016, WFP delivered 3.5 million MT of food to 74 countries, of which 2.2 million MT travelled by sea. On any given day, WFP operates 5,000 trucks, 20 ships and 70 aircraft. Food is stored in a network of 650 warehouses worldwide and across thousands of retailers and distribution partners globally.

With my 20 years of experience with the WFP and particularly in my present capacity as the Chief of Food Safety and Quality Unit of the organization, I am made cognizant of the food safety challenges posed by a multi-modal international supply chain, particularly exacerbated by exposure to harsh variations in climates - from sub-zero temperatures in Canada and France to high 40's and 50's in Sahel within the span of a few months or sometimes even weeks.

WFP has moved in the last 15 years from providing more-stable "raw" grains products such as cereals and pulses like Maize, Sorghum, Wheat and Lentils to a more sensitive food basket that includes processed foods such as fortified flours, nutritious foods such as ready to eat nutritious pastes to treat malnutrition in infants and young children.

The integrity of these "evolved" products is subject to extremities of food storage in challenging conditions down to the last mile, without the protection of temperature or humidity control and sometimes lacking even the basic pest control programmes. In the best case scenario, this may lead to minor loss in nutrient profile of the product; in the worst, it may compromise the food safety of the product. In this lies a paradox on whether to spend public money to feed people or to provide temperature control in warehouses when the people we serve barely have a roof over their heads.

The next challenge in stored product protection lies in identifying the best possible packaging options to assist in maintaining the product's integrity. Losses incurred within the supply chain, though minimal, significantly comprise factors related to packaging failure; spillage due to improper sealing, product exposure due to inadequate packaging material, and so forth. Globally, food packaging has seen one of the fastest growth rates and innovations in the last decade and WFP is catching up with the best solutions to optimizing packaging while keeping costs within range.

One challenge pertinent to this forum is WFP's work in capacity building in developing contexts. The organization continues to work with governments, private sector food processors and small holder farmers to improve farm to fork food supply chains as well as public procurement platforms. Several food safety issues emanate from the harvest and post-harvest handling, including grass root storage of the produce. In this, the developing countries are also the source of various innovations and yet, still playing catch-up with many practices that are considered basic in developed countries. Pest control, protection against pesticides, appropriate crop drying methodologies all play a part in reducing food safety issues such as elevated mycotoxin levels.

Similarly, food processing industry in some contexts is marred by basic issues such as improper hygiene practices and lack of adequate sanitation facilities, which proliferate food safety issues, accentuated downstream in storage.

Lastly, the inability to detect these issues originates in lack of knowledge and lack of proper infrastructure to be able to identify and test the key food safety markers, which are context-dependent. For example, lack of reliable data on the presence of aflatoxins in maize in one country in East Africa along with the absence of a reference lab within the same country to test aflatoxins has hampered general awareness amongst policy makers and thereby the creation of policies, monitoring tools and mitigation measures – which, by some research estimates, has allowed aflatoxins in the crop to run rampant in the country and may be a primary cause of stunting amongst children.

In our line of work at WFP, food safety needs to be addressed throughout the supply chain, starting from the source. Storage of foods, whether raw or processed, falls under the bigger umbrella of food safety across the supply chain from harvest to consumption.

WFP has been and continues to liaise with the private sector to allow industry best practices to be channeled through its work at the grassroots and to bring about a transfer in knowledge to the people in need.

Yet, I personally believe that the solution to protection of stored foods across the supply chain lies in innovation. WFP strives to innovate in new ways of shortening the supply chain, such as by purchasing more locally and regionally; in packaging through research and development; in storage by using elemental energy to cool temperatures in the warehouses and so on.

WFP is a voluntary funded organization with the mandate of achieving Zero Hunger globally. It serves people in conflict contexts, on the move, malnourished children, pregnant and nursing mothers and some of the most vulnerable populations in the world. We deliver food through barges on Baro river in South Sudan and on the backs of donkeys in Nepal; it is stored under tents and in iron containers. While we strive to deliver the maximum food to these beneficiaries, the onus is also on the organization to provide safe food for consumption in an ever changing context.

Food waste and food losses - Importance of international partnerships and research

Friedrich Wacker

Bundesministerium für Ernährung und Landwirtschaft (BMEL), 10117 Berlin, Email: UAL62@bmel.bund.de

More than 800 million people are still starving worldwide and around two billion humans are suffering from "hidden hunger". And the world population continues to grow, thus increasing the demand for food. Additionally, changed consumption patterns in emerging economies and an increased global demand for sustainable raw materials for the non-food area are leading to increased demand and competition for agricultural products. On top of this, global challenges such as climate change are putting considerable pressure on agriculture to adapt. At the same time, food waste and losses is one of the greatest challenges of our times. Around one third of all available food is spoiled or wasted before it is consumed. To improve the nutritional situation and to reduce food waste and losses worldwide in the long term, international cooperation of agricultural and nutritional research institutes, industries and the society is fundamentally important. The German Federal Ministry of Food and Agriculture (BMEL) supports long-term national and international partnerships with the objective to enhance the direct benefit of German research, innovation and technologies to develop high-performance, nutrition-sensitive and sustainable agri-food systems worldwide. The focus of BMEL is on an effective and efficient cross sector information and knowledge exchange to create a bridge between science and the practical application of research results by the society, industry and policy makers.

Stop the brain drain – Why we need stored-product protection research for food safety

Cornel Adler

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Abstract

In the history of human development, stored-product protection (SPP) is probably older than the invention of agriculture because even what was hunted and gathered needed to be stored to provide food for the bad days. One may think that the human race had enough time to find out everything that could be found out on SPP. But this is not the case. SPP problems often require a solution custom-made for the given product or storage situation, climate, socio-economic background, etc. Modern SPP research in the Americas, Asia, Europe, or Oceania was often started as a result of World War I or II, when hunger was an issue. But, with the absence of hunger, we witness another scary development: SPP research is dying out, institutions are closed down, e.g., CSL UK 2009, SGRL Australia 2009, DPIL Denmark 2010, INRA France 2015. Yes, research costs money. But, do we take into account that climate change may already have led to increased numbers of conflicts and increased mobility? That a lack of food safety can tear apart all advances of civilization and culture in the brink of a moment? Why are there no calls for SPP research under Horizon 2020? What happened to the Millenium Goal to cut down hunger by 50%? The FAO states that one third of our grains are lost between harvest and consumption. It is high time to improve food storages and SPP methods using all knowledge and technology available in order to reduce losses, it is high time to support international SPP research!

Keywords: Storage, research, food-safety, policy, starvation, risk-prevention, innovation, needs

Introduction

If we imagine human development before the onset of agriculture, hunters and gatherers needed to store and protect their food in order to survive bad weather conditions or seasons of scarcity. Thus, stored-product protection was needed even before the development of agriculture. One could expect that all of this time that man had to deal with difficulties such as moulds or two-, four, six-, and eight-legged competitors should have been enough to solve the problems, but this is not the case. According to the Food and Agriculture Organization of the United Nations (FAO 2011), one third of the harvest is not consumed but lost or wasted. While tropical and subtropical warm climates keep insect development and post-harvest losses high at all seasons, in temperate climates and industrialized countries a large portion of the harvest is wasted at the retail and consumer level (FAO 2011). If we try to grasp this gigantic loss, we need to think of one third of arable land, of plowing labour, of seeds, seeding, plant protection activities and products, irrigation, plus harvesting and storing in vain. If we take the grain harvest as an example, estimated by world-grain.com for 2016 at roughly 2.569 billion tons, one third, which roughly equals 850 million tons, will be lost. How is it possible that the human species allowing such a waste is called *Homo sapiens*? In less provocative terms, we could possibly agree upon the fact that there is a hidden treasure. Reducing such losses may help feed an ever growing population in the coming years, increase the productivity of agriculture, allow for more environmentally sustainable agricultural practices, and still leave some protected landscapes to maintain species diversity.

Climate, food security, and mobility

According to Diamond (1997), the availability of plant-based nutrition in early agriculture-driven societies allowed the development of different professions (farmers, hunters, warriors, doctors, chiefs). A society that cannot any longer supply sufficient food may easily fall apart. We should take into consideration that climate change and extreme climates with or without the influence of "El Niño" already cause drastic changes: It has been reported that in 2007 extreme climates caused unusually low harvests in Russia and the Ukraine which together with other factors (fires in Oceania, speculation) led to high grain-prices in the world market. This in turn caused hunger riots in

northern Africa and the so-called Arabic spring. The mass demonstrations of frustrated youths led to the demise of governments. Religious fundamentalism, war lords, and terrorist groups are more successful among young people that do not find a job nor see a future. We are facing times of increasing unrest and aggression in regions where adverse conditions drive desperate people to leave their homes and search for survival elsewhere. In Germany, we thought for a long time to be far away from such struggles, but in the past few years, we had to learn that considerable numbers of refugees made it all the way to our front door.

Thus, what does this have to do with stored-product protection research? Stored-product protection research could help reduce losses in both developing and industrial countries. Improved harvests and improved income from agriculture could help farming communities to be more productive and resilient. Europe claims the will to help improve living conditions in Sub-Saharan Africa and elsewhere. Reducing post-harvest losses would be among the most promising policies. Joint research could help to reduce not only losses but also improve quality of the harvested goods, e.g., by reducing infestation and mycotoxin levels. An example of this is hermetic storage in Purdue Improved Crop Storage (PICS) bags (Anon. 2008, Baoua et al. 2012). Stakeholders and researchers in many tropical countries also have innovative ideas, e.g., on how to improve solar drying, the use of plant parts or extracts, wood ash, zeolites, or other dusts. From Argentina came the innovation of hermetic grain storage in silobags (Bartosik 2012).

Less SPP research in industrialized countries

True stored-product protection research in the Americas, Asia, Europe, or Oceania was often started as a result of World War I or II, when hunger was an issue. But, with the absence of hunger, we witness another scary development: Over the past decades reducing numbers of researchers attended our SPP conferences. Especially in industrialized countries, less and less resources seem available for this kind of research. Research could help to improve storage, but from the Netherlands not one public stored-product protection scientist ever came to a meeting. There is no stored-product protection research done in Belgium, Sweden, or Norway. Denmark made its small Danish Pest Infestation Laboratory part of Aarhus University in 2007, where it was closed around 2010. The stored product protection group of the French Institute National du Recherche Agronomique (INRA), that hosted the IWCSPP in 1990, ceased to exist by the end of 2015, when the last colleague, Dr. Francis Feurat-Lessard, retired. The Stored Grain Research Laboratory in Canberra, Australia, that hosted the IWCSPP in 1994, was closed down in 2009, even though Australia is the 5th biggest grain exporter after Russia, the EU, the US, and Canada. The UK hosted the IWCSPP in 2002 and closed down its Central Science Laboratory after severe cuts in 2009. The few remaining scientists under the new roof of the Food and Environment Research Agency (FERA) cannot any more attend international conferences like this one. Obviously, many countries do not regard stored-product protection research as a priority and rely on other countries to develop the necessary innovation.

Fig. 1: Estimated numbers of public stored product scientists world-wide

We need stored-product protection research in industrialized countries because new ways of transportation, like bulk storage of cocoa in shipping containers and large horizontal storages, may be more cost effective on one hand but cause challenges like condensation, heating, and even damage by fire. We need research to develop and build better storage structures and to develop structures for the food processing industry that takes into consideration the latest information on insect behaviour. We need improved processing machines that give less opportunity for stored-product insect infestation. We need more research because with new and improved knowledge on pests, preventive methods, monitoring, and control can change. We need more research to learn which new species may find its way into our products or which known species is changing relative importance. Because biology never remains stagnant, we should be aware of changes. New materials can help us to improve packaging technology. Hermetic seals and vacuum could avoid or control pest infestation. Solar drying and aeration cooling could render storages unsuitable for arthropod survival at moderate costs. New camera equipment and computer chips can improve automatic pest detection, and new physical means like laser-technology can allow new methods of pest control (see IWCSPP 2018 publication Adler et al. "Starwars in food stores"). Improved lures with highly attractive volatiles could turn traps from monitoring tools into pest control equipment. A combination of acoustic detection with biological control could render the latter more effective and economically feasible (see IWCSPP 2018 publication Mueller-Blenkle et al. "A new approach to detect insects acoustically in grain storage").

How come we use computers, mobile phones, and other high-tech equipment in our every-day life. But our staple food is stored in storages that are often worse than those of our great-grandparents because the farmer is paid too little money per ton of grain. For decades, farmers were told to invest little into storage structures. Now it could make sense to implement IPM strategies, to propagate

preventive methods such as grain cleaning, drying, cooling, and pest-proof storage structures. Now we have fewer and fewer pest control options. But can we offer sufficient data to convince farmers?

What happened to the United Nations (UN) Millennium Development Goal to cut down hunger by 50%? How can stored-product protection research be helpful to reach this goal? And is there sufficient research done?

The European Union (EU) did not make stored-product protection a topic in its calls for Horizon 2020 even though early on a number of colleagues wrote to their respective national contact points. So far, just mycotoxin-research is funded, but that insects locally increase moisture and thus facilitate mycotoxin formation is not taken into consideration. Who decides research funding policy, and who has sufficient oversight? Is there a way to make research funding a more flexible tool?

At least within Germany, there were national funds available for research projects within the last six years. But international cooperation mainly depended on personal scholarships by sources such as DAAD or Humboldt Foundation.

What needs to change?

As stored-product protection researchers, we are usually analyzing a specific problem and searching for specific improvements or solutions. But if I would lift my head to look at the greater picture, I would like to utter the following wishes:

1. EU: Please make stored-product protection research part of the funding for FP9!

2. EU and member states: Please provide funding and facilitate research cooperation between European and non-European stored-product protection scientists (travel grants, smaller and larger projects), while keeping administrative hurdles at a minimum.

3. FAO and UN World Food Programme (WFP): Please help initiating and coordinating stored-product protection research according to your needs, in organizing exchange of ideas and concepts. Participate more regularly in scientific conferences.

4. UN: Please develop an improved method on how to reach consensus and a clearer perspective on how to tackle pressing challenges (e.g., overpopulation, malnutrition and starvation, scarcity of fresh water, pollution).

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Counting losses to cut losses: quantifying legume postharvest losses to help achieve food and nutrition security

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Abstract

Projections suggest that by 2050 global food production will need to have increased by 70% to meet food demands associated with the world's population growth. Such forecasts, alongside growing awareness of the socio-ecological costs of food loss, and political ramifications of food crises have seen postharvest loss (PHL) reduction reappearing as a development priority. Particularly so in sub-Saharan Africa, a region deemed highly vulnerable to the impacts of climate change, where 307 million people are already affected by severe food insecurity, and the population is projected to double by 2050. Targets for reduced PHL are emphasised in the African Union's Malabo Declaration and Sustainable Development Goal 12.3. However, crop postharvest systems are complex and losses occur in various ways at different activity stages and due to a host of diverse reasons. To better target and prioritise loss reduction investments and policies we need to understand how much food is being lost postharvest, where, and why. The African Postharvest Losses Information Systems (APHLIS), brought a rigorous knowledge management approach to cereal PHLs. We are now expanding this to include key legume and other crops and estimates of the nutritional and financial values of these losses. The scientific literature was screened to build profiles of the PHLs occurring along the value chains, and combined with contextual information, to provide science-based estimates of PHLs where direct measurements are not available. We discuss these legume PHL profiles and the related opportunities and knowledge gaps.

Keywords: Legume crops, postharvest losses, PHL metrics, loss estimates, African Postharvest Loss Information System (APHLIS)

1. Introduction

With the world's population expected to reach 9.8 billion people by 2050, two-thirds of whom will be living in cities (UNDESA, 2017), projections suggest food production will need to have increased by 60% if the growing and changing food demands are to be met (Alexandratos and Bruinsma, 2012). Such forecasts, alongside a developing awareness of the socio-ecological costs of food production, food loss, and political ramifications of food crises have seen postharvest loss (PHL) reduction reappearing as a development priority (World Bank et al., 2011; Gustavsson et al., 2011; Foresight Review, 2011; FAO, 2013; Hodges & Stathers, 2013; Affognon et al., 2015; Mvumi and Stathers, 2014; Sheahan and Barrett, 2017). This is particularly so in sub-Saharan Africa (SSA), a region deemed highly vulnerable to the impacts of climate change (Niang et al., 2014), where the population is projected to double by 2050 (UNDESA, 2017), and where 307 million people already suffer from severe food insecurity (FAO et al., 2017). Sustainable food security will not be achieved through focusing on reducing postharvest losses alone. Increased food production must be achieved with less impact on the environment, alongside actions to modify resource intensive consumption patterns and population growth, improve governance systems, and reduce food loss and waste (Godfray and Garnett, 2014).

Postharvest losses are not just a loss of valuable food, but also of all the resources invested in producing the food. As climate change impacts, population growth, environmental awareness, and competition for water for agriculture increase, so does the pressure to reduce losses. This recent recognition of the importance of and socio-ecological benefits of postharvest loss (PHL) reduction has led to significant investments in improved postharvest management, particularly storage technologies, by governments and donors across SSA. Major targets for reducing PHL have been set. African Union member states in the June 2014 Malabo Declaration for Africa Accelerated Agricultural Growth and Transformation for Shared Prosperity and Improved Livelihoods agreed to reduce current levels of PHL by 50% by the year 2025 (African Union, 2014). In 2015, all member states of the United Nations adopted a set of Sustainable Development Goals (SDGs). SDG 12 aims to ensure sustainable consumption and production patterns, and includes target 12.3 of 'by 2030, halving per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses' (UN General Assembly, 2015).

Crop postharvest systems cover a range of different activity stages and are typically spread spatially and temporally across different locations and actors, and are thus both complex and dynamic. They include the harvesting, transport from field, drying, threshing/shelling, cleaning and sorting, storage, packaging, further transport, marketing, processing, and consumption of the crop. Losses can occur in a multitude of ways at each activity stage and due to a host of diverse reasons (e.g.,

grain left in field at harvest, spilt during transport, or consumed by pests during storage, etc.). To decide how to reduce PHLs, and which investments and policies to implement, it is important to understand not just how much food is being lost postharvest, but at which activity stages these PHLs are occurring, how, and why.

The 2008 food crisis acted as the trigger for development agencies involved in improving food security across SSA to realise they needed a more detailed and accurate understanding of the level of postharvest loss of staple food crops occurring (World Bank et al., 2011; Hodges & Stathers, 2013). This led to the European Commission funding the development of an online African Postharvest Losses Information System (APHLIS) www.aphlis.net, which was launched in 2009, bringing a rigorous knowledge management approach to cereal PHL estimates (Rembold et al., 2011).

To create APHLIS, the scientific literature on cereal PHLs in SSA was screened and weight loss data extracted to build PHL profiles for nine key cereal crops. Seasonal data were then supplied by a network of experts for each province of 37 SSA countries on: the quantity of each of the cereal crops produced, whether rain had occurred at harvest, whether the devastating maize storage insect pest the larger grain borer (LGB), *Prostephanus truncatus* had been present, % of the crop marketed versus stored on-farm, typical storage durations, farm-scale proportions, and climate types. An algorithm was then used to adjust the PHL profile according to the seasonal factors supplied for each location to produce a contextualised science-based estimate of PHL occurring at each PH activity stage for each of the focal crops. This system provided an overview of PHLs by crop across countries and years. The PHL estimate was then presented as % weight loss and quantity of crop lost. The data were used by development agencies for refining their food security assessments. As such, APHLIS provides governments and international organisations and bodies with science-based estimates of cereal PHLs by crop, postharvest activity stage, province, and year, filling a valuable information gap for the majority of locations where direct measurements of PHLs have never been made. As transparency regarding how the loss estimates had been calculated was viewed as important, the APHLIS system enables the data and original studies behind the calculation of each PHL estimate and a rating of the reliability of each loss figure in a profile to be identified, and updated or improved where necessary (Hodges et al., 2014).

A sizeable body of literature exists that discusses and debates postharvest cereal loss assessment methods. Much of the work has focused on different methods for measuring weight losses occurring during cereal storage, which is viewed as a critical loss stage with crop storage in SSA typically occurring at farm-level and often for periods of up to 10 months. However, a focus on just the physical weight loss occurring at different PH stages underestimates the overall value and multi-dimensional nature of PHL, as the quality as well as the quantity of the crop can diminish postharvest. Qualitative losses include: the reduced financial value of damaged, contaminated, or aged produce; nutritional loss which may not always be directly proportional to the weight loss, as rodents and some insect species selectively feed on specific parts of the grains, such as the germ and thus may particularly remove fats or vitamins; reduced seed viability; commercial losses if control treatments have to be purchased, or legal costs are faced; and reputational losses (Boxall, 2001). Including qualitative as well as quantitative losses in PHL calculations would result in substantially higher figures and give a more accurate representation of their socio-economic impact. However, qualitative losses are more complex to measure and the perceived importance of loss in quality may be dependent on the: surrounding food availability situation, location, expectations and standards, intended use of the product (i.e., whether consumed as a whole grain, dehulled or milled product, or marketed), how easy they are to observe, knowledge about what caused them, etc. (Compton et al., 1998; Hoffman et al., 2013; Jones et al., 2014; Kadjo et al., 2016), and limited work has focused on them. They can also be complex to express, as many are not typically considered in monetary terms, i.e., well-being, farmer's time, wasted natural resource inputs, etc. As APHLIS further develops, elements of quality loss are being incorporated to help provide a more complete understanding of PHLs.

The original APHLIS focused on cereal grains. Whilst cereal grains are the main food staple crops in many areas of SSA, root and tuber and legume crops are also crucial staple foods; legumes are a major source of dietary protein in diets of the poor in SSA. In recognition of this, APHLIS is now expanding to include key legumes and other important staple food crops such as cassava. In the current paper, we present the legume PHL data and the process of developing legume PHL profiles and the related opportunities and knowledge gaps.

2. Materials and Methods

To create the PHL profile in APHLIS for each focal crop, the scientific published and 'grey' literature was screened, and reliable high quality data of the PHLs occurring in a specific context extracted and entered into a database along with details of where, when, at which PH stage, and how the loss figure was determined. This followed the method developed by Hodges et al. (2014), and ensures the PHL estimates are based on the best data available. Where limited SSA PHL data exists, the search was widened to include other countries with similar legume production and PH systems and climate types.

This complex multi-stage process involved a thorough search of the literature, followed by screening of the titles and abstracts of each potential PHL study identified during the search to determine whether quantitative data on PHLs was reported. The full versions of studies considered likely to contain quantitative PHL data were accessed and read. The loss assessment and sampling methodology, type of study, and presentation and interpretation of the results were critically examined to determine how reliable the measurements or estimates were likely to be, to determine whether the data should be included, and, if so, the quality rating of the study's data (high, medium, low, exclude). This screening process was based on that used by Hodges et al. (2014), and was similar to that followed by Affognon et al. (2015). If quantitative PHL figures had been collected during the study, they were extracted and entered into the appropriate crop group database (i.e., cereals, legumes, root, tuber, and banana).

For each PHL figure used, the accompanying data on the context in which that PHL occurred was recorded. This included the:

- crop type;
- PH activity stage that the loss figure occurred in (i.e., harvesting, field drying, stripping, transport to home, further drying, threshing, storage, transport to market, market);
- method used to obtain the loss figure (i.e., measured vs guestimate, and details of the loss assessment method, sampling technique, and accuracy of interpretation of results);
- type of study (i.e., field survey, field trial, or on-station trial);
- geographical location where the data were from;
- Koppen climate zone where the data were from;
- farm type and technology used (i.e., smallholder or larger-scale farmers and whether they were using an improved postharvest management method applicable to that PH stage);
- relevant details about the method and study (i.e., if storage stage, what storage container, treatment, duration, and sampling process that the loss was associated with);
- decision to include or exclude the study, and, if included, the quality rating of the study's data score (i.e., high, medium, or low)

Due to their importance as protein sources in the food systems of many African countries, the focal legume crops included are cowpeas (*Vigna unguiculata*), groundnuts (*Arachis hypogaea*), common beans (*Phaseolus vulgaris*), bambara nut (*Vigna subterranea*), pigeon pea (*Cajanus cajan*), and soy bean (*Glycine max*).

Many of the legume PH studies focus on the storage stage, but had recorded data on the % of insect damaged grains as opposed to the % weight loss, likely as a time-saving measure. Where these

studies were of 'high' or 'medium' quality rating, the percentage damaged grain data were converted to percentage weight loss using crop specific conversion formulae from published studies, e.g., for cowpeas (y = -0.0025x^2 + 0.3551x -3.31, x = % damaged grain, y = % weight loss (Wright and Golob, 1999)). While the actual conversion rate between % damage and % weight loss is likely to be influenced by variety, storage insect pest species present, etc., it was judged to be beneficial to convert the % damaged grain data in order to increase and widen the geographical source of the number of PH loss figures being used to build the storage loss part of the PHL profile.

The dataset was then manipulated to provide an overview of what data of what quality exists for each legume crop, climate zone, and PH stage. Where major gaps in the available data exist, in terms of missing information on some of the postharvest stages for some legume crops, decisions are then made as to whether it is appropriate to use data from a similar legume crop for that stage or to include 'low' quality rated data as well as 'medium' and 'high', until higher quality studies for the PH stage of the specific crop are undertaken. This overview stage allows decisions to be made regarding which data will be used to create a profile of the PHLs occurring at each postharvest stage of the value chain for each crop. Details on key loss-causing factors at each stage are also collected and screened to determine what contextual data could be collected to indicate to what degree the main loss-causing factors occurred which will then be used in the algorithm to adjust the loss estimate for that particular context.

3. Results

3.1. Quantity, quality, focal PH activity stage and crop of legume PHL data

Although accessing and screening of the legume PHL literature is still ongoing, to date legume PHL figures from 63 studies have been identified, resulting in a dataset of 694 legume PHL figures.

Analysis of these figures reveals that 525 (76%) were categorised as of 'high' or 'medium' quality rating, and, of these, 75% were measured figures. When these 'high' and 'medium' quality legume PHL figures were grouped by PH activity stage, the majority were related to storage losses, with 57% giving data on losses during farm-level storage and a further 20% on losses during market storage stage (Table 1). Where storage data were provided as % damaged grains, it was converted to % weight loss; 58% of farm-level storage loss figures and 52% of market storage figures required conversion. Limited data on the losses occurring during the other PH stages exist, and data from cowpeas, groundnuts, and common beans dominate: 36, 28, and 19% of the legume PHL figures, respectively. Inclusion of lower quality data would increase the number of data points from the different PH activity stages but would reduce the reliability of the estimates produced using the profile.

Table 1 Number of legume postharvest loss figures obtained by crop and postharvest activity stage

Postharvest activity stages	Bambara	Common beans	Cowpea	Groundnuts	Pigeon pea	Soy bean	Total
Harvesting, field drying, pod stripping				24	2	4	30
Transport from field				6			6
Further drying				11	1	3	15
Threshing / shelling, winnowing			12	16	2	3	33
Storage on-farm	18	101	110	48	21	3	301
Packing, sorting, grading				8	1	1	10
Transport to market				13	1	1	15
Market	20		67	13	4	3	107
Processing				6	1	1	8
Total	38	101	189	145	33	19	525

3.2. Climatic and geographical nature of legume PHL data

The climate, in addition to the crop, activity timing, practices, and technologies, influences the level of PHL. When the 'high' and 'medium' quality PHL figures are viewed by the climate type of the

location where they occurred (using the Koppen climate classification), 46% are from tropical savannah (Aw) climate zones, 21% from warm semi-arid areas (BSh), 11% from humid subtropical climates (Cfa), and 8% from tropical monsoon areas (Am). These four climate types cover the majority of the crop producing areas of SSA.

Geographically, the legume PHL data came from a number of countries across sub-Saharan Africa, with 56 % of the studies coming from West African countries, particularly Nigeria, Ghana, and Niger (Figure 1). Relevant data from India, Brazil, and Thailand have also been included.
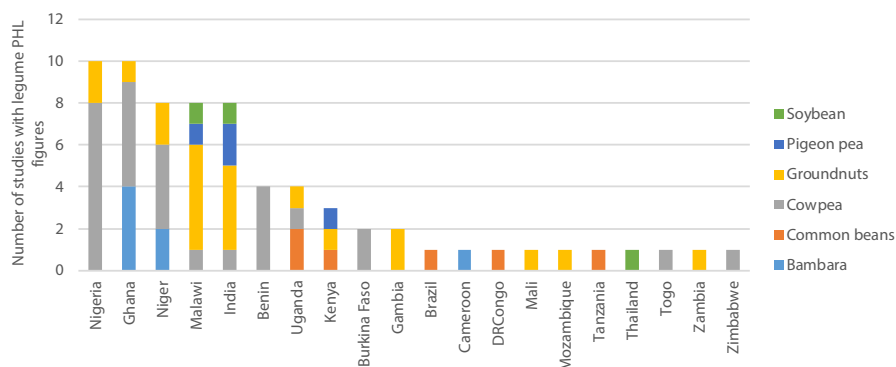
Figure 1 Number of studies with legume PHL figures by country

3.3. Age of legume PHL data

Analysis of the reporting year of the legume PHL data shows that 63% of the studies were published since 2000, reflecting the renewed interest in PHL reduction in SSA (Figure 2). Some high quality studies of legume PHL from before 1980 were also included.
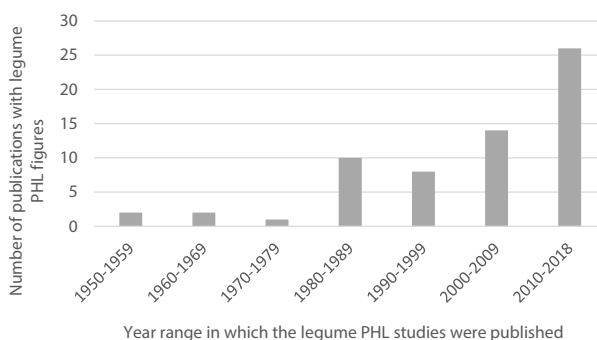
Figure 2 Age range of the legume PHL loss data

3.4. Storage losses from different treatments

The PHL data set includes 301 storage loss figures, and these are from a range of different storage treatments, with 36% from legumes stored untreated in sacks or outdoor granaries; 27% from legumes stored admixed with ash, sand, clay, botanicals, or above fire places; 17% from legumes stored in hermetic bags or other hermetic containers; and 3% from legumes stored admixed with synthetic pesticides (Figure 3). Where storage loss data were from surveys of a number of farmers who were using different treatments, including untreated, botanicals, and synthetic pesticides, it was recorded as 'range of treatments'. Most (70%) of the storage loss data came from legumes stored using the more traditional practices, e.g., untreated shelled or in pods, or admixed with ash,

sand, clay, botanicals, or kept above fire place. The majority of these storage loss data came from studies on cowpeas (39%), or common beans (36%). A preliminary comparison of the average loss levels occurring in the different treatments when loss figures were extrapolated to a standardised five month storage duration, revealed that the traditional practices resulted in average weight losses more than twice as high (>9% loss) as the improved treatments (<4% loss).
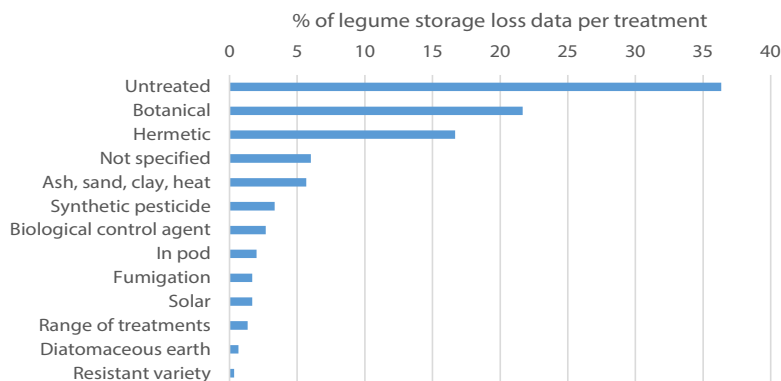
Figure 3 Overview of storage treatment methods from which the storage legume loss figures were obtained

While further calculations have been made using these data towards the calculation of PHL profiles for the different legume crops, it is premature to present this until further decisions are made by the legume technical expert panel regarding the sharing of data between crops and climate zones for cases where insufficient data exists on a particular crop at each PH stage in different climate zones.

During compilation of this legume PHL dataset, the loss causal factors have also been recorded, and these are being used to identify the seasonal or contextual factors that additional data is needed on to contextualise the losses to each location; these include: combining the PH activity timing with meteorological data (e.g., particularly for harvesting and drying), knowledge of the proportion of the focal population using different PH techniques, storage duration and number of harvests/ year, and the proportion of the crop marketed and the timing.

4. Discussion

The legume PHL scientific data are dominated by studies on cowpeas, groundnuts, and common beans, although other legume crops (e.g., pigeon pea) are widely grown and consumed in SSA. There is also more legume PHL data from West Africa than East, Southern, or Central Africa. Most research studies have focused on the storage losses which occur either on-farm or at the market place, and particularly those caused by insect pest damage. Very limited study has occurred of the losses which occur in legumes during harvesting, field drying, pod stripping, transport, further drying, threshing/ shelling, winnowing, sorting, or processing. A similar situation was observed with the cereal PHL data used to create APHLIS (Hodges et al., 2014), the durable crops included in the meta-analysis of PHL data from six SSA countries (Affognon et al., 2015), and a review of PHL in Organisation of Islamic Cooperation member states (Tomlins et al., 2016). However, whilst storage losses are clearly an important element of overall PHL and are the target of many PHL reduction investments, it is unlikely that a focus on reducing losses only during the storage stage will achieve the target of halving PHL by 2030. There is a need for losses at other PH activity stages to be reduced concurrently, and a more holistic and integrated view of pre- and postharvest crop systems.

To deepen understanding of legume PH systems and losses, studies of the non-storage stages are needed to provide greater insight into the proportional amounts being lost at each PH stage of the value chain and the reasons for these losses and opportunities for reducing them. This is important information to ensure available PHL reduction resources are being wisely targeted. Ideally, a PHL study should track the crop and its associated losses from field maturity stage onwards following it

through the different activity stages which will typically occur at and between different locations and times after harvest, and as it changes hands between actors along the value chain. However, such studies are rare, as the logistics of doing such a study at any scale, unless based close to the farms, are complex and costly. Such data would produce a more comparative understanding of where, why, and at what scale losses occur postharvest, and help by removing problems of lack of consistency between measurement methods, aims, geographies, data reporting styles, etc., which are well-known challenges. Many studies use 'guestimates', whereby farmers or other stakeholders are asked to provide verbal estimates of what percentage of their crop they lose postharvest, with the better ones of these studies asking for PHL estimates at each of the different stages and triangulating the responses with rankings, etc. However, these are perceived estimations, and highly subjective, and should not be confused with measured loss assessment. They are of course comparatively cheap and easy to obtain, but their accuracy is not well-understood and will vary by study, and the data obtained with them demonstrably prone to errors. If we think carefully about a simpler question of what % of the food in our home we lost or wasted during the last year or last 5 years, without measuring it and without having kept records, how accurate an estimate would we make? The growing research focus on food waste in developed countries has found quantitative estimates made from memory regarding the weight of food purchased and discarded are very prone to error (see Jorissen et al., 2015; FLW Protocol, 2017 for discussion). For more rapidly quantifying storage losses *in situ*, several visual scales have over the years been developed for different crops (e.g., maize cobs – Compton et al., 1991; cassava – Compton et al, 1992; millet – Hodges, 2013) (Compton and Sherrington, 1999; Hodges, 2013; Hodges et al., 2014).

Combining qualitative with quantitative losses provides a more realistic idea of the level and value of PHLs. However, the rejection criteria for produce varies by location, wealth group (Kadjo et al., 2016) and season (depending on food availability and typical quality) (Compton et al., 1998; Jones et al., 2014). Not all quality attributes are visible (i.e., aflatoxins, pesticide residues), and some studies suggest unobservable maize quality attributes affect farmers' food purchasing decisions and explain the large premium farmers place on maize they have grown themselves relative to that available for purchase (Hoffman and Gatobu, 2014; Kadjo et al., 2016).

There is a frequent misunderstanding that the weight loss occurring during storage is the same as the % of damaged grains, but this is not the case. The physical weight loss of grain is a fraction of the % of grains damaged, typical ratios for % weight loss : % damaged grains are: maize grain 1:8; sorghum 1:4; and paddy rice 1:2 (Adams and Schulten, 1978; Harris and Lindblad, 1978). Therefore a storage weight loss of 12% can mean the damage to the grain is so severe that unless there is extremely limited food availability, the grain would neither be eaten nor could it be sold, thus resulting in a total PHL of all the grain not just a 12% weight loss. There needs to be improved communication and understanding of this important topic, which could be helped with visual imagery. Currently APHLIS presents % weight loss data, and combines this with crop production figures for the different provinces of the focal countries to calculate what amount of each crop that province or country is losing. Preliminary work has begun in the APHLIS+ project to calculate the nutritional loss occurring as a result of these PHLs, and the financial value. Presenting PHLs in terms of dollars lost, or annual requirement of nutrient X for Y million people lost, or the number of extra acres of land farmed or cleared and associated quantities of seed, fertiliser, and water lost is likely to help increase public engagement with and concern about PHLs. However, the difficulty and complexity of including the more qualitative dimensions of PHL should not be underestimated. Attempts to estimate the economic impact of mycotoxins in SSA, for example, were thwarted by the lack of good data (Wu et al., 2011).

Looking beyond the use of APHLIS to calculate science-based estimates of PHL occurring at the different PH stages, there is interest in developing APHLIS to enable it to capture a more nuanced understanding of PHL and how these are or could change over time. Such development could enable it to become a useful support tool for PH investment scenario planning or a PHL M&E tool, for governments or Malabo Declaration or SDG 12.3 Global Food Loss Index M&E frameworks. For

example, the disaggregated storage loss data could be used to calculate changes in PHL as users adopt different improved crop storage practices. This could also be done for PHLs during non-storage stages if sufficient data were available. Governments wanting to better understand PH practices and technology use across their populations could ensure such questions were included in nationwide surveys such as the Living Standards Measurement Survey. However, it should also be noted that some 'improved' PH technologies or practices might be adopted to make a PH process less laborious or costly as opposed to reducing the quantity lost, and this may be of greater importance to the user.

Some improved pre or postharvest technologies or practices may actually increase PHLs, and these complex trade-offs need understanding; for example, some hybrid maize varieties had higher yields but were softer with poorer husk cover resulting in higher storage losses (Tyler, 1982, Boxall, 2001), mechanised harvesting and handling can result in higher levels of damaged grain which can render it more susceptible to attack by certain insect pests (Boxall, 2001), storage of milled rice is more susceptible to insect damage but takes up 25% less space than paddy (Boxall, 2001), double cropping may lead to increased annual production but may alter activity timings and disturb the traditional capability to conserve grain and lead to farmers putting wet season crop into store at higher moisture contents with markedly increased risks of spoilage (Wright, 1995; Boxall, 2001), and stricter food safety requirements and standards may result in increased removal of unsafe food from the food supply (Sheahan and Barrett, 2017). By contrast, a study in India reported rice showing signs of insect attack carried a price premium as it was taken as an indicator that the paddy was not freshly harvested and would taste better (Begum, 1991 cited by Wright, 1995). These examples highlight the importance of interaction and coordination between initiatives and a more holistic understanding of the whole interconnected agri-food system.

The rapid population growth and urbanisation occurring in SSA, the rise of the middle class (defined as those with purchasing power parity of 2 to 20 dollars a day (Ncube et al., 2011), and which is projected to reach 75% by 2040 (Tschirley et al., 2015)), and the growing consumption of food-away-from-home are also driving change in the agri-food systems. There are fears this will involve the consumption of more highly processed food, associated obesity, and unsustainable imports (USDA, 2013; Popkin, 2014), while hopes include demand for higher value and value-added agricultural products driving the creation of entrepreneurs and economic growth (Reardon et al., 2013; Badiane, 2014). Recent studies have found the share of dried legume and cereal grains in the diet reduces within the middle class, and the shares of fresh fruit, fresh fish, and eggs rise strongly, along with purchased maize meal replacing hand-pounded or custom-milled grain; and highly processed milk and vegetable oils and prepared food away from home rising sharply with income (Tschirley et al., 2015). This nutritional-transition will transform the agri-food system and very likely influence PHLs as diets diversify from staple roots, tubers, and grains to preferred cereals and increasing purchasing and consumption of more perishable dairy and meat products, vegetable oils, and fresh vegetables and fruits, which are known to have higher PHLs than cereal and pulses (Gustavsson et al., 2011). This will also come with environmental consequences, as many of these products are more land and water intensive to produce (Godfray and Garnett, 2014).

If PHLs are to be reduced by 50%, as per the Malabo and SDG 12.3 declarations, and make a serious and sustainable contribution to achieving food security in SSA, there is a need for: investment in deepening our understanding about and knowledge and awareness of the level, type, and reason for PHLs occurring along the value chain; institutionalised education of farmers and other stakeholders in postharvest management through practical hands-on learning opportunities (Hodges and Stathers, 2012) and ensuring postharvest management is woven into agricultural and agri-business curriculums; alongside supporting the promotion of appropriate and effective technologies and their distribution systems.

The APHLIS system has an important role to play in the postharvest system by providing science-based estimates of PHLs occurring at the different PH activity stages, for its focal crops by sub-national regions and years. These are useful to governments and development partners for

informing investment decisions and tracking progress. Other crops can be incorporated into APHLIS if sufficient PHL figures exist in the scientific literature, and APHLIS could be expanded to cover other geographical regions, e.g., Asia or the Middle East. The APHLIS team are always looking for new, carefully measured PHL figures to incorporate into APHLIS to keep increasing its accuracy and relevancy; please contact us if you have or plan to gather such PHL figures from SSA for any of the cereal, legume, or root and tuber focal crops.

Acknowledgement

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Food fights for life: Food diplomacy for food security

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Abstract

Stored food production is critical to food security. Food security refers to the physical availability of, the economic and physical access to, and the ability to utilize food (FAO, 2008, available at; http://www.fao.org/docrep/013/al936e/al936e00.pdf). Stored food production is a vital link in that chain: enabling the protection of (surplus) harvest to be made available when needed. Indeed, the means of stored food production constitutes an incentive for (surplus) harvest itself. However, food, food security, and alongside both, food *diplomacy* are not only practical concerns and challenges but also political. Furthermore, the politics of food are intrinsically related to health security, water security, and climate security, issues with increasing effects across the globe if at different orders of magnitude. Food insecurity may be measured higher in arid regions without adequate water and harvests and storage, but it also exists in 'urban deserts' without affordable access to (fresh) produce. In this presentation, I outline a cartography to depict the interconnections between local and global food securities using the characterization of diplomacy *of* food and *for* food, and food science *for* diplomacy. The aim is to enhance exchange of ideas and experiences to benefit food security – and reduced waste – in both food secure and food insecure settings.

Introduction

Food security is one of a litany of global challenges. Food security refers especially to the security *from* threats to food insecurity – including health threats, threats posed by climate change, and additional shocks such as economic upheavals (see Venezuela) and armed conflict. States alone, even if and when they want to act, can only do so much. That states themselves can constitute a threat to food security underscores a limit to this arrangement. "*The policy authority for tackling global problems still belongs to the states, while the sources of the problems and potential solutions are situated at transnational, regional or global level.*"However, food (science) for diplomacy, by contrast, can promote health for security in both developing and developed states, especially when it emerges from developing country contexts and is communicated with developed states.

The politics of food go far beyond states though states arguably remain control of the in- and outflows of food. These include direct actions such as state-based agricultural subsidies; (in)direct mechanisms of state and private sectors, that influence the export of lifestyle, including food, paradigms; and also more diffuse influences such as preferential trade agreements and humanitarian aid programs which exert economic as well as social pressures at all levels of the food chain – from planting decisions to food choice. Food politics is also subject to external shocks, including health crises, such as the outbreak of Ebola in West Africa which led to decreased sowing as well as harvesting and subsequently to increased food insecurity in the region. Thus while any viable and sustainable response to food insecurity in view of such challenges requires state action, it also depends upon inter-national, as well as global and local action.

It is at the formal and informal diplomatic levels that knowledge exchange to recognize; design; maintain, cope; and adapt models and modes to rising threats to food insecurity, including innovative insight into improved stored food protection takes place. Indeed, continuously improved stored food production technology integrated with political decision-making can actively support food security. The alternative to continued knowledge exchange and innovation, especially as set against the continuing and intensifying challenges that increasingly impact food security worldwide, though in some instances appearing as inevitable, is resistance and withdrawal (Gaire, K.R., 2015). Yet 'doing nothing' is also a political and policy choice. It does not imply stagnation, however. It can lead to significant upleaval: for instance to population displacement, further contributing to instabilities and insecurities. In order to avoid uncharted change, planned – if not predictable – responses are necessary.

Three ways to chart possible diplomacies to interconnect local and global food securities are summed up as 'diplomacy of food, diplomacy for food, and food science for diplomacy'. First, diplomacy *of* food includes the elevation of food to an issue of international, notably security, concern. Second, diplomacy *for* food is broader, and includes diplomatic efforts on the parts of states to increase awareness not only of food crises but of solutions. These include diplomatic efforts by state and non-state actors to facilitate access to food according to the criterion of availability, access, and utilization. Third, food (science) for diplomacy in turn includes research and innovation enabling the development and production of, food – and food storage – interventions.

Food Rights and Responsibilities

The balance of food rights and responsibility is underscored by the discourse of human security, introduced in the 1994 Human Development Report, (UNDP, 1994), which emphasizes state and non-state responsibility to promote and protect the rights of its human beings, including to food. It proceeds on the assumption that the achievement of health, while vital, is but one of a litany of local / global challenges facing policy makers. Other prominent competitors for attention include state security writ large, as well as additional aspects of human security – economic, environmental, and food, to name a few.

This has led to the notion on the one hand that food rights are tied to state responsibility and to state security. Yet in practice, food security at the global level has mostly been left to World Food

Program and FAO, both dependent upon Member State financial contributions and votes to authorize distribution and support. On the other hand, the global human security narrative has advanced the claim that health rights are universal, their implementation the responsibility of the international and global communities. As globalization – in communication technology, travel, and climate change –accelerates, so too does the urgency of identifying and addressing rights, including those to food, and responsibilities globally and locally. This is especially critical as policy issues compete for priority: the crises of climate change, energy security, food production and the financial system "represent serious potential threats…in international politics, the prospects for global" "diplomacy, and the effectiveness of global health governance mechanisms."(Lee, et al, 2011, Filder, 2007).

Diplomacy of food

This section analyses the elevation of food to an issue of international, notably security, concern. It focuses on the role of diplomacy and diplomatic efforts to put food security on the international agenda.

Diplomacy for food

This second applies an analysis that is broader that that introduced above. It extends to diplomatic efforts on the parts of states to increase awareness not only of food crises but of solutions. These include efforts by state and non-state actors (NSAs) to facilitate access to food according to the criterion of availability, access, and utilization. As such, it represents a move from advocacy to action, notably on the part of states and NSAs. It traces the shift from food delivery to food production and trade (for example through the implementation of debit cards to enable local purchase and to spur local production).

Food (science) for diplomacy

This section looks briefly at tried and tested examples as well as new research and innovation enabling the development and production of, food – and food storage – interventions.

Discussion

These insights need to be shared: food security requires food diplomacy at all three levels, local, national and global, to recognize and respond to food insecurities across the board; and to critically exchange knowledge based on empirical evidence and (political and social) experience to surmount threats to such insecurities even at different orders of magnitude. This discussion also includes the anticipated impacts of climate change and migration on changing food needs and patterns.

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On farm grain storage – potential opportunity or risk- meeting the demands of food safety and quality, an Australian perspective

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Abstract

Traceability, product identity, food safety and quality assurance are increasingly required by end users and customers. The Australian on farm storage system has a unique opportunity to deliver grain to meet these requirements, provided the system is set up and managed to ensure the end product meets the market requirement.

Australian grain growers are becoming more aware of the changing nature of markets and their requirements, and the importance of managing storage to meet food safety requirements. With the increasing change in storage dynamics in Australia from a central receival system to a range of storage entities, of which on farm storage is becoming a major player, there is a growing need for the grains industry to ensure all who can affect grain quality and food safety are aware of and can meet their obligations.

There are many challenges for Australian growers to manage; including managing existing facilities, investing in new facilities, managing insects, managing grain quality and ensuring treatments are used in accordance with best practice. Despite these challenges, there are many opportunities and potential for the on-farm storage system to meet the demands required of them to deliver a quality and food safe product to the end-user.

This paper discusses the on-farm grain storage system, management of and the opportunity and risks for growers and end users to work together to ensure a quality and food safe product is delivered to the end-user.

Traceability, product identity, food safety and quality assurance are increasingly required by end users and customers. The on farm storage system has a unique opportunity to deliver grain to meet these requirements, provided the system is set up and managed to do this in collaboration with the end user and market.

Whilst grain growers are aware of the changing nature of markets and their requirements, it is fair to say food safety and how they might affect this is relatively new in their thinking. With the increasing change in storage dynamics from a central receival system to a range of storage entities, of which on farm storage is becoming a major player, there is a growing need for the grains industry to ensure all who can affect grain quality and food safety are aware of and can meet their obligations.

There are many challenges for growers to manage; including managing existing facilities, investing in new facilities, managing insects, managing grain quality and ensuring treatments are used in accordance with best practice.

Despite these challenges, there are many opportunities and potential for the on-farm storage system to meet the demands required of them to deliver a quality and food safe product to the enduser and customer.

This paper discusses the on-farm grain storage system, management of the system and the opportunity and risks for growers and end users to work together to ensure a quality and food safe product is delivered to the enduser and customer.

Introduction

With the increase in on-farm grain storage there has been a corresponding increase as to the impact it has on the supply chain. Traceability, food safety, product identity, biosecurity and quality assurance are required to differing extents by industry and the market place. Australia has long had a reputation for providing "clean and green" agricultural products, however there is an increasing demand from both domestic and international markets to prove that food products are safe, and the term food safety is increasingly common when describing market requirements.

Both the domestic and export grain markets continually change, however the need for suppliers to be customer focussed and respond to and manage changes in the market place, places the on-farm grain storage system in a unique position to meet and capitalise on these requirements. There is no question that grain growers are investing in their storage systems and have a unique opportunity

to put in place and improve their existing system to meet the current and future requirements of the supply chain and market.

The on-farm storage system can be developed into one which can manage product identity, quality, traceability, food safety and changing market demands, giving growers flexibility and choices in how they market and deliver their grain through the supply chain. Whilst some may view this as a negative, the growth in on-farm storage continues and can store and deliver grain precisely as the market needs.

The types of systems invested in give growers the ability to segment and manage grain in storage to provide a quality product to the market. Certainly the on-farm system needs to ensure it implements best practise and invest in technologies and training to support best practice, however there are many examples where growers are doing this and many more are seeing the examples of this and considering their own business opportunities.

The grains industry has developed a number of codes of practise as guidelines for Good Agricultural Practice (GAP), and has developed a QA program based on food safety principles using the internationally recognised HACCP (Hazard Analysis Critical Control Point) system. Graincare (the grains industry developed QA system) is well positioned as a HACCP based quality assurance program to deliver a food safe assured product to the market.

There are many potential opportunities and risks when sourcing grain from the on-farm system, however ensuring that the market and the supplier are clear about their needs and responsibilities and establish beneficial outcomes for both, the on-farm market is well placed to provide a quality, safe food product for the enduser.

The on-farm storage system

Traditionally the on-farm storage system was based around smaller silos up to 70 tonne capacity, sheds and in some areas ground storage such as pads and bunkers. In the past 10 years the size of silos has increased substantially, and there has been a trend to larger flat bottom style silos ranging in capacity from 500 – 5000tonnes capacity. The use of temporary systems such as silo bags has increased significantly, and are usually used as a short term option.

Grain in unsealed storage is typically treated with a contact grain pesticide treatment to protect the grain whilst in storage. Resistance to commonly used grain protectants has meant growers are looking for alternatives to control resistant insect pests.

In 2014 the only registered spray treatment to kill insects was taken off the market, currently the only way to kill an infestation is by fumigation. This is forcing growers to seriously consider their investment strategy in new systems because fumigation can only work properly in a sealed gas-tight structure.

With the deregulation of the export market growers storing grain became more aware of the PRF (pesticide residue free) requirement of the export market. Growers have also seen this transfer into domestic markets where they are increasingly being asked for grain to be stored without being treated with contact pesticide protectant treatments. This has meant growers have had to invest in gas-tight sealable storages to effectively fumigate grain to kill insects. There has been a significant increase in the investment in sealable storage, and in 2010 an Australian standard (AS 2628) for gas-tight sealable storage was gazetted providing growers with a benchmark for purchasing gas-tight sealable silos.

In Western Australia grain storage protectants are not registered for grain treatment in on farm storage. Growers are only permitted to fumigate grain, as such, sealed storage has been widely used in on-farm storage for over 30 years

Best practice when storing grain requires growers to implement an integrated approach, using physical and chemical interventions to manage quality and control insects. Over the past 20 years there have been significant changes in the storage types, design and ways growers have applied an

integrated approach. Implementing good grain and system hygiene ensures insect numbers are limited, understanding insect species and their ecology assists in managing pests, and using chemical treatments and fumigants correctly ensures insects can be controlled when needed. Cooling grain using ambient aeration systems has increased in the past 10 years and is gaining widespread acceptance as a way of managing insects and quality by reducing grain storage temperatures.

Growers are increasingly becoming aware of the need to understand the quality of their grain, particularly to ensure grain out turned from their system meets market specifications. One of the advantages of on-farm storage is the ability to segregate grain more readily by using a combination of small, medium and larger storages.

Provided growers are willing to invest in a system which meets market requirements, they are in a unique position to provide a package which delivers product identity, traceability, can meet the needs of food safety requirements and best practise. There is no question that the on-farm storage system can build on and become a larger component of the supply chain, providing confidence and integrity to the market

Food Safety – Can on-farm storage meet this requirement?

The on-farm storage system is well placed to demonstrate that the product stored is safe for consumption. The grains industry has produced a number of codes and guidelines for growers and industry to enable this. "Growing Australian Grain – Safely Managing Risks with Crop Inputs and Grain On-farm" is a guide for growers and advisors to help manage risks with inputs, grain handling and safety on farm.

Grain Trade Australia has produced in collaboration with industry the Australian Grain Industry Code of Practice for the post harvest/post farm sector. Both of these documents enable growers to begin the journey to manage the risks associated with grain production and storage. The grains industry has also developed GrainCare which is a HACCP based quality assurance system which directly enables the grower to demonstrate they meet food safety requirements and are independently audited and assessed.

With the development of a modern, fit for purpose on-farm storage system, which can manage quality, identity preservation, outturn and food safety risks, there is a growing opportunity for the supply chain and market to access grain post farm gate with the confidence that supply chain integrity is maintained.

Conclusion

There is no doubt that the on-farm grain storage system is an integral and growing part of the supply chain. Growers need to ensure they understand their role in the supply chain, and invest in technologies, systems and training which enable them to implement best practise in their grain storage system.

Ensuring that the integrity of the supply chain is maintained requires all parties to do their part and give feedback to all stakeholders. Growers can and will respond to the needs of their market, providing a product which can provide traceability, product identity and assure the product meets food safety requirements. Managed correctly, the on-farm storage system can be a growing opportunity for markets to access quality products direct from the grower, minimising the risk to the end user and supply chain.

Strengthening national food safety for improved food security in Nigeria

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Abstract

A review of literature concerning the quality and safety of eight key staple products in Nigeria, West Africa, was made. These products included stored rice, maize, cashew, yam, cassava, millet, sorghum, and beans. Food safety notifications, both national and international concerning mycotoxins, pesticides, and quality in these foods are highlighted. Across these commodities, a significant number of non-conformances were found, arising from a combination of factors including lack of technical knowledge, supply chain management, and public institutional and policy challenges. The paper discusses the subsequent impact on health, well-being, and the economy. Current strategies aimed at improving food quality and safety in the country was also examined. Recommendations in addressing some significant issues are given.

Keywords: Food security, Nigeria, cowpea, safety, HACCP

The accepted definition of food security is when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life. In Nigeria, only recently, is food safety seen as an integral part of food security. According to a report by the Nigerian Federal Ministry of Agriculture and Rural Development (FMARD), the country is beset with an inability to either meet domestic food requirements or export agricultural products of the desired quality or safety standards. The agricultural sector in Nigeria suffers from inadequate infrastructure and resources, inadequate financial investments, weak food control systems, obsolete food regulation systems as well as inability to enforce compliance to international standards. The country lacks effective functioning, comprehensive food inspection mechanisms. Laboratory support is also woefully inadequate. Most supply chains in the country are inefficient, with poor traceability systems (The Agriculture Promotion Policy, 2016-2020), and thus national food control is weak.

Cassava, is one of a number of targeted export crops for 2016-2018 by Nigeria's Federal Ministry of Agriculture and Rural Development FMARD. In addition, rice, cowpea (beans), and maize are three of the five targeted domestic crops prioritized for 2016-2018. In terms of nutritional losses and safety, numerous studies have shown many marketed samples of rice across the Nigeria to contain harmful mould causing mycotoxins which is a public health concern (Makun et al., 2011; Egbuta et al., 2015). Maize samples across the country have been found to contain harmful levels of mycotoxins, particularly aflatoxins (Egbuta et al., 2015). The extent to which cashew nuts pose a real food safety risk owing to contamination during storage and marketing is not clear as there are few reported studies. Yam and cassava, however, are commonly processed into dried products using traditional methods. Dried yam derivatives such a 'Elubo' is common amongst the Yoruba tribe as a weaning food for babies. There have been many instances where Elubo has been found to contain elevated levels of mycotoxins, lead, and iron. Gari, a popular cassava derivative has again been found to contain aflatoxins in particular. Millet and sorghum samples across the country have also been shown to contain harmful mycotoxins in a number of studies. The Standards Organisation of Nigeria (SON) has drafted Codes of Practice for cowpea. However, maintaining cowpea quality is posing a significant challenge for farmers and traders, who may store for up to a year. Cowpeas vary according to the size of the grain, color of the skin, texture of the skin, and amount of damage resulting from insects. Consumers prefer beans with few insects present. This has led to the use of unauthorised pesticides in some cases. Due to the detection of high quantities of the unauthorised pesticide dichlorvos, the European Commission Implementing Regulation (EU) 2015/943 temporarily suspended the import of dried beans from Nigeria to the EU in 2015. The ban is still in place.

The Rapid Alert System for Food and Feed (RASFF) of the European Union which highlighted the safety concerns of Nigerian cowpeas was put in place to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation to food or feed. The legal basis of the RASFF is Regulation EC/178/2002 which highlights the principles and requirements of food law, and procedures relating to food safety. Concerning agricultural exports, processed or unprocessed, Nigeria does not export many products in significant volumes, with the exception of raw cocoa. Nevertheless, there were around 200

(RASFF) food-related notifications between the period January 2013 and March 2018 originating from Nigeria, over 50% of which were classified as ' serious', ~40% 'not serious' and around ~10% 'undecided'.   Cowpea (or beans) were responsible for over 40% of the serious notifications and resulted in border rejections. The non-conformances mainly concerned the presence and levels of unauthorised chemicals such as dicholorvos, cyhalothrin, chlorpyrifos, dimethate, proferiofos, and trichlorphos in cowpea though the National Agency for Food and Drug Administration and Control (NAFDAC) has produced guidelines and regulations for the import, manufacturing and distribution of pesticides and other chemicals, food additives and fats and oils, and port inspections (http://www.nafdac.gov.ng/). Certification and inspection of food and produce is carried out by the Standards Organisation of Nigeria (SON), Agricultural Quarantine Service (NAQS), NAFDAC, the Federal Produce Inspection Service (FPIS), or a combination of agencies. In addition, the Nigerian Food Safety and Applied Nutrition (FSAN) Directorate's mandate is to ensure that food manufactured, imported, exported, distributed, sold, and marketed in Nigeria meets the highest standard of Food Safety reasonably available and protect public health and consumer interests. It is evident that the environments in most rural areas which is where significant production, postharvest handling, and processing takes place, the monitoring and enforcement of safety standards, and marketing and storage conditions are not conducive to protecting the highlighted products from contamination.

A group of international food safety experts and regional representatives met in 2012 to determine the requirements for an African food safety authority, similar to the EU's European Food Standards Agency (EFSA), along with a communication system such as the RASFF. The effectiveness of RASFF is achieved by having a simple structure: it consists of clearly identified contact points in the Commission, EFSA, EFTA surveillance authority and at national level in member countries. One of the issues highlighted for the failure of the Nigerian government to address over fifty warnings on exported cowpeas before the ban was imposed, was the uncoordinated reporting structure to the various responsible agencies responsible for food safety (TAIEX Report, 2016). A representation of structure of the RASFF is depicted below (Figure 1).
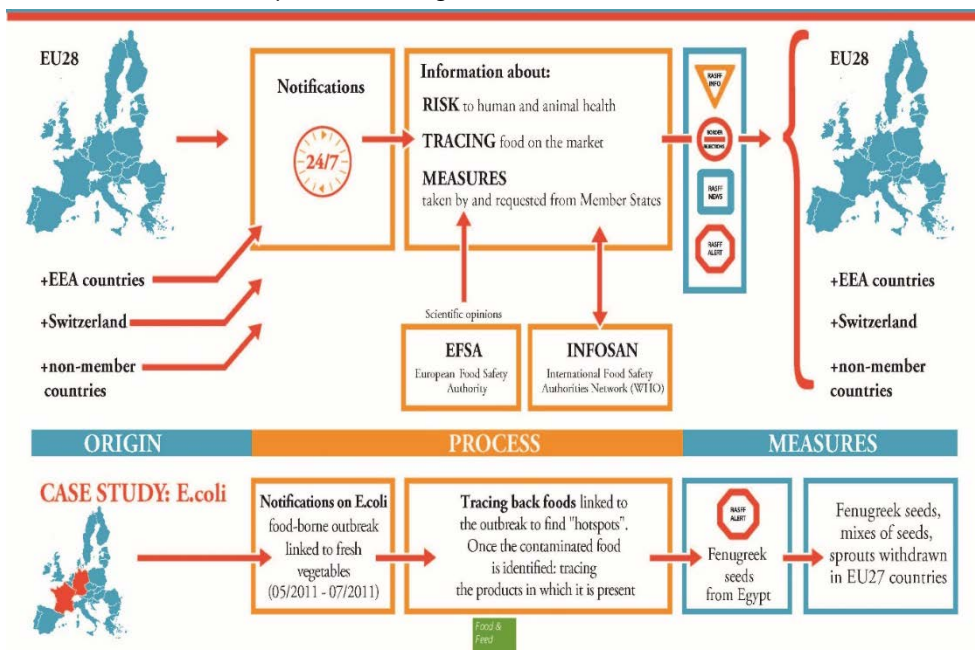
Figure 1: Workings of the European Rapid Alert System for Food and Feed

Source:https://ec.europa.eu/food/sites/food/files/safety/rasff/images/030614_how_does_it_work.jpg

Other serious RASFF alerts originating from Nigeria came in form of notifications relating to aflatoxin B1 (in nutmeg, groundnut, dried ugu leaves, dried bitter leaf, dried ginger, suya pepper, and sesame seeds in particular), various strains of Salmonella (in raw ginger, melon seeds, and sesame seeds in particular), *E. coli* (in ogbono, *Irvinia gabonensis*), and colouring Sudan Red in palm oil.

Unsafe food poses major economic risks. For example, the *E. coli* outbreak in Germany in 2011 was estimated to cost US\$ 1.3 billion in losses for farmers. Food-borne disease outbreaks, such as cholera, typhoid, lassa fever, chemical contamination like lead and mercury as well as mycotoxin poisoning, is thought to be responsible for thousands of deaths in Nigeria. However, the exact numbers will not be known owing to poor surveillance and reporting mechanisms. However, Odeyemi (2016) estimated that well over 35 million people (~20%) in Nigeria are affected by foodborne illnesses annually. The true economic and health impact of these illnesses is yet to be properly quantified.

Many African countries including Nigeria are becoming increasingly interested in regional and international trade, with demands to strengthen their Sanitary and Phytosanitary (SPS) capacity, and are consequently trying to address their national food safety issues. As a result, a common framework for the countries is being developed. International cooperation and technical support for African countries in areas of agriculture, food security and food safety is centred around the Comprehensive Africa Agriculture Development Programme (CAADP). In spite of such efforts, it is unlikely significant changes in Nigeria will be made over the next 5 years. This is reflected in the absence of a realistic budget set aside by successive governments to transform the food security situation in the country, including a detailed timebound, auditable, and accountable implementation strategy.

Over 70% of the food in Nigeria is produced in rural areas where farmers and traders often have not gone beyond secondary school education. The Nigerian government must therefore develop a practical and workable strategy to sensitize and educate such stakeholders on good hygiene practices. Achieving food safety begins with ensuring good agricultural practices in production at the farm level. Further, open markets and vendors with basic facilities should be in place. Many foodborne illnesses are well known to be preventable when adopting proper handling, processing, and storage methods for foods guided by HACCP principles. Therefore, the provision or accessibility of appropriate infrastructure to facilitate this such as clean water, power supply, good processing facilities, and physical market design, alongside regular basic HACCP and food handling training of food handlers and vendors should be made in order to significantly reduce the number of incidents of foodborne illnesses and deaths and support the national economy.

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Insect Pests and Fungal Pathogens in Maize Stored in Ghana

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Abstract

Insect infestations and mycotoxin contamination contribute to postharvest degradation and crop loss in sub-Saharan Africa, including maize stored in Ghana. Surveys were conducted to assess the prevalence of insect pests and fungal pathogens in stored maize from the major and minor cropping seasons (September to December and January to April, respectively) that was stored on-farm and in retail markets in Ghana. Results show differences between the major and minor storage seasons for on-farm sites and retail markets. The presence of internal feeders such as *Sitophilus zeamais* (Motschulsky) was positivly correlated with insect-damaged kernels and percentage weight loss. Levels of aflatoxin were generally greater than the established threshold of 15 ppb early in the major crop storage season, while fumonisins were generally lower than threshold levels of 4.0 ppm in on-farm sites and in the retail markets.

Keywords: maize, storage, management, insects, mycotoxins

Introduction

Stored-product insects are a major threat to food security in sub-Saharan Africa, with loss estimates due to insects and associated mycotoxins ranging as high as 70%, depending on the specific commodity, storage site, and management strategies (Hell and Mutegi, 2011; Affognon et al., 2015; Kumar and Kalita, 2017). Major insect pests include the larger grain borer, *Prostephanus truncatus* (Horn), maize weevil, *Sitophilus zeamais* (Motschulsky), lesser grain borer, *Rhyzopertha dominica* (Fauvel), and Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Darfour and Resentrater, 2016). Interactions between insect infestations and subsequent prevalence of mycotoxins are known to occur (Lamboni and Hell, 2009). Many African countries have set tolerances for mycotoxins; for example, the allowable limits for aflatoxin and fumonisin in Ghana are 15 ppb and 4.0 ppm, respectively (Ghana Standards Authority, 2013). In 2015 to 2016, surveys were conducted by the Department of Crop and Soil Sciences of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, to assess insect pest populations and mycotoxin content of maize stored on-farm and in commercial markets. The United States Agency for International Development (USAID), through the U.S. Government's Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss (PHLIL) funded this work conducted in Ghana.

On-farm sites

The survey of on-farm sites was conducted in the Middle Belt crop production region of Ghana, which is the primary grain-producing region of the country. Complete details of this survey are described in Danso et al. (2017). In this study, a total of 51 farm sites were sampled from three separate geographic areas within the Middle Belt of Ghana: Ejura, Sekyedumase, and Amantin. Sampling was conducted on maize on stalks just before harvest, maize piled on the ground as unshelled cobs pending threshing (shelling), or maize shelled and then stored for distribution to grain markets. For the field sampling and sampling from ground piles, maize cobs were collected from different areas within the piles, then dehusked and mixed into 500-g replicate lots, and the kernels stored for subsequent analysis and processing. Sampling for the third category, dried maize, was done by collecting 2-kg samples, then sub-dividing and mixing into 500-g lots as described for the first two categories. For each sample, temperature, moisture content, and relative humidity (r.h.)

were assessed using a John Deere moisture meter (Armstrong et al., 2017). Samples were sieved to collect live insects. Separate subsamples were taken to analyze for aflatoxin and fumonisin, using a standard Romer Labs test kit (romerlabs.com). Complete data analyses are given in Danso et al. (2017) and Armstrong et al. (2017), and will be summarized here in general terms.

Data for each species were summed over the entire year and analyzed first by Chi-Square analysis (SAS Institute) to determine differences between the three sites at each geographic area, and then summed by the months associated with major season storage (September to December) and with minor season storage (January to April) to determine differences between storage season. The predominant species collected were *S. zeamais* and *S. cerealella*, with ground piles as the site where most were found (Table 1) in the respective geographic areas. The other species in order of abundance were *Carpophilus dimidiatus*, *Cathartus quadricollis*, *Cryptolestes ferrugineus*, and *Tribolium castaneum*, with ground piles again being the site where most *C. dimidiatus* and *C. quadricollis* were found (Table 1). *Cryptolestes ferrugineus* and *T. castaneum* were the least prevalent species (Table 1).

Table 1. Total numbers of *S. zeamais* (SZ), *S. cerealella* (SC), *C. dimidiatus* (CD). *C. quadricollis* (CQ), *C. ferrugineus* (CF), and *T. castaneum* (TC) collected from three types of on-farm areas where maize was stored after harvest (Field, Ground Piles, and Post-drying) in Ejura, Sekyedumase, and Amantin during September to April. Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, *P*< 0.05).

Location	Site	SZ	SC	CD	CQ	CF	TC
Ejura	Field	85b	71b	37b	48c	7a	0a
	Ground pile	200a	128a	76a	126a	9a	8a
	Post-Drying	181a	35c	26b	70b	15a	8a
Sekyedumase	Field	69b	48b	52a	40b	2a	0a
	Ground pile	149a	76a	70a	75a	6a	2a
	Post-Drying	141a	25c	63a	41b	10a	4a
Amantin	Field	112c	86b	17b	59a	6a	7a
	Ground pile	236a	125a	46a	76a	9a	13a
	Post-Drying	189b	58c	49a	65a	9a	6a

More *S. zeamais* were collected in the minor season compared to the major season in all three locations, but the only difference for *S. cerealella* occurred in Amantin (Table 2). More *C. dimidiatus* were collected from the major season compared to the minor season, while differences were mixed or not significant for the other four species (Table 2). Temperatures in all locations and sampling sites ranged from about 27.0 to 34.5°C during the period of the experiment. Moisture content was more variable, but in general ranged between 15 and 27% during the major season, and declined from September to December, with the low moisture content levels predominantly in the post-drying samples (as expected) (Danso et al., 2017). MC in the minor season ranged from about 9 to 17%, with less variation between sites, but MC was usually lowest in the post-dried samples. Neither temperature nor moisture content were correlated with the insect populations (P < 0.05). Numbers of *S. zeamais* were positively correlated with percentage of IDK and with kernel weight loss (P < 0.05). This was the primary species contributing to IDK and weight loss, as it is an internal feeder.

Average aflatoxin levels at all three locations were well above the tolerance level of 15 ppb during the major season, but ranged between 0.6 and 3.6 ppb during the minor season (Table 3). Fumonisin levels were below the tolerance level of 4 ppm.

Market Sites

The survey of market retail sites was also done in the Middle Belt of Ghana, in the geographic regions of Ejura, Techiman, and Amantin. The maize that was sampled was bagged mixed-variety white maize. Samples were taken monthly from September to April by randomly selecting 100-kg polypropylene or jute bags, inserting a 1.2-m grain probe into the bag (Seedboro, Chicago, IL, USA), and withdrawing a sample of approximately 350 g. Three samples were taken from the bag, mixed, and 500 g weighed out for sampling for insects. In selected months, a second 500-g sample was

collected for mycotoxin analysis, as described above. The maize was sampled from the same market location, but not from the same bags each time as this was an active retail market. Maize was also sampled for temperature, moisture content, and r. h. Collection procedures, sample preparation, and methodology for collecting insects, is the same as described above. More detailed descriptions of methodology are found in Danso et al. (2018), along with complete depictions of the results. Data from this study are re-analyzed and summarized here to present important findings from the market survey.

Table 2. Total numbers of *S. zeamais* (SZ), *S. cerealella* (SC), *C. dimidiatus* (CD). *C. quadricollis* (CQ), *C. ferrugineus* (CF), and *T. castaneum* (TC) collected in Ejura, Sekyedumase, and Amantin during the Major and Minor seasons (data for the three sites combined). Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, *P*< 0.05).

Location	Season	SZ	SC	CD	CQ	CF	TC
Ejura	Major	117b	111a	97a	95b	10a	1b
	Minor	349a	123a	42b	148a	21a	15a
Sekyedumase	Major	109b	73a	106a	72a	13a	2a
	Minor	250a	76a	79b	84a	5a	4a
Amantin	Major	121b	54b	94a	85b	7a	2b
	Minor	416a	215a	18b	115a	17a	24a

Table 3. Average aflatoxin values (ppb, means ± SE) during the major and minor seasons in Ejura, Techiman, and Amantin. Data from Danso et al. 2017. All comparisons by season were significant (*P* < 0.05, SAS, Tukey's Honestly Significant Difference Test).

	Major Season	Minor Season
Ejura	39.2 ± 9.1a	3.2 ± 0.1b
Sekyedumase	24.8 ± 0.8a	3.6 ± 3.6b
Amantin	23.4 ± 4.0a	3.6 ± 0.2b

There were six predominant stored-product insect species collected from the market samples: *S. zeamais*, *C. ferrugineus*, *C. quadricollis*, *S. cerealella*, *T. castaneum*, and *C. dimidiatus*. Data for each species were summed over the entire year and analyzed first by Chi-Square analysis (SAS Institute) to determine differences between markets, and then summed by the months associated with major season storage (September to December) and with minor season storage (January to April) to determine differences between storage season. The order of species abundance, in terms of total numbers, is arranged from left to right in Table 4, with *S. zeamais* as the predominant species. Varying levels of these six species were found in all markets, with no consistent differences between markets (Table 4).

Table 4. Total numbers of *S. zeamais* (SZ), *C. ferrugineus* (CF), *C. quadricollis* (CQ), *S. cerealella* (SC), *T. castaneum* (TC), and *C. dimidiatus* (CD) collected from three different maize markets in Ghana during September to April. Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, *P*< 0.05).

Market	SZ	CQ	SC	TC	CF	CD
Ejura	816b	192a	112c	121a	100b	80a
Techiman	960a	139b	180a	125a	67c	37b
Amantin	930a	116b	207a	85b	144a	62a

Data were then summarized to compare total numbers during the major versus the minor season. For 14 out of the 18 comparisons (6 species x 3 markets), there were more insects collected during the major versus the minor season, and only one instance where there were more collected during the minor versus major season (*C. quadricollis* in the Amantin market) (Table 5).

Table 5. Total numbers of *S. zeamais* (SZ), *C. ferrugineus* (CF), *C. quadricollis* (CQ), *S. cerealella* (SC), *T. castaneum* (TC), and *C. dimidiatus* (CD) collected from each market during the major season vs the minor season. Sum totals within columns for each market followed by different lower-case letters are significantly different (Chi Square, *P*< 0.05).

Market	Season	CF	SZ	CQ	SC	TC	CD
Ejura	Major	91a	702a	113a	90a	77a	77a
	Minor	8b	116b	73b	22b	43b	3b
Techiman	Major	60a	786a	82a	80a	58a	31a
	Minor	8b	174b	57b	100a	67a	6b
Amantin	Major	136a	811a	28b	140a	49a	58a
	Minor	7b	119b	88a	67b	36a	5b

Again, temperature combined for all markets during the storage months was about 27 to 32°C, well within favorable limits for insect population development. Moisture content combined for all markets ranged from a high of approximately 15% in September to a low of about 9% in December, then began to increase until April. However, most of the *S. zeamais* collected during the major season storage were collected in November and December (see Danso et al., 2018), the months with the lowest MC. There was no correlation between temperature or MC and insect pest populations ($P < 0.05$). Average aflatoxin levels at all three market sites were far above the tolerance level of 15 ppb during the major season, but less than 4 ppb during the minor season (Table 6). Fumonisin levels were below the tolerance level of 4 ppm.

Table 6. Average aflatoxin values (ppb, means ± SE) during the major and minor seasons in markets in Ejura, Techiman, and Amantin. Data from Danso et al. 2018. All comparisons by season were significant ($P < 0.05$, SAS, Tukey's Honestly Significant Difference Test).

	Major Season	Minor Season
Ejura	66.2 ± 14.6a	3.4 ± 0.4b
Techiman	58.9 ± 14.2a	2.9 ± 0.2b
Amantin	28.0 ± 8.7a	3.1 ± 0.2b

Conclusions

Temperature was generally within 27 to 32°C during these studies, which is within the optimum range for development of the collected species (Howe, 1965; Fields, 1992), and hence was not correlated with the insect populations. The predominant stored-product insect collected in the on-farm and market sites was *S. zeamais*, but it was surprising that no *P. truncatus* were collected given the extensive presence of this species in stored maize, particularly cob-stored maize, in western Africa. Few *R. dominica* were collected as well, and this species is also listed as one of the main storage pests in Africa. More *S. zeamais* were collected during the minor season compared to the major season in the on-farm sites, but the reverse was true for the market sites. During the minor season, the maize is left on stalks for long periods to dry, in contrast to the major season, which is a possible explanation for the greater incidence of S. zeamais during the minor season. The greater infestation in the market sites during the major season versus the minor season may be because the maize in the market sites could have already been infested when the maize was brought to those sites. In addition to the seemingly greater insect populations in maize with high MC, drying of newly-harvested maize is also essential to reduce fungal contamination. Results show improved storage management, which includes integrated pest management strategies for drying and storing maize, may be necessary to limit economic losses and ensure food security.

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Low-Cost Instrument to Measure Equilibrium Moisture Content of Bagged and Bulked Grain

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Introduction

Storage of grain in bags is common in Africa, Asia, and many other less developed countries. Because of this an *in situ* grain bag probing method is well-suited for moisture content (MC) measurement. A low- cost meter was developed under a USAID project to reduce post-harvest loss (PHL)(Fig 1). The meter, referred to as the PHL meter, measures the MC of maize and other grains based on relative humidity (RH) and temperature (T) measurements obtained by a small digital sensor located in the tip of a tubular probe that can be inserted into bags of grain or other grain bulks (Armstrong et al., 2017). Measurements are used in equilibrium moisture content (EMC) equations programmed into the meter to predict MC. A handheld reader connected to the probe provides a user interface.

Keywords. Equilibrium moisture content, Grain storage, Maize, Moisture content, Moisture meter, Post-harvest

The PHL moisture meter was evaluated based on laboratory studies in the U.S. and field studies in Ghana. Meter readings from field studies were compared to two commercial meters, a John Deere Chek-Plus-SW08120 grain moisture tester and a DICKEY-john GAC®2100 Agri meter. The John Deere portable moisture meter is a low cost meter by developed country standards (~US$250, 2016 price); the GAC 2100 benchtop moisture meter is an approved moisture tester by the U.S. Grain Inspection, Packers and Stockyards Administration (GIPSA) and has been a highly regarded and used electronic meter. Laboratory studies indicated that the PHL moisture meter may require up to six minutes to take a measurement due to the time required by the probe tip and sensor to equilibrate to grain conditions. Methods to reduce the measurement time by measuring temporal equilibration rates were developed. These can be programmed into the reader to shorten measurement time for many conditions. The accuracy of the PHL moisture meter was comparable to the GAC 2100 moisture meter for maize below 15% MC_{wb}. Average differences showed a positive offset of 0.45% for the PHL meter relative to the GAC 2100. The PHL meter provided an effective tool to probe bulk grain and bags.

A second generation (2G) PHL meter, Fig 2, has been developed with an emphasis on reducing manufacturing cost, improving the user interface, and increasing battery life. The 2G device also incorporates Bluetooth technology which can potentially reduce the cost further by eliminating the user screen.
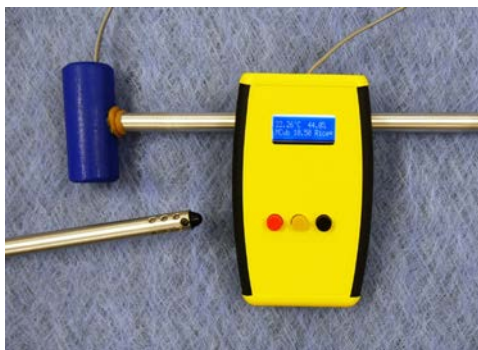
Fig. 1 PHL moisture meter based on measuring equilibrium moisture content.

Figure 2. Second generation PHL moisture meter

To expand the scope of the PHL meter, two crops, sesame and chickpea, were studied and included into the meter software by developing EMC equations specific to these crops. Both of these crops are important crops for Ethiopia as both are major exports providing small farmers and the country much revenue. There is a lack of information on fundamental equilibrium moisture content (EMC) relationships for these products which would help facilitate better monitoring and storage. For this reason EMC adsorption and desorption prediction models based on temperature (T) and relative humidity (RH) were developed for the modified Chung-Pfost and modified Henderson models for kabuli chickpea (KC), black sesame (BS), and white sesame (WS) seeds. Samples for adsorption and desorption tests were conditioned to various moisture content (MC) levels for EMC tests. Samples of approximately 500 g were placed in multiple sealed enclosures equipped with T and RH sensors, placed in an environmental chamber, and exposed to three temperatures (15, 25, and 35ºC). For KC samples, the MC_{db}% ranges used for model development were 11.6-19.5% and 8.9-16.9% for adsorption and desorption, respectively; for BS, the range was 5.0-8.7% and 4.3-6.9%, respectively, and for WS, 4.2-8.7% and 3.5-7.6%, respectively. Nonlinear regression was used to determine model coefficients for the modified Henderson and modified Chung-Pfost equations. Prediction statistics for KC adsorption and desorption models yielded a SEE of 0.53 and 0.68% MC_{db}, respectively; for BS, SEE was 0.23 and 0.13%, respectively; and for WS, SEE was 0.28 and 0.25%, respectively. Model coefficients were incorporated into the PHL moisture meter.

EMC equation development for additional crops is needed which may include pulses, other grains and processed food products that span harvesting, drying, storage, conditioning, and processing operations.

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Stored Grain Protection: cases studies in Portugal

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Abstract

Considering the edibility of insects' species associated with storage ecosystem, chemical control methods can be easily replaced by environmental and economically sustainable alternatives.

Use of biogenerated atmospheres is an inexpensive method that tolerates insect presence. In Portugal, during one year, hermetic bags were used to store paddy under 65-75-85% relative humidity (RH) and 14-17-24ºC temperatures. Brown rice infested with *Sitophilus zeamais* adults was placed inside the hermetic bags.

Biogenerated atmosphere was naturally produced inside the hermetic bag, at 85% RH, with low O_2 and high CO_2 contents, showing that *S. zeamais* can survive but has no progeny at 14º-17ºC, or attained 100% mortality before producing progeny at 24ºC. The most abundant fungi isolated were *Alternaria alternata* and *Epicoccum nigrum*. The results showed the importance of the RH on changes in atmospheric gas content of paddy, due to biological agents' activity.

Analysing the edibility of insects species associated with stored grain, preliminary studies were carried out to evaluate the nutritional value of immatures stages of *Tribolium castaneum*. Larvae of *T. castaneum* had a content of 21.4% protein, 9.1% lipids, 8.8% fiber, and a relevant content of eight essential amino acids and also manganese and copper. The edibility of insects must be consider given their high nutritional value, low emissions of Green House Gases (GHGs), low requirements for land, and by reducing and mitigating the need for chemical control.

Keywords: insect edibility, paddy, hermetic storage, biogenerated atmosphere, *Tribolium castaneum*, *Sitophilus zeamais*

1. Introduction

Rice in Portugal and in Europe in general is a seasonal crop. Among pest species of stored rice, *Tribolium castaneum* (Herbst, 1797) is common but *Sitophilus zeamais* Motschulsky, 1855 is considered the key pest. The most common practice to control insects of stored grain is the use of fumigants to prevent and suppress insect development. The development of resistance of these two insect species to fumigants has decades of history (Champ and Dyte, 1977), and consumer concern over the use of pesticides in food oblige the research for alternative methods of insect control in order to get food product free of pesticide residues. Also, insect protein can be a good alternative to livestock production because it is more efficient feed/food conversion, lower greenhouse gas and ammonia production, less land area needed, and has potential to be grown on organic byproducts (Huis et al., 2013). Concerning producing benzoquinone-containing defensive secretions by *T. castaneum*, IARC (International Agency for Research on Cancer) officially states that no epidemiological data are available on the carcinogenicity of 1,4-benzoquinone, which consequently is not classifiable as to its carcinogenicity to humans (IARC and WHO, 1999).

On the other hand, fungal mycotoxin producers are the major cause of loss during long-term storage periods without efficient control of temperature and, above all, moisture content of stored grain (Christensen and Kaufmann, 1969; Wicklow, 1995; Fleurat-Lessard, 2017). One of the most important phenomena caused by fungi is the "hot spot" previously associated with insect infestations but currently identified as a consequence of fungal development in situations of poor storage conditions, such as high moisture content. The microclimate generated, i.e., temperature and moisture, attracts the insect populations (Fleurat-Lessard, 2017).

Based on these assumptions, preliminary trials were developed to analyze the edibility of immatures stages of *T. castaneum*. Also, trials were carried out using hermetic bags to store paddy rice, as a green and inexpensive alternative method to control insects and fungal development, under 65-75-85% RH and 14°-17°-24°C. Moreover, rheological tests were performed on a MARS III controlled-stress rheometer to analyse the viscoelastic functions of the respective rice pastes.

The main objective is considering hermetic storage as a sustainable technology to reduce insect presence and fungal development, mitigating pesticide effect in food and feed.

2. Material and methods

2.1. Modified atmosphere

2.1.1. Sample preparation

Experiments were conducted in a warehouse located in Alcácer do Sal, Portugal. Three trials were carried out: T_1 the first trial, four months, temperature average 14°C (December to April); T_2 the second trial, seven months, temperature average 17°C (December to July); T_3 the third trial, four months, temperature average 24°C (July to November).

For experiments, GrainPro° SuperGrainbag° Farm™ were used to store two rice varieties: Ronaldo, a japonica variety, and Sírio, an indica variety. Paddy rice, was stored in jute bags, as the control.

In all experiments, the two varieties were stored as paddy and submitted to three different relative humidities: 67, 75 and 85% RH, at three different average temperature (14, 17 and 24°C). The RH and temperature were monitored by Hobo ® Data loggers, with probes placed inside the bags.

Both jute bags and SuperGrainbag° have a capacity of 50 kg, and for each treatment and variety three replications were carried out, for a total of 48 samples per trial.

At the end of all experiments, CheckpointII Portable O_2 and CO_2 Gas Analyzer was used to assess gas contents, at the bottom and top of each bag, totalizing six measures per treatment. The gas content is expressed in % by volume in air. After, the bags were opened and paddy samples were collected, samples were taken to be dehusked and milled to analyze water activity (a_w), with three replications per treatment, and rheology tests were performed. HygroPalm HP23 Rotronic was used to estimate a_w.

2.1.2. Bioassays

To evaluate insect response to each treatment, *Sitophilus zeamais,* the maize weevil, was chosen because it is the main pest of rice in Portugal (Carvalho et al. 2012). *Sitophilus zeamais* was originally collected from Portuguese rice mills and reared in climatic chambers, at 25±2°C and 70% RH, at laboratory of Instituto Superior de Agronomia, University of Lisbon.

For experiments, 20 g of brown rice were infested with ~20 one-week-old *S. zeamais* adults and placed inside paper bags. One paper bag was set up inside of each paddy bag, totalling three replications per treatment.

2.1.3. Mycoflora analysis

From samples collected at the end of T_1 and T_2 trials, three samples were collected in sterilized containers and taken into the laboratory. In the laboratory, the rice samples were subdivided into samples with 100 grains. The surfaces of these grains were disinfected with 1% sodium hypochlorite for five minutes, as described by Pitt and Hocking (2009) and Magro et al. (2008). Ten disinfected grains were placed on Petri dishes with 20 mL of Potato Dextrose Agar (PDA) medium with chloramphenicol (1%). There were ten replicates for each sample.

2.1.4. Rheology measurements

Rheology tests were carried out for T1 trial and performed on a MARS III controlled-stress rheometer (Haake) coupled with a temperature controlling Peltier unit, using 35-mm-diameter serrated parallel plates and 0.5-mm gap. Aqueous flour suspensions (10% w/w) were held 5 min at 20ºC, between the plates, before testing. Stress sweeps were performed to ensure that all measurements were within the viscoelastic region. Then, the rheological study using SAOS (Small-Amplitude Oscillatory Shear) was performed according to previously optimized conditions (Torres et al., 2014).

2.2. Tribolium castaneum analysis

Moisture content was determined by placing approximately 2 g of each sample in a drying oven at 60ºC for at least 48 hours until constant weight. Protein was determined using the Dumas method as described by Saint-Denis & Goupy (2004), using a LECO FP-528 (LECO, St. Joseph, USA) calibrated with EDTA. The conversion factor of 6.25 was used to calculate total protein values. Total fat was determined by extraction with petroleum ether of around 0.5 g of sample using a Soxtec HT apparatus, and total fibre was determined according to the Weende method by extraction of non-fibre components from 0.5 g sample with sulphuric acid (0.2 M) and potassium hydroxide (0.2 M), followed by acetone washing in a FibreTec apparatus. Amino acids determination was performed by HPLC (Agilent 1100 HPLC, Agilent, Palo Alto, USA), using a Phenomenex Gemini ODS C18 110 Å column (4.6 × 150 mm, 5 µm, Phenomenex Inc., Torrence, USA) and a fluorescence detector according to the method described in Henderson et al. (2000). Mineral elements were determined by flame atomic absorption spectrophotometry (Unicam Solaar M, Thermo Electron GmbH, Dreieich, Germany) following acid digestion of 0.5 g of dried sample in 7.5 mL nitric acid and 2.5 mL hydrochloric acid using an SCP Science heat block (1.5 hours at 110 ºC).

2.3. Data analysis

All the computations and graphs were performed with software R (R Core Team, 2017). Function lm was used to fit and test for significance of the linear models.

3. Results and Discussion

3.1. Hermetic storage and *Sitophilus zeamais*

The average temperatures were similar for the same experiment and type of rice, but the individual days showed different values that are summarized in Table 1: T_1 had a temperature average of 14ºC, and was never higher than 20ºC; T_2 had an average temperature of 17ºC with 43 days above 20ºC and 13 days above 25ºC; and T_3 had a temperature average of 24ºC with 103 days above 20ºC of which 60 days were over 25ºC and 34 days above 27ºC.

Table 1- Number of days of each experiment, mean temperature, and number of "hot days".

Trial	[T_1]	[T_2]	[T_3]
* days	139	215	121

Temperature average (ºC)	14±1	17±4	24±3
* days with Temp>20ºC	0	43	103
* days with Temp>25ºC	0	13	60
* days with Temp>27ºC	0	0	34

*days- the number of days with a determined mean temperature.

Tables 2 and 3 report the results of the population growth of *S. zeamais* in each type of rice, Indica and Japonica, under different conditions of temperature, RH, atmosphereic composition, and water activity. When we consider the progeny, the first 20 weevils adults used, per replication, were eliminated from results, to better understand if there were offspring produced during trials. Under hermetic conditions at low O_2 and high CO_2 contents in trials in which the RH was 85%, at all temperatures there were no progeny although the initial insects were still alive. In trials at RH of 75% under hermetic conditions at medium oxygen and CO_2 contents, progeny were found, and the number of F1 individuals was dependent on temperature. In trials at RH of 67% under hermetic conditions, there was no change of the atmospheric content, and at 14 and 17ºC there were fewer adults alive than the trials at 75% RH. The population showed a significant increase when the temperature average was 24ºC.

Table 2 – Indica rice. Average number of *Sitophilus zeamais* adults: alive, dead, and progeny (alive + dead-20) for each pair of temperature and relative humidity (RH) conditions. Values correspond to averages across the replications, with small bags containing rice initially contaminated with 20 insects. The amounts of oxygen (O_2, %), carbon dioxide (CO_2, %) and water activity (a_w, %) at the end of each experiment are also shown.

Trial	Temp (ºC)	RH (%)	CO_2	O_2	a_w	*S. zeamais* alive	*S. zeamais* dead	Progeny
[T_1]	14	67	1.2	20.4	0.5	4.3	44.5	28.8
	14	75	3.9	18.3	0.5	13.7	37.3	31.0
	14	85	20.6	4.4	0.5	10.3	7.7	0.0*
[T_2]	17	67	1.5	19.4	0.4	8.5	33.8	22.3
	17	75	9.2	10.5	0.4	24.5	46.0	50.5
	17	85	23	0.8	0.5	0.7	20.3	1.0
[T_3]	24	67	1.4	19.9	0.6	84.0	93.3	157.3
	24	75	6.5	14.4	**0.7**	86.3	64.0	130.3
	24	85	21.8	2.6	**0.7**	0.0	20.0	0.0

*at end less than 20 insects were found

Table 3 – Japonica rice. Average number of *Sitophilus zeamais* adults: alive, dead, and progeny (alive + dead-20) for each pair of temperature and relative humidity (RH) conditions. Values correspond to averages across the replications, with small bags containing rice initially contaminated with 20 insects. The amounts of oxygen (O_2, %), carbon dioxide (CO_2, %) and water activity (a_w, %) at the end of each experiment are also shown.

Trial	Temp (ºC)	RH (%)	CO_2	O_2	a_w	*S. zeamais* alive	*S. zeamais* death	Progeny
[T_1]	14	67	1.3	20.3	0.6	1.3	69.7	51.0
	14	75	3.5	18.7	0.5	12.5	41.0	33.5
	14	85	17.3	7.4	0.6	27.7	9.3	17.0
[T_2]		67	1.6	19.3	0.5	3.3	32.3	15.7
		75	4.0	16.9	0.5	36.5	20.0	36.5
	17	85	26.7	0.2	0.4	0.0	25.3	5.3
[T_3]	24	67	1.4	19.8	0.6	108.0	71.0	159.0
	24	75	3.8	17.8	0.6	16.0	43.0	39.0
	24	85	22.0	3.8	0.7	0.0	20.0	0.0

3.2. Hermetic storage and mycoflora analysis

Thirteen species of fungi were found on paddy hermetically stored during T_1 trial with average temperatures of 14ºC, and 20 species were found after seven months of storage with average temperatures of 17ºC [T_2]. Fungi identified in more than 20 grains were *Alternaria alternata*, *Aspergillus flavus, A. fumigatus, A. niger, Epicoccum nigrum, Nigrospora.* sp, and *Rhizopus* sp. After four months of storage at 14ºC (T_1), the majority of the fungi identified were associated with field species.

After seven months of storage and higher average temperature (17 ºC) (T₂), *Aspergillus spp.* became more abundant and dominant relative to field fungi species. From all samples collected, the a_w was lower than 0.6 and was considered secure for the non-development of mycotoxins, as the husk is most certainly a barrier to protect grain from water changes (Fig.1).

3.3. Hermetic storage and rheological measurements

Rheological tests were carried out at end of trial T₁. Figure 2 shows the gelling point (when G' overcomes G'') of 10% (w/w) pastes of japonica and indica rice flour heated from 20 to 90ºC, from paddy flours kept under the three different RHs. For the japonica rice paste, there is a slight decrease in the gelling point temperature with the increase of RH: hermetic storage at 67% RH and control started gelling at about 87ºC; hermetic storage under 75% and 85% RH, started gelling at 85 and 82ºC, respectively. For the indica rice pastes, the results were reversed: the control and hermetic storage at 85% RH started gelling at 85ºC, and both hermetic conditions of 67% and 75% RH paddy pastes started gelling at 82ºC.

Figure 3 shows the mechanical spectra of the paddy pastes by variety and RH conditions. The paste obtained from rice stored at lower RH values, i.e., 67%, is slightly more structured than rice stored at higher humidity and control, i.e., the values of the viscoelastic parameters G' (storage or elastic modulus) and G'' (loss or viscous modulus) are slightly higher for the pastes from rice stored under lower RH conditions, showing a better structured paste (Nunes et al., 2006).

3.4. *T. castaneum* chemical analysis

Singh and Sinha (1977) studied the changes in protein levels in the developmental stages of *Sitophilus oryzae* (L.) and *S. granarius* (L.) reared on whole wheat at 30°C and 70% RH. In S. *oryzae* life cycle, the average values of this nutrient (percentage of dry body weight) ranged between 50.0 (L4) and 78.3% (adult). In *S. granarius* life cycle, the comparable protein values ranged between 47.0% (prepupa) and 75.7% (adult). The protein contents in S. *oryzae* increased up to the prepupal stage, declined slightly in pupae, and increased again in adults; whereas in *S. granarius* it constantly increased through the life cycle stages up to adult emergence.

The larvae of *T. castaneum* also is particularly rich in several essential elements confirming that the enrichment of different flours with this insect will improve its global nutritional value.

The values for the nutritional analysis presented in this work (Table 4) are typical of other insects from the same order, with a relatively high protein content, although the total fat is lower than the usual values, taking into account that these values are highly dependent on species and on the feed type (Ghosh et al., 2017).

Regarding the amino acid analysis, the larvae of *T. castaneum* have a higher amino acid content compared to the regular flour confirming that the presence of this insect will enrich it in several amino acids. Leucine was the most abundant essential amino acid followed by valine and threonine. Among these three essential amino acids, threonine is strictly indispensable since it is not transaminated and its deamination is irreversible (Belluco et al., 2013).
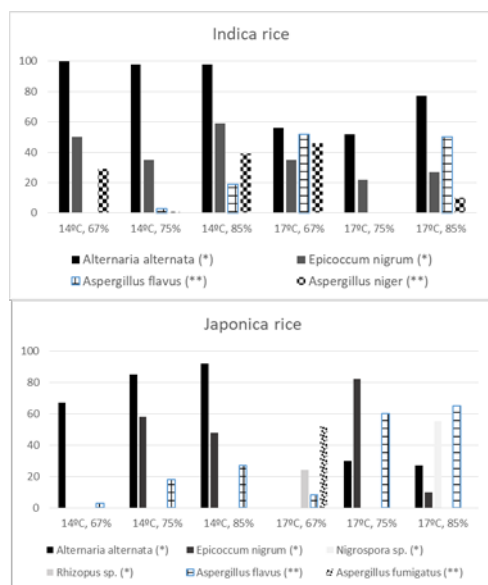
Figure 1 - Fungi incidence in 100 grains tested in indica and japonica rice stored for four months at 14ºC temperature average and stored for seven months at 17ºC temperature average. (*) field fungi (**); storage fungi.
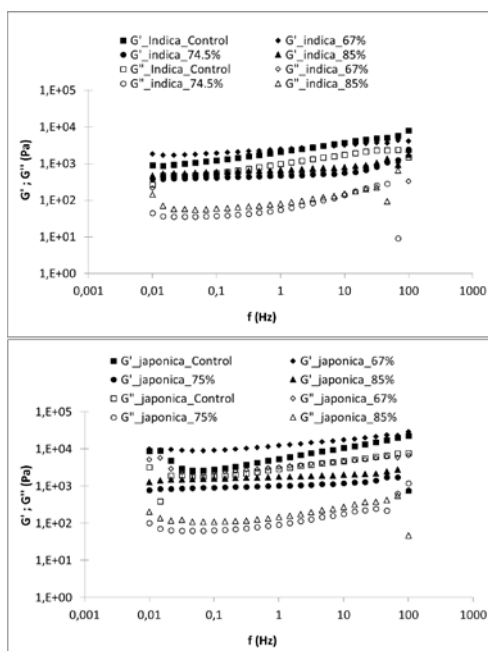
Figure 2- Indica and japonica rice: Mechanical spectra at 20ºC (G'- elastic modulus; G''- viscous modulus).

Table 4 – Chemical and nutritional composition of *Tribolium castaneum* larvae.

Water content (%)	42.23 ± 2.11	Total fat (%)	9.07 ± 4.09
Protein (%)	21.37 ± 0.45	Fibre (%)	8.76 ± 1.07
Amino acids (%)		**Mineral elements (mg/kg)**	
Histidine	0.64 ± 0.06	Fe	71.3 ± 8.1
Threonine	0.97 ± 0.06	Cu	6.1 ± 1.2
Valine	1.00 ± 0.07	Zn	48.2 ± 1.7
Lysine	0.84 ± 0.10	Mn	6.7 ± 0.5
Methionine	< LQ	Mg	41.5 ± 1.8
Leucine	1.23 ± 0.07	Na	199.5 ± 25.2
Isoleucine	0.71 ± 0.06	K	219.1 ± 8.6
Tryptophan	< LQ		
Phenylalanine	0.90 ± 0.07		

LQ: limit of quantification, 9 pmol/μL
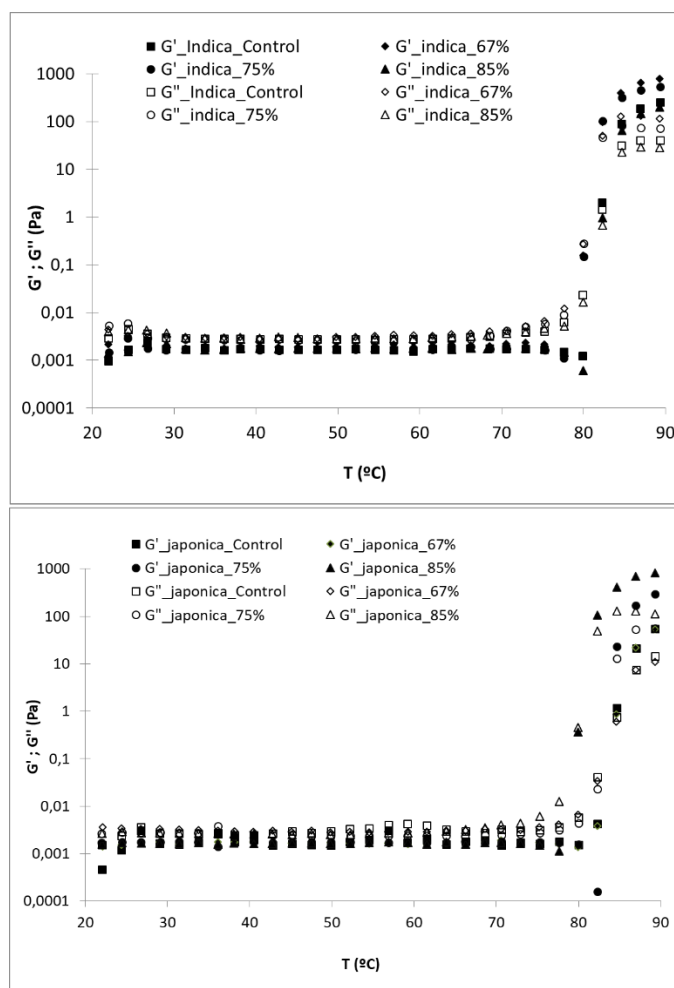
Figure 3 - Indica and japonica: **Starch gelatinization**. Heating from 20ºC to 90ºC at 2ºC/min (G' elastic modulus; G'' viscous modulus).

López-Vergé et al. (2013) evaluated the protein content of *Tenebrio molitor, Ephestia kuehniella*, and *Tribolium confusum* on a dry matter basis, and the results obtained in crude protein ranged from 42.47 to 58.77% and in ether extract ranged from 24.99 to 34.13%, supporting the idea that they can be incorporated into the feed diet. The same authors carried out a feeding trial in order to compare the effect of adding larvae of insect species *Sitophilus zeamais* to the diet on the performance parameters. Animals fed the insect-infested diet had higher final body weight (*P*=0.015) and higher average daily feed intake (ADFI, P=0.015) compared to animals fed the untreated (control) diet (López-Vergé et al., 2013).

Reuters announced in November 23, 2017, that a Finnish baker launched bread made from crushed crickets explaining that it «*offers consumers a good protein source and also gives them an easy way to familiarize themselves with insect-based food*».

4. Conclusion

Concerning the studies of hermetically stored paddy under different environmental conditions, the results showed that RH is the key factor for the modified atmosphere, attained only on trials under

75 and 85% RH at any average temperature, mainly due to respiration of paddy and fungi. As the time of storage increased, more fungal species developed, mainly associated with stored products. However, considering the determined a_w value was always below 0.6, the environment was considered safe from mycotoxin development. The modified atmosphere produced inside the hermetic bag with low O_2 and high CO_2 contents at 85% RH and at average temperatures of 14 and 17ºC demonstrated that *S. zeamais* can survive but produce no progeny. At the same conditions but higher average temperature of 24 ºC, ~100% mortality of *S. zeamais* occurred but progeny still were produced.

The increase in respiration rate at higher RH values, reduced the viscoelastic functions and changed the starch gelatinization point of indica and japonica rice.

The enrichment of different flours with larvae of *T. castaneum* can improve its global nutritional value.

It is our intention to break the misconception that insects are always harmful and to contribute positively to the concept of edible insects in the sector of stored products. It can be used for the incorporation of their protein as food/feed ingredients, and also to allow some tolerance to their presence during the storage period in order to suppress the use of chemical control methods, contributing to the sustainability of the environment and human health and animal welfare.

Following this concept, hermetic storage can be one of the sustainable and green methodologies to be used on grain storage. Storing paddy hermetically at low RH, did not change atmospheric content but maintained the viscoelastic functions of the rice pastes, low fungal incidence, and reduced insect population growth.

Acknowledgments

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Survey of dermestids of the genus *Trogoderma* in grain storages in Spain

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Several *Trogoderma* species of the family Dermestidae are important pests of stored products. Among them, *Trogoderma granarium* Everts, is one of the most harmful pests of cereal grains for all countries that are major exporters of agricultural commodities and for their trading partners (Athanassiou et al., 2016). Therefore, in most countries a very strict quarantine legislation exists to prevent the introduction of this pest (Myers and Hagstrum, 2012).

Trogoderma granarium is considered an endemic species in the southern Mediterranean region, and it has been intercepted or eradicated in many European countries. Nevertheless, global warming and the increase in international trade of raw materials are favoring its expansion. The establishment of *T. granarium* can likely occur in countries with more than 4 months per year with an average temperature higher than 20ºC (EPPO, 2011). However, temperatures in storage facilities can be higher than in open field, so there is also a risk of establishment in colder climatic areas.

According to the EPPO, *T. granarium* is present in Spain with a restricted distribution. But, while it has been detected in the country, there is no evidence of its establishment. It was found in 1952, but, after that record, there have been no new records of its presence (Banks 1977, Rebolledo and Arroyo 1993). Therefore, it is important to know whether *T. granarium* is present or not in Spain to take the necessary measures for its eradication or management. In the present study, a survey of the species of the genus *Trogoderma* has been conducted to determine the species present in grain storage facilities in Spain and their phytosanitary importance.

In 2016 and 2017, we sampled with traps baited with the pheromone of *Trogoderma* spp. in fifteen warehouses and grain silos along the Spanish Mediterranean coast. Monthly samples were collected in each sampling location using five PC floor traps placed in the storage facilities and three probe traps inserted in the grain piles. Taxonomic keys were used for the identification of the specimens found, as well as *T. granarium*-specific molecular markers by conventional PCR analysis.

A total of 3,276 *Trogoderma* specimens were captured in almost all locations sampled. However, no *T. granarium* were found. The majority of them were *T. inclusum* Leconte, and some were *T. variabile*

(Ballion), which can be distinguished by the male genitalia (Green, 1979) (Fig. 1). Captures were particularly abundant from June to September. These species are considered secondary pests affecting stored grain, processed dry foods, and animal feeds. Some other pest species were captured with both type of traps, including the Coleopteran *Anthrenus* sp., *Sitophilus* sp., *Rhyzopertha dominica* (F.), *Oryzaephilus* sp., and *Tribolium*. sp. According to our results, the presence of *T. granarium* in the sampled areas is not confirmed. Therefore, this species does not seem to be established in Spain.

Fig 1. Male genitalia of *T. inclusum* (left) *T. variabile* (center) and *T. granarium* (right):

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Performance Assessment off a Commercial Scale Solar Biomass Hybrid Dryer for Quality Seed Maize Production

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Abstract

Though several maize varieties have been developed and introduced over the years in Ghana, farmers still face challenges of access to quality seed maize. Among the major constraints is lack of proper drying systems to quarantee quality of seed produced. Peculiar to most parts of Africa, drying of maize in the open, on bare ground along shoulders of roads is still a common practice in Ghana. In this study, a 5-tonne capacity solar biomass hybrid dryer was developed for drying maize for seed and food/feed in Ghana. Effect of drying air temperature in the dryer on the physiological quality and germination of maize kernels was investigated. Maize grains were dried in the open sun simulating farmers practice and using the dryer at 4 varying levels (L1, L2, L3 and L4) with corresponding heights (0.6m, 1.2m, 1.8m and 2.4m) respectively. Harvested maize at 22.8% moisture content

was dried at the varying levels until reaching overall mean moisture content of 12.8 ± 0.2% (wb). Results showed that, drying air temperatures in the dryer increased in accordance with height with lowest mean temperature of 44.4 ± 4.6°C recorded at L1 and mean maximum of 52.8 ± 5.4 °C at L4. The increase in drying temperature at L4 increased kernel stress crack index by an average of 14% and reduced germination by 33%. However, drying temperatures at L1-L3 and in the open sun had no significant effect (p > 0.05) on the germination potential of maize grains. This satisfies the dryer's potential to be used for drying maize grains for high quality seed production on commercial scale.

Keywords: Solar biomass hybrid dryer; drying; maize grain; germination.

1. Introduction

Maize (*Zea mays*) is an important cereal food crop extensively cultivated worldwide for food and as livestock fodder. In Ghana and sub-Sahara Africa, maize is the most important cereal crop produced and is also the most widely consumed staple food. The production of maize in Ghana is dominated predominantly by small-holder resource poor farmers mainly under traditional production practices and rain-fed conditions resulting in yields well below attainable levels (Amanor-Boadu, 2012). Maize yields in Ghana average approximately 1.9 metric tonnes per hectare, however, achievable yields as high as 6 metric tonnes per hectare are possible, if farmers use improved seeds, fertilizer, mechanization and irrigation (MoFA, 2013). However, availability of quality improved seeds is one of the main challenges faced by farmers in Ghana resulting in their over reliance on their own seed stock for production. After harvest, it is common for maize grains to have moisture contents considered inadequate for safe storage for seed. Under such situation, there is clearly a need for reduction of this characteristic to preserve the physiological quality of seeds for at least eight months, impeding possible chemical and physical changes that may come about during storage up to sale of the seeds (Carvalho et al., 2016). Drying maize after harvest from high moisture content (20-30%) to low safe storage moisture content (12-14%) is therefore necessary to ensure storability and preservation of maize grains as seed lots in warm and humid countries like Ghana. Post-harvest activities such as drying and storage are among the key areas along the maize value chain that is of critical importance to small-holder farmers/traders in Africa (Akowuah et al., 2015). However, traditional drying methods where farmers rely on leaving their crops to dry in the field or in the open sun next to farmers' homes or along shoulders of roads either on bare ground or on tarpaulins are unhygienic and can be detrimental to the quality of the dried seed grain. During unfavorable weather condtions, drying can take up to 10 to 14 days before a safe storage moisture content of 12-14% is reached. Inadequate drying especially among peasant farmers in rural communities poses a serious threat to food safety and security in Ghana, since it creates favourable conditions for fungal growth and insect damage during storage (Folaranmi, 2008) leading to substantial losses grains for seed or food. A significant percentage of the current post-harvest losses and aflatoxin contamination can be attributed to improper and/or inefficient drying of foodstuffs such as maize and groundnuts (Togrul and Pehlivan, 2004). However, the introduction of heated air mechanical dryers is not desirable by most small-holder farmers in Ghana due to high drying charges. Also, drying air temperatures as high as 70 -100 °C may be reached with these dryers. These temperatures are considered excessive by most farmers for seed drying. The most severe constraints are on beans (35 °C), rice (45 °C), and all grains if they are to be used for seed (45 °C) (Weiss and Buchinger, 2015). The viability of grain is therefore directly linked to the temperature attained during drying. Seed embryos are killed by temperatures and therefore for seed grains, low temperature drying schemes must be used. Seed grain may be dried in any type of dryer provided it is operated at a low temperature and preferably with high flow rates than generally used (Weiss and Buchinger, 2015). Hassan (2010), reported maximum average germination percentage and viability of dried paddy seed of 86% and 98% respectively after drying paddy seeds at 44 °C in a hybrid solar drier. Tonui (2014), attested to the effect of rapid drying under high temperatures in mechanical drying which often results in stress cracks, reduction of the milling quality of grains, discolouration and reduced germination potential. Fast drying due to high temperature are also likely to induce seed cracking including internal fissures due to trapped moisture. Conventional solar dryer's may be a solution to

these challenges but due to its high weather dependency, its usage is limited during rainy periods, cloudy weather conditions and at night. Due to this, the commercialization of solar dryers has generally not been successful leading to limited or non adoption of such systems by farmers in Ghana (Sekyere et. al., 2016). However, Kaaya and Kyamukangire, (2010) reported that, maize grain dried using biomass-heated natural convection dryer did not significantly reduce the kernel viability.

In this study, the performance of a 5-tonne capacity solar biomass hybrid dryer (SBHD) that integrates both solar and biomass energy for seed maize drying on commercial quantities is investigated. Specifically, effect of drying temperature on germination rate and stress crack on grains were determined.

2. Materials and Methods

The experiment was conducted in January, 2017 using a 5-T capacity SBH dryer purposely constructed for a commercial seed maize distributor at Wenchi in the Brong Ahafo Region of Ghana.

2.1 Dryer Description

Fig. 1 shows the chematic and constructed views of the 5-tonne capacity SBHD at the site. The SBHD is based on a greenhouse structure design with an overall dimension of 10.7 × 6.5 × 3.3 m. It is partitioned inside into three drying sections i.e., the Right Section (RS), Left Section (LS) and a Middle Section (MS). Each of these stations have four levels of drying shelves/racks. The drying shelves are arranged from top to bottom in order of Level 4 (L4), L3, L2 and L1 respectively. The dryer is coupled with a biomass burner enclosed with a crossflow type heat exchanger to raise the temperature of ambient air that is blown into the SBHD with a 0.374-kW axial-flow fan that draws in drying air through the heat exchangers and passes it through the drying beds. The SBHD was test-run during the minor maize production season under load conditions during the month of January 2017 which is one of the driest month in the year.

Fig. 1: *Solar Biomass Hybrid Dryer; schematic view (left) and constructed view (right)*

2.2 Experimental Procedure and Setup

To assess the performance of the SBHD, white shelled maize at 22.8 % moisture content wet basis was obtained from a local farmer and dried in the dryer and in the open sun. Drying occurred using experimental cages (0.3m × 0.3m × 0.05m). Each cage was filled with maize seed samples weighing 1kg and were replicated three times at each level within the dryer. A control set-up of equal maize seeds (1kg) in triplicate was also dried in the open sun as practiced by most rural farmers in the area. The experimental trays containing the maize samples were taken out every hour and weighed to monitor the moisture reduction of the maize samples. The moisture content at the ith time during the drying period was calculated using Euqtaion 1 until the required final moisture content was attained.

Moisture content (%); $MC_i = (1 - W_o \frac{(1-Mo)}{Wi})$ ………………….. Equation 1

MC_i = moisture content at i^{th} time (%)

M_o = initial moisture content in decimal

W_o = initial weight of samples in grammes (g)

W_i = weight of samples at ith time in grammes (g)

The drying process started at 09:50 am to 16:50 pm, over a period of seven (7) hours. During this period, Tinytag TGP-4017 data loggers (Gemini Data Loggers, Chichester, U.K.) were installed near the experimental cages to monitor and register variations in heat and humidity in the drying environment. Five data loggers were positioned, as shown in Fig. 2, at each of the four levels and the average used to represent the drying conditions at each level. Drying conditions in the dryer were compared with the ambient conditions.
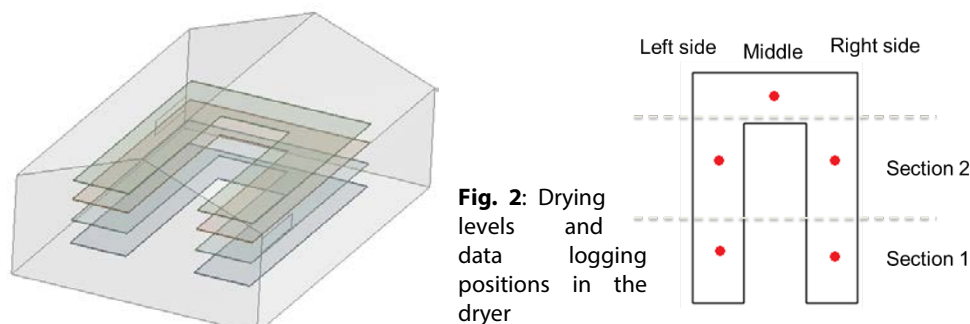
Fig. 2: Drying levels and data logging positions in the dryer

2.3 Germination Test

The germination test was carried out in accordance with the criteria established in the Rules for testing seed (ISTA1985). Four 100-seed sub-samples for each drying level and the open sun were randomly selected and planted in germination trays fill with soil fetched from a river bank. The setup was kept under room temperature conditions. Emergence counts started from the fifth day after planting and continued on the sixth and seventh days. The percentage of normal seedlings was calculated eight days after the test was set up. The percentage germinated seeds were calculated using Equation 1 as stated by Azadi and Younesi (2013).

$$\% \text{ Germination} = \frac{\text{number of seeds germinated}}{\text{number of seeds set for germination}} \times 100\% \ldots \ldots \ldots \text{ Equation 1}$$

2.4 Stress Crack Test

Maize grains were selected by random sampling from each station per layer of dried grains. For each sample, 100 seeds free from insect attack were counted and analysed for stress cracks. The samples were placed on a light box and checked for single, double, multiple or no cracks. The stress crack index (SCI) for each of the analysed sample was determined using Equation 2 as proposed by Kirleis and Stroshine (1990).

$$\text{SCI} = (1 \times \text{single crack}) + (3 \times \text{double crack}) + (5 \times \text{multiple crack}) \ldots \ldots \ldots \text{ Equation 2}$$

2.4 Data Analysis

The experiment was conducted in a complete randomized design and data obtained were subjected to analysis of variance (ANOVA) using GenStat statistical software version 12 at a significance level of 5% (p≤0.05). Results were presented in tables and graphs using Microsoft Excel using the mean values obtained.

3. Results

3.1 Temperature variations during drying

The temperature profile recorded during the drying of the maize grains for seed in the SBHD is presented in Fig. 3. Drying air temperature at level 4, increased during the first four hours, from 41.1 ˚C to a maximum of 58.9 ˚C by noon and reduced to 43.2 ˚C at the end of the drying process. Over the drying period of 7 hours, a mean temperature of 52.8 ± 5.4 ˚C was recorded at L4. Similar variations in temperature trend were oberserved for L3, L2 and L1 in the dryer with mean temperatures of 49.8 ± 5.6˚C, 45.4 ± 5˚C and 44.4 ± 4.6˚C respectively. Comperably, the overall mean temperature inside the dryer (L1-L4) of 48.1 ˚C was higher than the ambient temperature by 12 ˚C. This could be attributed to the solar insolation during the time of the experiment (Tibebu et.al, 2016). Also, the hot air from the biomass furnace rises due to the force convection from the blower with the heat accumulated at the upmost part of the dryer leading to the recorded higher temperatures at L4.

3.2 Changes in maize grain moisture content during drying

As the drying temperatures increased, the moisture content of the maize grains reduced. Maize grains at 22.8% moistute content was dried to an overall final average moisture content of 12.8± 0.2% (wb) within 7hrs. However, samples at L4 and L3 reached average moisture content of 13% within 5 and 6hrs of drying respectively while samples at L1 and L2 reached final moisture content of 12.7% by the 7th hour. Comparably, samples dried in the sun reduced from 22.8% to 12.9% in 7hrs. The result showed that overall drying rates of 1.3%/hr were acheievd in the dryer compared to 1.2%/hr for samples dried in the open sun. The high moisture reduction rate achieved in the dryer could be attributed to the additional heat from the biomass furnace attached to the dryer which facilitated the faster drying of grains to the required moisture content. Fig. 4 shows the variation of moisture content of maize samples dried at different levels in the SBHD as compared to the samples dried in the open sun.
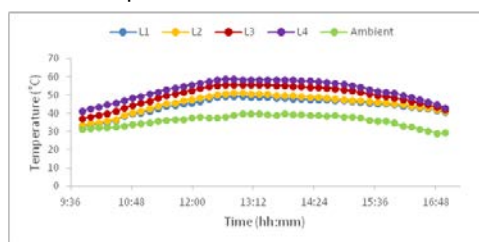
Fig. 3: Temperature trend at different levels in the SBHD vs ambient
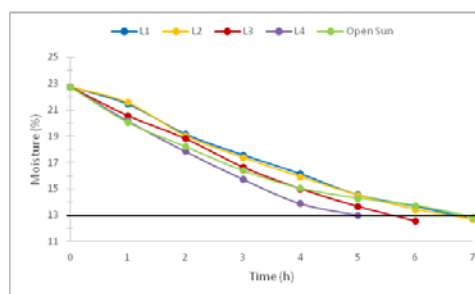
Fig. 4: Moisture content across levels in the SBHD vs open sun

3.3 Effect of drying temperature in SBHD on maize kernel viability

Drying maize using the SBHD did not significantly reduce the viability of the kernels. However, there was a 9% reduction in overall germination potential of grains dried in the SHBD compared to grains dried in the open sun. From the temperature effect, it was observed that, due to the increase in temperature of drying air at the upper level (L4) of the SBHD, the percentage of normal seedlings decreased compared to the lower levels (Table 1). Germination rate of grains dried at various levels with mean temperaures of 52.8 ± 5.4 ˚C, 49.8 ± 5.6˚C, 45.4 ± 5˚C and 44.4 ± 4.6˚C were 44, 61, 64, and 78% respectively (Table 1).

Tab. 1: Percentage germination of maize dried using SBHD[*]

Layer	Mean temperature (°C)	Germination (%)
Level 4	52.8 ± 5.4	44 c
Level 3	49.8 ± 5.6	61 b
Level 2	45.4 ± 50	64 b
Level 1	44.4 ± 4.6	77 a
Open Sun	35.99 ± 3.2	71 ab
LSD (p≤0.05)		11.95

[*]Within a column, means followed by the same letter are not significantly different (P>0.05)

3.4 Stress Crack Analysis

Percent stress-cracked kernels in different categories for maize grains dried at the various levels in the SBHD are presented in Table 2. It was observed that, there was much variation among the different stress-crack categories for grains dried at the different levels in the dryer. In all categories, grains dried at level 4 (L4), had higher stress crack values compared to grains dried at L3, L2, L1 and the open sun. Among the stress-crack categories, the percentage multiple stress cracks were the lowest at all the levls followed by the doubles and the singles in that order. To establish variations in drying temperature effect on the quality of the grains, the SCI was determined for grains dried at the various level. As shown in Table 2, the highest SCI of 160 was recorded for Level 4 followed by L3, L2, L1 and the open sun with SCI of 70, 24, 22 and 14 respectively

Tab. 2 shows the stress crack analysis for the dried samples at each level comapred to the samples dried in the open sun. It is revealed that temperature has an effect on the final quality of the dried maize in terms of the grains susceptibility to cracks. Samples at L4 had the highest stress crack index (SCI) of 160 due to relatively high average temperature experienced at that level during the trial. This high SCI value indicates a high susceptibility of grains dried at L4 to cracking. With relatively low temperatures experienced in the ambient, low SCI value of 14 was observed.

Tab. 2: Stress crack analysis of sampled maize at different levels

Level	Stress Crack Categories				SCI
	No crack (%)	Single (%)	Double (%)	Multiple (%)	
Level 1	90	5	4	1	22
Level 2	90	4	5	1	24
Level 3	68	18	9	5	70
Level 4	38	26	23	13	160
Open Sun	94	2	4	0	14

4. Discussions

The maximum temperature recorded during drying in the SBHD was 58.9°C which was recorded after 4 hours at the top shelve (Level 4). Similar temperature recordings of 55.8, 51.1 and 49.4 °C were recorded for the lower drying shelves (L3, L2, and L1) respectively. Comparably, ambient temperature of 39°C was recorded at the same time. The high temperatures recorded in the dryer compared to the ambient temperature could be attributed to the transparent cover material used in construction of the SBHD which has the ability to retard the heat from escaping by acting as a heat trap for infrared (thermal) radiation thereby forming a confinement for the heated air. Similar results were obtained by Achint et al., (2017) during corn drying in a solar cabinet dryer. The additional heat from the biomass furnace also contributed to the high temperature trend in the dryer compared to the ambient temperature agreeing with the findings of Kaaya and Kyamuhangire (2010).

In the solar biomass hybrid dryer, as the drying temperature increased the drying time and grain moisture content decreased irrespective of the grain position (L1 –L4) in the dryer. Mean moisture content decrease steadily from 22.8% to 12.2% (wb) during the 7 hour drying period. This agrees with the findings of Agona et al., (1998), who reported that drying of maize cobs in a biomass natural

convection dryer takes between five and six hours to reduce the moisture content to 14%. Similar results were also obtained for the drying of eggplant (Ertekin and Yaldiz, 2001), and coroba slices (Corzo et al., 2010).

The effect of temperature across levels on the stress crack of the dried maize grains was distinct. Increase in drying air temperature across the drying trays/shelves in the SBHD did not significantly affect the quality of grains dried at the lower shelves with only 10% of grains samples dried at L1 and L2 showing visible signs of stress cracks. However, grains dried at the top tray (L4) had over 60% of dried grains samples showing visible signs of stress cracks with about 50% recorded under the single or double stress crack category and little over 10% found in the multiple range category. The maximum grain temperatures reached in the dryer at the top trays (L4) may have contributed to this occurrence. Similar works (Lewicki and Pawlak, 2003; Sadjad and Saeid, 2014) have showed similar results where increase in drying temperature increased moisture gradient, creating internal tensions, cracks, breakages, and fractures in dried corn. Chakraverty (1988), also reported that these changes create internal stresses, resulting in cracks, which lead to damage and fractures in the structure of agricultural products.

Evaluation of seedling performance also manifested the negative effects of increase in drying temperature on the physiological quality of maize seeds. It was observed that, maize grains dried at the upper level (L4) of the SBHD where the highest mean temperature (52.8 ± 5.4 ˚C) and highest SCI (160) where recorded had the lowest maize seed percentage germination of 44%. Similar observation was made by Ullmann et al., (2015) in evaluation of sweet sorghum seeds in which a reductionin in germination was recorded , especially at temperatures above 40 °C. As suggested by Afrakhteh et al. (2013); Menezes et al. (2012), and also observed in this study, this could be attributed to the high drying temperature at the upper level (L4) resulting in the formation of stress cracks in the seed coat and microfissures in the cotyledons thereby affecting the quality of the seeds.

However, maize dried at the lower levels (L1-L3) of the SBHD did not significantly reduce the germination potential of the grains as the recorded percentage germination of 61, 64 and 77% (L3, L2 and L1 respetively) were close or above the acceptance percentage germination of 70% recommended by the Plant Protection Act (1976). It was observed that, a 10 ˚C drop in drying temperature across the levels from L4 to L1, led to about 33% increase in germination rate of maize seeds. Similar observations were made by Kaaya and Kyamukangire, (2010), who reported that, drying maize using a biomass-heated convection dryer did not significantly reduce germination potential. Agona et al., (1998), also reported that grain dried using the natural convection dryer technology is good for seed production.

The suitability of the solar biomass hybrid dryer for the production of seed maize was investigated. The dryer has the potential to be utilised for drying maize grains for seed on commercial basis but users are encouraged to use the lower drying shelves; Level 1 through to Level 3 since maize grains dried at these levels met the standard commercial limit recommended for sale of maize seeds in Ghana. Moreover, the upper level (level 4) could be potentially utilized for drying maize grains for food or feed.

Acknowledgement

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Evaluation of AgroZ Hermetic Storage Bag against insect pests on stored maize

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Abstract

A study on AgroZ airtight bag was conducted against two major storage insects under simulated farmers' storage practice. Two (2) lots of 50kg white maize of Pioneer variety were put into AgroZ bag and polypropylene woven bag to serve as a control. Four replications of each bag type were used. In each bag, 50 adults of unsexed larger grain borer, *Prostephanus truncatus*, and maize weevil, *Sitophilus zeamais*, each were introduced. AgroZ bag had one liner placed inside polypropylene bag to provide support and handling convenience. Each liner had been tested for air tightness before use. The AgroZ bags were securely tied to ensure airtightness thus leading to a hermetic environment. The bags were then randomly placed in a barn on pallets in a randomised complete design (RCD). Sampling was done every 4 weeks up to 24 weeks. A 500g sample was initially taken using a compartmented long spear probe from each bag for baseline data, and subsequent ones at 4, 8, 12, 20 and 24 weeks. Repeated sampling from the same storage device reflected farmer practices of opening the device at regular intervals to draw grain for use as household food. Gas analysis in AgroZ bags showed oxygen level dropping rapidly to 7% within 4 weeks and later increased gradually to 10% at 12 weeks. Conversely, carbon dioxide level increased sharply to 10% and declined gradually to 9% over the same period. The number of insects and percentage damaged grains between AgroZ bag and polypropylene bag significantly differed from 12th week to 24th week. AgroZ bag outperformed the polypropylene bag commonly used by farmers and conveniently protected maize from insect infestation within the 6-month storage period.

Introduction

Maize (*Zea mays* L.) is a major grain staple in Sub-Saharan Africa that provides calories and income for many households (Zia-Ur-Rehman, 2006). Whereas the crop is harvested every season, substantial amount of the grain is lost to insect pests during storage because to control these pests remains a challenge to resource-poor smallholder farmers. This is aggravated by lack of effective, appropriate and affordable storage devices (Baributsa *et al.*, 2014). As a result of insect feeding, damage, and contamination, the volume of stored grain and quality, grain value, and marketability is reduced (Affognon *et al.*, 2015). To avoid the risk of losing the harvested crop to insect pests, some farmers sell their maize early at low price while others treat it with dilute insecticidal dusts, but satisfactory protection is never achieved (Obeng-Ofori, 2011). The major insect pests of stored maize are the larger grain borer, *Prostephanus truncatus* (Horn), and the maize weevil, *Sitophilus zeamais* Motschulsky (DeGroote *et al.*, 2013). The former is the most damaging pest, and in endemic areas causes weight loss estimated at 30% while the maize weevil causes 10-20% weight loss when untreated maize is stored in traditional structures (Boxall, 2002; Mutambuki & Ngatia, 2012; Likhayo *et al.*, 2016).

Hermetic storage technology offers farmers' effective alternative for protection of stored maize against insect pests. The technology functions by creating modified atmospheres around the grains through physical and biological means to retard the activity and survival of insects in the stored grain (Anankware *et al.*, 2012). In recent years, the technology has received much attention by the private sector as the means of safeguarding stored grain because it is cheaper compared to metal silo and safe (Murdock et al., 2003). Currently, there are two hermetic bags available commercially, namely, Purdue Improved Crop Storage (PICS) and GrainPro bags. Whereas these bags effectively control storage insect pests of crops such as maize (DeGroote *et al.*, 2013), cowpeas (Moussa *et al.*, 2014) and beans (Mutungi *et al.*, 2015), the plastic film (liner) has been found perforated (Garcia-Lara *et al.*, 2013; Martin *et al.*, 2015; Likhayo *et al.*, 2016) thus compromising the integrity of the bags. With changing behaviour of insect pests, it is imperative to continually evaluate novel and newer hermetic bags to address this concern. Hermetic grain storage bags of 100kg or less capacity offer smallholder farmers the desired flexibility and control of their produce. AgroZ bag is a low permeability plastic bag developed by A to Z Textile Mills Ltd (Tanzanian manufacturer) for the storage of maize and pulses against postharvest insect pests.

In an effort to contribute to the Public-Private Partnership, the manufacturer submitted samples of AgroZ bags to KALRO-Kabete for local validation through a structured evaluation. The aim of this study was therefore to validate the efficacy of the bag in protecting stored maize against the larger grain borer and other important storage insect pests under field conditions.

Materials and methods

The field trial was conducted at Kiboko sub-centre, about 200 km from KALRO-Kabete, along Nairobi Mombasa highway. The choice of the site was due to prevalence of the larger grain borer and a barn where simulation of farmers' storage condition was adopted. Two (2) lots of 50kg white maize of Pioneer variety (H614D) that had been fumigated was put into AgroZ bag and polypropylene (farmer) bag to serve as a control. Four replications of each bag type were used. In each bag, 50 adults of unsexed *P. truncatus* and *S. zeamais* each (based on 1 adult insect per kg) were introduced. AgroZ bag had one liner placed inside polypropylene bag to provide support and handling convenience. Prior to placing the liners in polypropylene bags, they were tested for air tightness or leakage by filling the air to form a pouch before compressing them with both hands. The AgroZ bags were securely tied to ensure airtightness thus leading to a hermetic environment. Any bag that leaked was discarded. The bags were then randomly placed in a barn on pallets (dunnage) in a randomised complete design (RCD). Sampling was done every 4 weeks up to 24 weeks. A 500g sample was initially taken using a compartmented long spear probe from each bag for baseline data, and subsequent ones at 4, 8, 12, 20 and 24 weeks. Repeated sampling from the same storage device reflected farmer practices of opening the device at regular intervals to draw grain for use as household food.

Each grain sample was sieved to separate dust from insects and grain. Grain moisture was determined using Dickey-John Multi-Grain® moisture tester (Dickey-John Corporation, Aurburn, IL, USA) (wet basis). Moisture content was measured at the beginning of experiment and at every sampling time. Three readings of each sample were taken, and the average recorded. The sample was then divided using a riffle divider until four sub-samples of approximately 65g were obtained. Grains in three of the sub-samples were sorted into undamaged, damaged, discoloured, and broken grain categories which were counted and weighed. The damaged grain was expressed as a percentage of the total grain in the sub-sample. The fourth sub-sample was reserved for reference. Percentage grain moisture content, number of live adult insects, and percentage grain damage were parameters used to judge the efficacy of each treatment. Upon termination of the trial, the hermetic bags were inspected for perforation (holes) made by adult *P. truncatus* and the number of holes recorded.

Statistical analysis

The number of insects was $\log_{10}$ (count +1) transformed, while percentage moisture content and damaged and discoloured grain data were square-root transformed in order to stabilize the variances. The transformed data were analysed using General Linear Model procedure of GenStat Release 12.1 (VSN International Ltd 2009), with bag type and storage period as main effects. Insect counts, grain moisture content, and percentage damaged grains at each time-point post-treatment were the response variables. Significant differences between the means were separated by Tukey test at $P<0.05$. However, for ease of understanding untransformed means are presented. The means of gas composition levels at each sampling time-point were computed.

Results

Effect of bag type on grain moisture content

There were significant differences ($F_{1, 33} =16.43$; $P<0.001$) in grain moisture content between bag type and storage period ($F_{5, 33} =21.82$; $P<0.001$) but the interaction was not significant ($P>0.05$). Although significant differences were observed, the moisture content did not change markedly between bag types throughout the entire storage period and, remained below the recommended limit of 13.5% (Table 1). The grains stored in polypropylene bag (12.1%) and AgroZ bag (12.3%) recorded almost same moisture contents after 24 weeks of storage, respectively.

Table 1: Mean percentage (±SE) grain moisture content changes during storage

Bag Type	Storage period (weeks)					
	0	4	8	12	20	24
AgroZ	12.3 ± 0.2cd	11.9 ± 0.0a	12.4 ± 0.0cd	12.6 ± 0.0d	12.3 ± 0.0cd	12.3 ± 0.0bcd
Polypropylene	12.2 ± 0.0bcd	11.6 ± 0.0ab	12.1 ± 0.1bc	12.5 ± 0.0cd	12.1 ± 0.1bc	12.1 ± 0.0bc

Means within the same column or row followed by the same letter are not significantly different at P = 0.05 level (Tukey test)

Effect of bag type on adult insect counts (both live and dead)

In this study *P. truncatus*, *S. zeamais*, and *Tribolium castaneum* (Herbst) were detected. At the start of the trial, the maize did not have emergent infestation. Interaction effect between treatment and storage duration was significant ($F_{5, 33}$ =73.6; P<0.001). AgroZ bags supressed increase in insect population compared to control (polypropylene) bags. On all sampling occasions starting at 12 weeks, least number of adult insects was recorded in the grains stored in AgroZ bags (Table 2). Significant numbers of insects became evident starting from 12th week of storage in the polypropylene (4) bags (Table 2). At the end of the trial, holes perforated by *P. truncatus* were detected in AgroZ bags. Two replicates had 3 and 4 holes each while the other 2 had no holes hence only half of the plastic liner bags used were perforated.

Table 2: Mean number (±SE) of adult insects (both live and dead) per grain sample

Bag Type	Storage period (weeks)					
	0	4	8	12	20	24
AgroZ	0 ± 0f	2 ± 0de	1 ± 0ef	2 ± 1d	2 ± 1de	1 ± 0def
Polypropylene	0 ± 0f	1 ± 0ef	2 ± 2de	4 ± 0c	10 ± 0b	16 ± 1a

Means within the same column or row followed by the same letter are not significantly different at P = 0.05 level (Tukey test)

Effect of bag type on grain damage

There were significant interaction differences ($F_{5, 33}$=185.3; P<0.001) between treatments and storage duration. Grain damage for the treatments is presented in Table 3. At the start of the trial, the maize showed little damage. From 12 weeks' storage duration, no further grain damage was detected in AgroZ bags (Table 3). In contrast, grain damage in the control bags increased steadily from 8th week of storage and reached 11.7% at the end of the trial. If by the 24th week the damage was adjusted by subtracting the baseline damage, actual damage for AgroZ bag was only 1.1% compared to 10.4% for polypropylene bags.

Table 3: Mean percentage ((± SE) grain damage due to insect infestation during storage

Bag Type	Storage period (weeks)					
	0	4	8	12	20	24
AgroZ	1.6 ± 0.2hi	2.0 ± 0.2gh	2.2 ± 0.1efg	2.5 ± 0.2ef	2.8 ± 0.2de	2.7 ± 0.2de
Polypropylene	1.3 ± 0.2i	1.8 ± 0.1ghi	3.1 ± 0.2d	5.7 ± 0.1c	8.4 ± 0.2b	11.7 ± 0.2a

Means within the same column or row followed by the same letter are not significantly different at P = 0.05 level (Tukey test)

Changes in gas composition in AgroZ bag

Although the storage period was 24 weeks, gas composition levels in AgroZ bags was only measured for 12- week storage period (Figure 1). Gas composition levels determined after closing the bags at the onset of the storage were 20.7 ± 0.0% oxygen and 0.9 ± 0.0% carbon dioxide. Oxygen level dropped rapidly to 6.7 ± 0.1% within four weeks and thereafter increased gradually to 10.7 ± 0.1% at 12 weeks. Carbon dioxide level on the other hand increased sharply to10.3 ± 0.1% then declined gradually to 8.9 ± 0.1% within the same period.
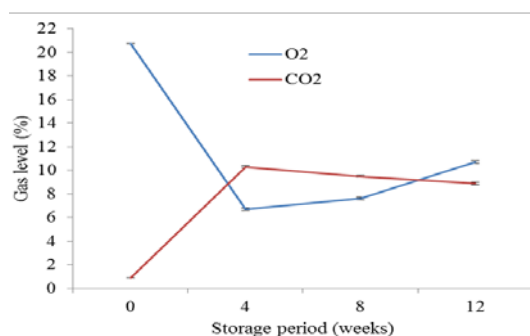
Figure 1: Oxygen and carbon dioxide levels in AgroZ bag over 12 – week of maize storage. Data shown are means ± standard error for four replications.

Discussion

Smallholder farmers store their maize grain to assure supply between the harvests. However, factors such as use of improved susceptible varieties and the spread of the exotic storage insect pest like the larger grain borer (*P. truncatus*) could negatively impact effective storage practices. Despite farmers applying insecticides and traditional protective measures, fewer achieved satisfactory control of the insect pests. Grain damage due to insect infestation is a serious concern that threatens food security and livelihood of rural farmers.

The modified environment created by respiration of th maize grains and insects effectively suppressed insect survival and as a consequence stopped grain damage. Low oxygen levels and enhanced carbon dioxide of inter-granular atmosphere is the basis of insect infestation suppression in hermetic storage. Evidently, extreme oxygen depletion and carbon dioxide build-up levels were not achieved in the AgroZ bags probably due to opening of the bags during sampling. The depletion of oxygen and build-up of carbon dioxide is a function of, among other elements, storage containers; insect population; grain moisture; and gas-tightness. Development of a low oxygen environment is very slow in the absence of insects and predominance of dry grains (<13% moisture content), even in containers where high standard of gas-tightness is achieved. This is attributed to low aggregate oxygen demand in the containers (Moreno-Martinez *et al*., 2000). Other studies, however, reported gradual decrease of oxygen to 8.4% within 30 days in clean maize stored without insect infestation under hermetic conditions (Moreno-Martinez *et al*., 2000). Further, Ng'ang'a *et al*. (2016) reported that oxygen level dropped to 4.9% and carbon dioxide increased to 10.5% within the first seven weeks of on-farm storage of maize in PICS bags. The gas composition levels reported in this study did not differ markedly from those documented by these researchers suggesting hermeticity of AgroZ bags is comparable to that of PICS bags.

This study has demonstrated significant grain damage in maize stored in polypropylene bags compared to that which was stored in AgroZ Plus bags. A study by Njoroge *et al*. (2014) reported 3.4% grain damage when maize (variety H614D) was stored in PICS bags in the presence of *P. truncatus* at ambient conditions for six months. The same maize variety was used in the current study, and a difference in grain damage reported (0.9%) is very small to be important. Therefore, the grain damage recorded by AgroZ bags compares well with that of PICS bags. Although insect pest multiplication was not very high in the control bags as expected, the grain damage levels observed were mainly a result of insect infestation attributed to favourable ambient conditions. Conversely, multiplication of insect pests was drastically reduced in AgroZ bags because of the modified environment (low oxygen and high carbon dioxide levels) within the bags.

Upon termination of the trial, inspection showed physical damage (perforation) of AgroZ bags. These bags are made of tougher polyethylene (PE), 90 µm thick, with good gas and water barrier properties. Therefore, grain volatiles would not be released to the outside to elicit movement of

insects into the bags looking for food while the insects inside the bags died due to depleted oxygen levels (hypoxia). Although the holes were evident to the naked eye, their examination by use of hand-held magnifying glass showed that the scratch and tear were less marked around the holes on the side from which the insects perforated the liner, an indication of exit holes (Riudavets *et al.*, 2007). The holes might have been made by *P. truncatus* attempting to escape from the bags when exposed to oxygen-depleted environment. *P. truncatus* has the ability to bore through hard materials such as a 35mm thick plastic (Li, 1988). The holes were made near the bottom of the bags. The holed bags therefore failed to attain air-tight conditions resulting in ineffective control of the storage pests. The observation is in agreement with that made by Ognakossan *et al*. (2013 – not in References) when maize was stored in PICS bags for 150 days and 180 days in SuperGrain™ bags (De Groote *et al.*, 2013). Cowpea bruchid *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) was found to bore PICS bags during storage in Niger (Baoua *et al.*, 2012) but the hermetic condition was not completely lost because of the imperforated second liner.

Conclusion and Recommendation

AgroZ plastic bag effectively prevented insect multiplication, changes in moisture content, and grain damage as demonstrated in the field trial. Without perforations or a few as observed in the trial, the bag maintained air-tight condition leading to death of insects and hence translating into very minimal grain damage. Owing to this good performance, AgroZ bag is recommended as a storage grain protectant against storage insect pests.

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Impact of Rodent Infestation on Availability, Safety and Nutritional value of Maize Stored On-farm in Lowland Tropical Zone of Kenya

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Rodents are the second most important storage problem after insects during on-farm maize storage in Kenya, and the greatest storage problem in the lowland tropical agro-ecological zone. However, there is limited information on the actual magnitudes of food lost, and food safety issues associated with rodent grain damage. Such information would help to improve maize postharvest management. Farmer stores were monitored over 3 months under natural infestation conditions to quantify actual weight losses due to rodents. Rodent trapping was also carried out to determine rodent species associated with the losses and their population. Additionally, samples of rodent-damaged and non-damaged grain were analysed for total mould count (CFU/g), mould incidence, total aflatoxin contamination, proximate content, and amino-acid and fatty acid profiles. Cumulative weight losses ranged from 2.2 to 6.9% in shelled maize grain, and from 5.2 to 18.3% in dehusked cobs during 3 months of storage. Rattus rattus was the only rodent species captured over the whole trapping period with a trap success rate of 0.62 -10%. Total mould count and Fusarium spp. incidence were significantly higher in rodent-damaged grains than in the non-damaged ones (P= 0.001; P= 0.011, respectively), whereas no significant difference was observed for Aspergillus spp incidence (P=0.239) and total aflatoxin contamination (P = 0.077). Contents of methionine, valine, proline and all fatty acids were significantly lower in the rodent-damaged grains.

Postharvest losses of agricultural commodities in Trincomalee, Sri Lanka

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Abstract

In Sri Lanka, postharvest losses vary with the geographical area; higher losses are reported in warmer areas. A survey was conducted in Trincomalee district, one of the hottest areas in Sri Lanka, to ascertain the status of crop cultivation and postharvest losses of cultivated crops. Farming is the main livelihood of the people in the area. The main crops cultivated are paddy, red onion, chili, brinjal, tobacco and manioc; the average land extent possessed by a farmer family and the yield varies with the crop. Paddy, onion, and tobacco are stored for 6, 3, and 12 months, respectively. Paddy is stored indoor in bags, onion as racks (indoor), and tobacco as piles (indoor and outdoor) under shade conditions. During harvest, drying and storage losses occur in paddy and onion. *Sitophilus oryzae*, *Rhyzopertha dominica*, *Sitotroga cerealella*, and rats are the major problems during paddy storage. Pesticides are not used regularly by the farmers. Instead they practice traditional pest management methods.

1. Introduction

Postharvest losses of agricultural commodities are much higher in the tropics (Wijayaratne et al., 2018). Insects are major cause for these losses (Hill, 1990). Trincomalee is located in the northeast area of Sri Lanka, and belongs to the dry zone. Due to the limited availability of water, crop cultivation in this region is mainly seasonal. The harvested yield is stored for consumption during the off seasons. Despite the high postharvest losses of agricultural commodities in Sri Lanka, a detailed study has not been conducted recently in Trincomalee area. Therefore, this survey was conducted to determine the status of crop cultivation and postharvest losses of cultivated crops in two cropping areas in Trincomalee, Sri Lanka.

2. Materials and methods

Two geographical areas in Trincomalee district having storage practices, Nilaweli and Mahadiwulwawa, were selected for the study. From each location, 21 families were selected. Using a questionnaire, information on crop growth and postharvest practices were gathered.

3. Results
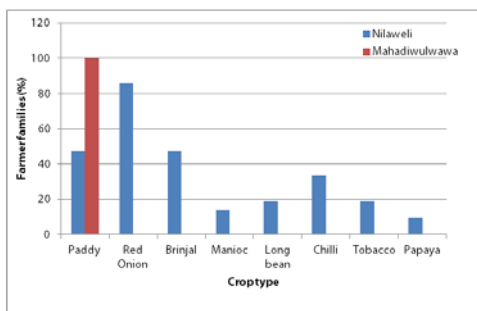
. **Fig. 1.** Crop cultivation (as a percentage of all families) in Nilaweli and Mahadiwulwawa areas.
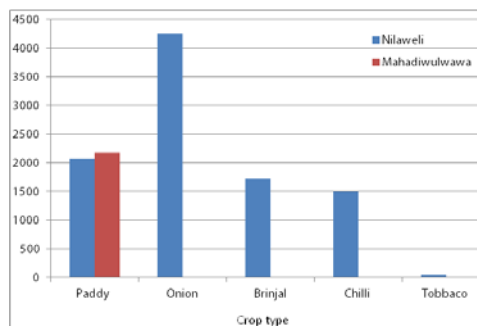
Fig. 2. Annual crop yield (kg) in Nilaweli and Mahadiwulwawa areas.
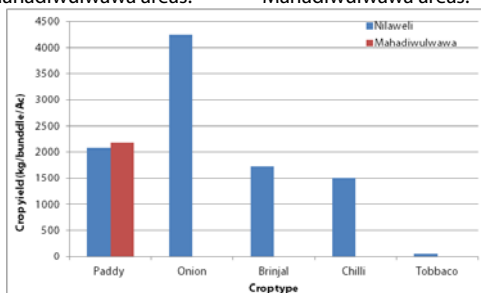
Fig. 3. Post-harvest losses in Nilaweli and Mahadiwulwawa areas.

Several economically-valuable crops are grown in Nilaweli area whereas paddy is the only economically valuable crop in Mahadiwulwewa area. Most of the families in Nilaweli area tend to grow red onion. As a crop grown in the two areas surveyed, the highest annual yield is obtained from onion cultivation. Yield losses during storage happen due to stored-product insects, rodents, and unfavorable conditions of food stores.

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Abundance of insects in rice mills in Polonnaruwa, Sri Lanka

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Abstract

Monitoring of insect population is a prerequisite for integrated pest management attempts. The complex structures/machines in rice milling facilities, however, limit surveying attempts aggravating the ignorance of insect fauna associated with such facilities. Furthermore, insect surveys conducted in Sri Lanka are very rare. The objective of the current study was to determine the presence, diversity, and abundance of insects in rice mills of varying capacity as found in a major rice processing area in Sri Lanka. A group of large-, medium-, and small-scale mills were used for the survey. Samples were collected from different locations in the mills, and the density of insects at each location was determined. Insect species and their abundance varied with the type of mill as well as with the location in the mill. This information is useful to design and implement pest management for the mills.

Keywords: small scale, medium scale, large scale, abundance, insect

1. Introduction

Rice is the staple food of Sri Lankans. In Sri Lanka, the annual paddy production in 2015 in one season was 1.9 million MT. Of this production 14% is from Polonnaruwa district (Department of Census and Statistics, 2015). Furthermore, rice milling is popular in Polonnaruwa area. Insects in rice mills are a challenge as they cause quantitative and qualitative losses in the milling products (Hagstrum and Subramanyam, 2006; Wijayaratne et al., 2018). However, lack of information on insect pest populations and their diversity in rice milling facilities restrict the development of integrated pest management programs. No proper survey has been conducted in rice mills in Sri Lanka on the presence of insects. Therefore, this survey was carried out to determine the presence, diversity, and abundance of insects in rice mills in Polonnaruwa district, Sri Lanka.

2. Materials and Methods

Using a questionnaire, information was collected from large-scale, medium-scale and small-scale rice mills on the abundance of insects and their diversity. Samples from different parts of the mill were collected: store house, rice polishers, destoner, dehusker, separator, silky machine, grader,

elevators, paddy cleaner, flour machine, and polisher. The insects were identified using morphological characters. The abundance of insects in each sample was determined.

3. Results
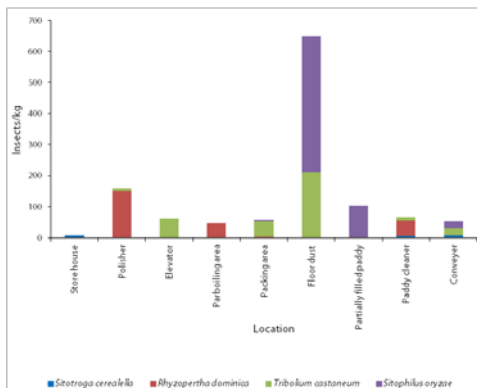
Figure 1. Abundance of insects at different places in the mill-large scale.
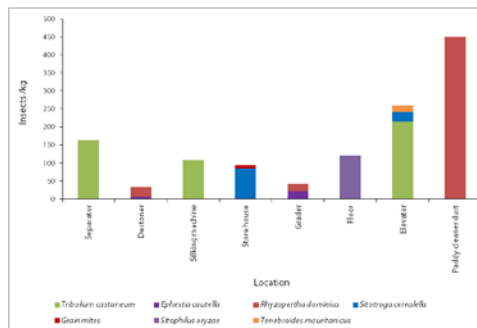
Figure 2. Abundance of insects at different locations in the mill-medium scale.
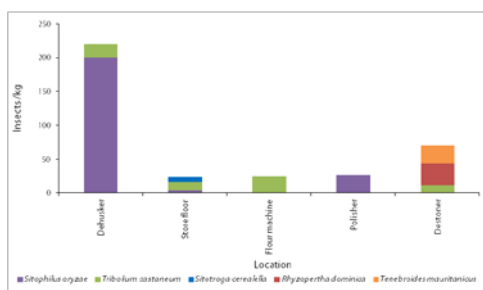
Figure 3. Abundance of insects at different locations in the mill-small scale

Insect diversity and their abundance varied with the type of the mill surveyed. As pest management methods, fumigation and vacuum cleaning are practiced in the large- and medium-scale mills whereas the small-scale millers rely on botanicals.

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Loss of animal feed due to infestation by Rhyzopertha dominica

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Abstract

Despite the use of natural food for livestock production, different animal feeds are currently available at the market. Long-term storage of these animal feeds lead to deterioration and contamination by insects. Therefore, it is important that the loss of these animal feeds be determined and methods to control the damage be sought. This study was conducted to determine the loss of eleven types of animal feed commonly used in Sri Lanka due to infestation by *Rhyzopertha dominica*, a major granivorous insect species.

Twenty newly emerged adults of *R. dominica* were introduced separately to each animal feed: fish feed, rabbit feed, dog feed, cat feed, chick mash, grower mash, layer mash, broiler starter, broiler finisher, bird feed (Bajiri), and rice polish. Each animal feed was maintained either aerated or air tight. These parent adults were maintained for 21 days in the media under ambient environmental conditions (30ºC, 65% relative humidity), and then removed. The progeny adults emerged in each feed sample were removed and the weight of the samples was determined at monthly intervals. In general, weight loss of animal feed varied with the feed type, duration of exposure, and aeration condition. Attention needs to be paid to protect those animal feeds that recorded higher losses due to *R. dominica* during storage.

Keywords: Animal feed, *Rhyzopertha dominica*, Weight loss, Duration, Aeration

1. Introduction

Traditional practice to feed farm animals is by natural vegetation, but different animal feeds are now available at the market as an alternative. During storage, these animal feeds can be infested by insects. The lesser grain borer, *Rhyzopertha dominica*, is a major pest of stored cereals (Chittenden, 1911), pharmaceuticals, leather stuffing, and packing materials (Riley, 1882; Chittenden, 1911; Winterbottom, 1922; Hoffman, 1933; Potter, 1935). There is a potential that animal feeds can be infested by *R. dominica*. The objective of this research was to examine the potential of *R. dominica* to damage eleven types of animal feed commonly used in Sri Lanka.

2. Materials and methods

Eleven types of animal feed were used in the study: fish feed, rabbit feed, dog feed, cat feed, chick mash, grower mash, layer mash, broiler starter, broiler finisher, bird feed (Bajiri), and rice polish. Twenty adults of newly emerged *R. dominica* were introduced to 20 g of each animal feed in separate vials and maintained either aerated or air tight under the ambient environmental conditions (30°C, 65% relative humidity). The experiment was conducted using four replicates. After 21 days, the parent adults were removed. The progeny adults were counted and the weight of each animal feed was determined at monthly intervals for five months.

3. Results
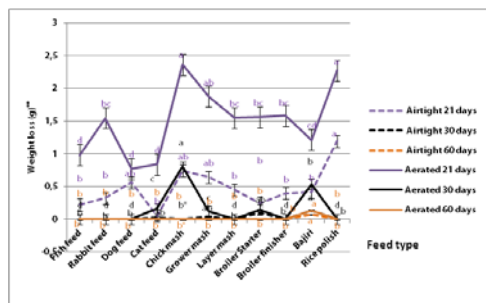
*Fungal contamination

**For a combination of a 'given aeration (aerated or air tight) and duration', weight loss followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA.

Fig. 1. Weight loss in animal feed at 21, 30 and 60 days following infestation under aerated/air tight condition.
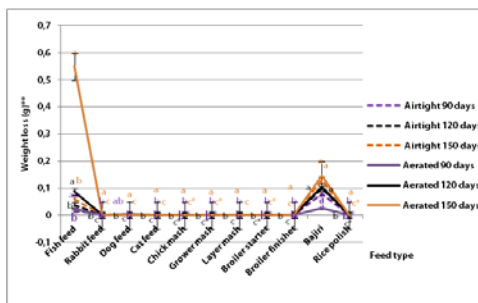
* Fungal contamination

**For a combination of a 'given aeration (aerated or air tight) and duration', weight loss followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA.

Fig. 2. Weight loss of animal feed at 90, 120 and 150 days following infestation under aerated/air tight condition.

4. Discussion

Aerated samples of a given animal feed demonstrated higher weight loss than air-tight samples. The maximum weight loss occurred in chick mass and Bajiri. The minimum weight loss was recorded in dog feed and cat feed. Discard of certain animal feed samples due to fungal contamination seemingly interrupted the smooth increase of weight loss when the duration increased.

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Quality and Safety Conditions of Flocked Oats (*Avena Sativa* L.) Stored in Bags

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Abstract

Oats (*Avena sativa* L.) have reached the healthy food market worldwide due to its special nutrients composition and fiber high quality. Therefore, quality & safety control is a must, both during the storage and commercialization stages. The current study evaluated the physicochemical characteristics (flakes size/variation %, pH, moisture content-mc, water activity-aw), living organisms (insects & mites / mycoflora - fungi load& genera identification), mycotoxins(ochratoxin A – OTA / zearalenone – ZON / aflatoxins – AFLs / esterigmatocistin – EST)andthe storage conditions of flocked oats stored inbags.Regarding the oats physicochemical characteristics, flakes particle size varied, however most of the samples present size uniformityand only one sample had high percentage of residue. That indicates high insects and other living organisms activity (consumption / proliferation) of oats starch and other nutrients. The analysis through

stereomicroscope showed intense presence of insects and mites. Samples were seen also sheltering those living organisms (27%), which are not allowed by regulation (no soils, parasites and larvae presence). As expected, mc (10.8-13.2%) and/or aw (0.61-0.90) varied, however they kept on the safer levels (< 13% / 0.90) insects/mites and fungi growth wise. With respect to pH, it varied from 4.1 to 5.85, indicating some rancidity/fermentation reactions taking place, thus changes in organoleptic parameters. The total fungi load ranged from $3x10^2$ to $1.29x10^5$ CFU/g, with *Aspergillus* and *Rhizopus* the genera more identified. Only one sample was toxin contaminated (OTA - 80 µg/kg). Insects are known vectors of fungal spores and can spread their hyphae on their dead/live skeleton, apart from mites that can trigger allergies in humans and animals. Therefore, current data demonstrate that despite the storage conditions control application, living organisms can occur in flocked oats (stored in bags) and it is necessary to apply decontamination methods to control/prevent their proliferation.

Key words: oats, storage, bags, insects, fungi, toxins.

Introduction

As part of the demand for a healthy diet, oats (*Avena sativa* L.) have gained more and more popularity due to its functional claims (mainly due to its composition). Oats have large amounts of beta-glucans and soluble fibers, which are able to reduce glucose absorption and increase intestinal transit. Several studies have shown its effectiveness in preventing diabetes and cardiovascular diseases, and in reducing glucose levels and blood pressure. It is important to emphasize that processing steps do not alter the concentration of oats nutrients (De Sá et al, 1998).

During grain storage, moisture and temperature reduction and control is required. Despite that, grains need to be harvested in the most efficient way so that there is no mechanical damage allowing insect infestation and fungi proliferation. Storage locations vary from warehouses, bags or silos, and also in more modern ways, such as hermetic silos (Marini et al., 2007).

Oats, like others cereals, are susceptible to a number of fungi, including those of field and storage, such as *Fusarium*, *Aspergillus*, and *Penicillium*. Fungi, under favorable conditions, can cause deterioration in grains and produce mycotoxins. These toxins can affect animal and human health, being more severe in some animals, such as swines and equines. We are exposed to fungal spores at all times as they are easily transported through the air. These fungal spores can be toxigenic - those that are able to produce toxins harmful to health. As these spores are not easily perceived due to their microdimensions, we only identify their presence in foods when they are well developed, spoiling their tissues and producing mycotoxins. More than 300 mycotoxins have been isolated in food, however there are five main ones, among them aflatoxins (AFLs), ochratoxin A (OTA), T-2 toxin, deoxynivalenol, and fumonisins (Scussel, 2002; Agais, 2005).

Some foods may contain mycotoxins and are apparently healthy, which leads us to consume these foods without the full certainty of safety. It is necessary to monitor the quality of the grains stored and marketed, so that the population is aware of what they are consuming.

Therefore, this work evaluated the quality and safety conditions of flocked oats stored in bags.

Materials and Methods

Material

Samples: flocked oats stored in bags.

Culture media and reagents: potato dextrose agar (PDA) and peptone bacteriology media were purchased from Himedia (Curitiba, Parana, Brazil) and chloramphenicol were from Vetec (Duque de Caxias, RJ, Brazil), phenolphthalein and sodium hydroxide from Merck (Darmstadt, Germany).

Equipment: autoclave, Phoenix (Araraquara, SP, Brazil); microwave oven, Philco (Sao Paulo, SP, Brazil); tweezers, Prolab (São Paulo, SP, Brazil); caliper, Digimatic (Mitutoyo, Tokyo, Japan); drying oven, Olidef-cz (Ribeirao Preto, SP, Brazil); a_w meter, Aqua- Lab4TE, Decagon (Sao Jose dos Campos, SP, Brazil); Peagameter, Model Schott-gerate CG 818 (Schott,, Mainz, Germany); laminar flow cabinet, Veco (Campinas, SP, Brazil); fume cabinet, Quimis (Diadema, SP, Brazil); rotary shaker, Marconi (Piracicaba, SP, Brazil); microbiological incubator, Quimis (Diadema, SP, Brazil); colonies counter,

Phoenix (Araraquara, SP, Brazil); sieve system, mesh (2-1mm) Beffer (Caieiras,SP, Brazil).Microscopes - light (LM), CH-Bl45-2, Olympus (Shinjuku, Tokyo, Japan); stereo microscope (SM), Opzt coupled to a color image-capture camera, model OPT14 MP, Opticam Microscopy Technology (Doral, Fl., USA).

Methods

Sample collection and preparation: samples (300 g) were collected from stored bags, then sealed, labeled, and transported to the Laboratory of Mycotoxicology and Food Contaminants for analysis; *(b) preparation* - each oat sample was homogenized and then divided into two main portions: (b.1) integral i.e., its original flakes characteristics (analysis: pH, mycology, and aw) and (b.2) ground, for mc and mycotoxins.

Granulometry of oat flakes: sample portions (100 g) were subjected to separation by a Screen System (sieves) with different apertures (Mesh: 9; 16; 200, corresponding to 2.0; 1.0 and 0.75 mm) (Lorini et al., 2015) then %/mesh calculated.

Physicochemical analysis: pH, acidity, and moisture content (mc) were determined by the international official AOAC methods (Peisino et al., 2015; AOAC 2005). The water activity (a_w)was determinedusingthe Aqualab apparatus at 25ºC (n=3) (Decagon, 2001).

Total fungi load and genera identification: the enumeration technique and genera identification of Da Silva et al. (2007) and Pitt (1979) were used.

Storage conditions: the environmentalconditions of the storage- ventilation / refrigeration, application of pest control system, cleaning of the premises - were evaluated (Souza et al., 2013).

Multi-toxin analysis: the method of Soares&Rodrigues-Amaya (1989) was applied for the determination of multi-toxins [AFLs (AFB1, AFB2, AFG1, AFG2), ZON, EST and OTA].

Results

From the data obtained, it was possible to observe that part of the flocked oats stored in bags showed that the flocculation process applied and the bags storage condition in which they were submitted were efficient. Despite that, some oat flakes presented different physicochemical conditions ideal for the development of insects, mites, andfungi.Table 1 shows the total fungal load, genera, and humidity of flocked oats samples (*Avena sativa* L.).

Insects and mites: they were detected in all oats samples (at different percentages), enphasizing the concern on the storage conditions and safety. Part of the samples (32%) presented insects and mites when analyzing under stereoscopic microscope. Thereboth living and dead insects present. Figure 1shows by sterescopy (a, b) *insects*and (b) *mites* isolated from oat samples.

Humidity: the mc of the samples analyzed varied from 10.8 to 13.2%, indicating a small variation of the products stored and process. With respect to a_w, the samples varied from 0.4782 to 0.5906 and the pH ranged from 4.1 to 5.85 indicating some rancidity and fermentation process, thus flavor alterations.

*Fungi:*as expected, the total load was high ranging from $3x10^2$ to $1.29x10^5$ CFU / g.The genera isolated were *Aspergillus* and*Rhizopus*, the first with possible toxin formation and the second only deterioration. Figure 2presents the light microscopy of fungi (a) *Aspergillus* and (b) *Rhyzopus*isolated from oat samples.

Mycotoxins: a single sample showed contamination by OTA (80 μg / kg) well above the regulations of several countries (OTA daily intake: 3; maximum tolerant level: 50 μg / kg – FAO/WHO, 2017).

Table.1 Physico-chemical characteristicsand fungi of flocked oats (*Avena sativa* L.) stored in bags

Flakes oats		Physico-chemical			Fungi		Mycotoxins* (ug/kg)			
Number Code		Humidity		pH	CFU/g	Genera	AFLs	EST	ZON	OTA
		mc (%)	a_w							
1	A	11.48	0.5429	5.75	ND	ND	ND	ND	ND	ND
2	B	11.97	0.5712	5.535	ND	ND	ND	ND	ND	ND
3	C	11.92	0.5547	5.853	1.05×10^4	*Aspergillus/ Rhizopus*	ND	ND	ND	ND
4	D	12.94	0.5514	5.6	3×10^2	*Rhizopus*	ND	ND	ND	ND
5	E	12.42	0.5159	5.39	7.0×10^4	ND	ND	ND	ND	ND
6	F	12.88	0.5904	4.095	6×10^{-2}	*Aspergillus/Rhizopus*	ND	ND	ND	80
7	G	12.11	0.5239	5.53	1.47×10^4	*Aspergillus*	ND	ND	ND	ND
8	H	10.81	0.4781	5.25	ND	ND	ND	ND	ND	ND
9	I	13.08	0.5728	4.61	1.29×10^5	*Aspergillus*	ND	ND	ND	ND
10	J	12.83	0.5416	4.595	8.85×10^4	*Aspergillus*	ND	ND	ND	ND
11	K	13.24	0.5061	5.665	ND	ND	ND	ND	ND	ND

Mc: moisture content a_w: water activity CFU/g: colony-forming units AFLs: aflatoxins OTA: ochratoxin A EST: sterigmatocistin ZON: zearalenoneND: not detected *LOQ: 2 ug/kg each
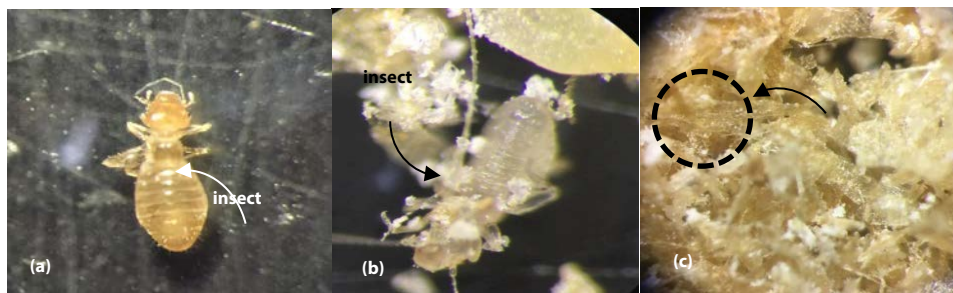
Fig.1 Insects and mites detected in flocked oats (*Avena sativa* L.) samples stored in bags understereomicroscopy [x60].
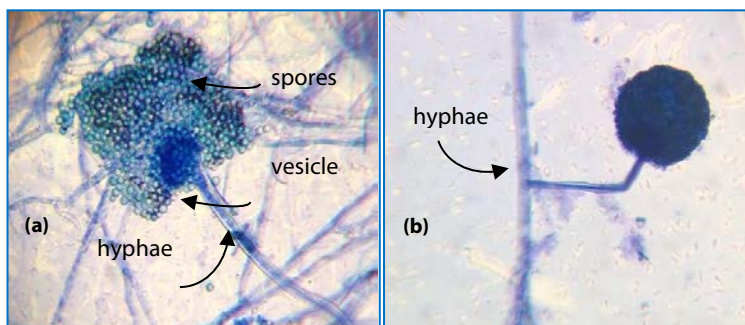
Fig.2 Reproductive structures of fungiisolated from flocked oats (*Avena sativa* L.)by light microscopy (a)*Aspergillus* and (b) *Rhyzopus*genera [x400].

Discussion

Insects and mites are vectors of fungal contamination, as spores and hyphae may develop and be carried / transported in their exoskeleton.In addition, mites can trigger allergies in humans. Franzolin (1998) has identified that when spores of *Aspergillus flavus* adhere to the body of mites, they do so as a means of spreading, causing viable spores to proliferate in grains stored incorrectly.

Soares et al. (2018) isolated fungi of the genus *Aspergillus* and *Penicillium* adhered to the exoskeleton of beetles *Alphitobiusdiaperinus*, considered a secondary pest in storage units.

The mc values detected in the samples were higher than those found by Sandrin (2013) in oat flakes where authors got 10.04%. Gutkoski &El Dash(1999) suggest that after hydrothermal processing followed by flocking, the oats flakes should reach 10% mc.When the mc is higher than 13% over time, the acidity index increases very fast, indicating grain deterioration (Gutkoski and Pedó, 2007; Rupollo et al, 2004). All samples analyzed showed high pH, which leads us to conclude that the deterioration and rancidity process started, generating a characteristic odor, however, only in one sample studied. The oil extracted from oats presents a great amount of unsaturated fatty acids, with linoleic being the main one. Unsaturated fatty acids have double bonds in their structure, making themmore unstable to the rancidity process. Hydrolytic and oxidative rancidity are factors that adversely affect quality (Gutkoski and El-dash, 1999). They can be caused by enzymes (active at acidic pH) present naturally in the grain or by contaminating microorganisms.The acidity of sample F (pH: 4.1), followed by samples J (pH: 4.60) and I (pH: 4.61), can be explained by that reason (enzyme catalyte activities). Samples I and J both presented microorganisms contamination, one of the causes of rancidity byoxidative enzymes.

Only 2.8% the samples were out of the standards required for total fungal load. Unsatisfactory conditions at the storage time, such as high temperature and humidity favor the development of fungi (Scussel, 2002).With the data obtained from the total fungal load it is concluded that there was a high contamination in storage due to the presence of *Aspergillus* (storage) and *Rhyzopus* (deterioration) genera. The presence of fungi in the product can cause spoilage, alter organoleptic properties, and present health risks (DallaVecchia&Castilhos-Fortes, 2007).

Influenced by extrinsic factors such as a_w, fungi can develop, causing serious problems for the grain. According to Iamanaka et al. (2013) the values that favor fungal development and toxin formation vary between 0.60 and 0.90. However, these values obtained from samples C (aw: 0.5547), F (aw: 0.5904), and I (aw: 0.5728) were favorable to the development of some genus of filamentous fungi. The mycotoxin production isdirectly related to the quality of storage. According to Gerez et al. (2014), the optimal conditions for *Aspergillus niger* to produce OTA was a_w of 0.995. Despite that, the toxin can be produced from a_w as low as 0.60, depending on other factors such as temperature and substrate composition.Other authors such as Esteban et al. (2006) also reported lower values of $a_w$for OTA production.

In a single sample (F) the highest $a_w$detected was 0.590.Consequently it was contaminated by OTA. Kuzdralinski et al. (2013), when analyzing oat grains, reported that 42 of58 samples were contaminated with OTA. In another study by Sacchi et al. (2009) authors did not find AFLs and ZON in their samples, corroborating what was found in the present study.

Conclusion

The samples showed uniformity in the flakes size. However, the presence of insects and mites, which exposes the grain to other types of contaminants such as fungi, was registered along with high colony forming units of *Aspergillus* and *Rhyzopus*. The high pH detected in the samples leads us to conclude that deterioration and rancidity were in the process of initiation. Other characteristics such as the levels of mc and a_w also favor the development of fungi and their metabolites.

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The impact of two drying methods on the quality of high-moisture rice

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Abstract

In this experiment, freshly harvested rice was dried by natural and mechanical methods. For natural drying, paddy rice was spread on a cement floor under a shelter at a thickness of 4cm, and it was turned twice a day. At a temperature of 19.3°C and a relative humidity of 58.8%, a total of 28 days was needed to reduce the water content from 23.11 to 14.38%. For mechanical drying, the Guwang 5HXG-15B circulating dryer was used, drying temperature was set to 42°C, and it took a total of 5 hours to reduce the water content from 23.1 to 11.8%. The changes in spore count, fatty acid value, germination rate, waist burst rate, whole polished rice rate, and taste value of rice mold after drying were studied. The results showed that compared with mechanical drying, the

drying rate of air-dried rice was slower, and the number of mold spores increased from 0.65×10^5/g to 3.05×10^5/g, a 3.7 times increase. The number of mold spores in dried rice was not significant. Dried rice fatty acid value of 25.1mg/100g for natural drying was higher than the value of 19.9mg/100g for mechanical drying. High temperature affected rice seed vigor: mechanically dried rice germination rate was 58.0%, far lower than the 87.5% for natural drying. The blasting rate, polished rice rate, and taste value of mechanically dried rice were 5.33%, 57.9%, and 83.7, respectively, which was 2.33%, 58.9%, and 89.3 for naturally-dried rice. The processing quality and taste quality were even worse. Therefore, the drying process of the optimized circulation dryer should be further adjusted to reduce its impact on rice processing quality and taste quality.

Key words: rice; natural drying; circulating dryer; processing quality; taste quality

1. Introduction

Rice is one of the world's major grains, and 50% of the world's population is rice-based. In China, as one of the three major grain consuming countries, the yield of rice ranks second only after corn. In 2017, China's rice cultivation area was 30 million hectares, and the output was about 210 million tons. The planting area and output all ranked first in the world. The moisture content of rice after harvest is usually high. At this time, the rice itself has a strong respiration. At the same time, the biological activity of grain reserves is intense. If it is not dealt with in time, it can easily cause adverse effects such as high temperature, germination, and mildew. According to statistics, China's high-moisture paddy after harvest was too late to reach the safe storage moisture content, resulting in losses of up to 5% in storage, transportation, and processing. Rice drying is a necessary processing step after harvest. Rice is a heat-sensitive grain, and unreasonable drying methods can cause changes in the physical and chemical characteristics of the main components of rice. Therefore, it is important to choose an effective and appropriate drying method. The natural drying method is a technique for ventilating and drying food by means of solar energy and natural wind in a natural environment. The natural drying method does not require special equipment. Although it is largely limited by weather conditions, it is still the main method of grain drying in China. With the rapid development of agricultural machinery, grain dryers have become more and more widely used. They can be roughly divided into continuous and circulating types. Compared to corn and wheat, rice has higher requirements for drying process due to its heat sensitivity, and circulating dryer with small batches and low temperature is more suitable.

In this experiment, removal of water was performed on high-water rice by using two drying modes: natural rice drying and circulating dryer. The changes of water and fungal spores in rice during the natural drying process were studied. The quality of the dried rice was compared between the two methods, and the differences in the changes were studied to provide reference for the effective and reasonable method of rice drying in the post-harvest treatment of rice.

2. Materials and Methods

2.1 Test materials

Test material was new rice that was harvested on September 13, 2017. The variety was Shenliangyou 5814, and the place of production was Xinjin County, Chengdu. The initial quality indicators of rice are shown in Tab.1.

Tab.1 rice intial quality indicators

Moisture（%）	Impurity rate（%）	Brown rice rate（%）	Thousand kernel weight（g）
23.11	1.83	76.36%	31.16

2.2 Test equipment

FA22048 Electronic Balance: Shanghai Jingke Tianmei Scientific Instrument Co., Ltd.; 101-1A Electric Heating Blast Drying Box: Beijing Zhongxing Weiye Co., Ltd.; JFSD-100 Crushing Machine: Shanghai Jiading Grain and Oil Instrument Company; JJSD Type Filter: Shanghai Jiading Grain and Oil Instrument Company; JLG-1 Huskers: Chengdu Grain Storage Research Institute, China Grain Storage; JNM-III Milling Machine: Chengdu Grain Storage Research Institute, Middle Grain Storage;

SMART Biological Microscope: Beijing Zhongxian Hengye Instrument and Meter Co., Ltd.; HPS-250 Biochemical incubator: Harbin Donglian Electronic Technology Development Co., Ltd.; JSWL rice taste detector: Beijing Dongfu Jiuheng Instrument Technology Co., Ltd. and Japan Satake Company; TH802A mechanical temperature and humidity meter: Meideshi Instrument Co., Ltd.; DTS418 type three-phase four Line Electronic Energy Meter: Changan Group Co., Ltd.

1.3 Test methods

1.3.1 Rice natural drying

Fig.1 Rice natural drying
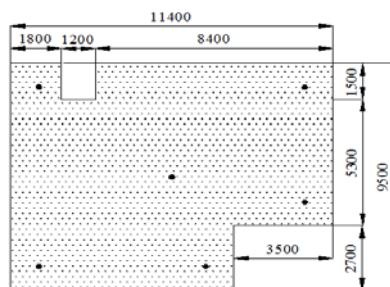
Fig.2 Sampling point layout

In accordance with the common farmer's pattern of grain drying, we chose a well-ventilated area to lay the wet rice on the cement floor under the shelter (Fig.1). The total weight of rice was 1.60t, and the area of the site was $11.4 \times 9.5 - 1.2 \times 1.5 - 3.5 \times 2.7 = 97.05 m^2$, and the thickness was about 4cm. Samples of rice moisture content and fungal spores were routinely taken at 8:30 daily. The location of the sampling points was set as shown in Fig.2. Six sampling points were located at five corners and at the center. The sampling point on the corner was 1.00m away from both adjacent sides. The six sampling points can more fully reflect the change of moisture in the rice during the drying season. In accordance with the method of foodstuffs for farmers, daily sun drying was carried out. [Taking into account the relationship between autumn temperatures, the use of wooden clogs is repeated every day at 9:00 and 14:00.What does this mean?] We placed a thermometer and hygrometer on the drying area and recorded the temperature and humidity changes from 9:00 to 17:00 every hour.

2.3. 2 Rice mechanical drying

We used a mechanical dryer to dry 10.00t of the same batch of wet rice. Dryer model GuWang5HXG-15B, a cross-flow circulation dryer.

2.3.3 Determination of indicators

2.3.3.1 Determination of moisture content was according to GB/T 5497-1985. In the early drying period, the moisture content of rice exceeds 18%. Therefore, it was necessary to use two drying methods. The specific steps were: Weigh out 20g of rice (accurate to 0.001g), and put it in a baking box with a diameter of 10cm and a height of 2cm. Bake at 105±2°C for 30-40min. Take out and cool to constant weight. (The difference between two weighings does not exceed 0.005g. This is the weight of the sample after the first baking). After smashing the first baked rice sample, use an aluminum box that is baked to a constant weight, weigh about 3g of the sample (accurate to 0.0001g), put the aluminum box cover on the bottom of the box, and put it into the drying oven . Take it out after 105±2°C for 3 hours, remove it, cap it, place it in a desiccator and cool it to room temperature. Take it out and weigh it, then re-bake it according to the above method, take it out and cool and weigh it once every 30min, baking it before and after. The difference between the two weights should not exceed 0.005g. If the latter weight is higher than the previous weight, the previous weight is calculated. The moisture content of rice was calculated according to the following formula:

$$\text{Moisture（\%）} = \frac{W \cdot W_2 - W_1 \cdot W_3}{W \cdot W_2} \times 100$$

Where: W - the weight of the sample before the first baking, g;

W_1 - weight of the sample after the first baking, g;

W_2 - weight of the sample before the second baking, g;

W_3 - Sample weight after the second baking, g.

2.3.3.2 The number of fungal spores was determined according to the fungal spore count method proposed by Cheng et al. (2009). The specific operation procedure was: Take 10.0 g of rice sample, add 30 mL of deionized water in a 50 mL test tube, add stopper, shake vigorously for 1 min, and filter with 300 mesh. The cloth was filtered and the filtrate was counted under a microscope for fungal spores.

2.3.3.3 Determination of waist burst rate According to GB/T 5496-1985.

2.3.3.4 Determination of germination rate According to GB/T 5520-2011.

2.3.3.5 Determination of roughness According to GB 5495-2008.

2.3.3.6 Determination of the rate of polished rice According to GB/T 21719-2008.

2.3.3.7 Determination of fatty acid value According to GB/T 5510-2011

2.3.4 Test site

Chengdu Qingbaijiang National Grain Reserve of Sichuan Province

3. Results

3.1 Natural drying rice moisture changes
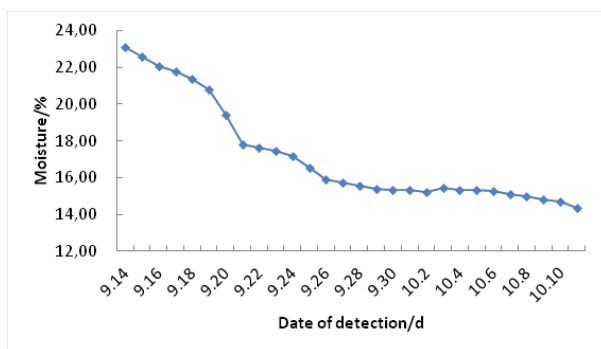
Fig.3 The curve of rice moisture

Tab.2 Daily precipitation and temperature and humidity of rice correlation analysis

		Temperature	Relative humidity	Daily water loss?
Temperature	correlation coefficient	1.000	-0.502**	0.529**
	Sig.	-	0.009	0.005
Relative humidity	correlation coefficient	-0.502**	1.000	-0.220
	Sig.	0.009	-	0.281
Daily water loss?	correlation coefficient	0.529**	-0.220	1.000
	Sig.	0.005	0.281	-

Note: ** indicates a significant correlation at the α=0.01 level, and * indicates a significant correlation at the α=0.05 level.

As shown in Table 3, there is a positive correlation between daily water loss and temperature, and the correlation is extremely significant. The daily water loss is negatively correlated with the average relative humidity, and the correlation is not significant. Therefore, under the conditions of natural

drying of rice in this experiment, the effect of temperature on the drying rate is greater than the humidity.

3.2 Rice fungi spores change during natural drying

During the natural drying, due to the slower rate of water loss, rice is in a state of unsafe moisture for a long time. Because the ambient temperature is not high, the external conditions are extremely suitable for the growth of fungi.

（a）Fusarium spore   （b）Aspergillus niger spore
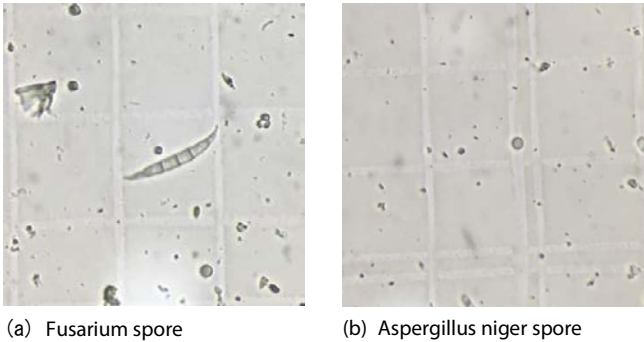
Fig.4 Microscopic view of fungal spores
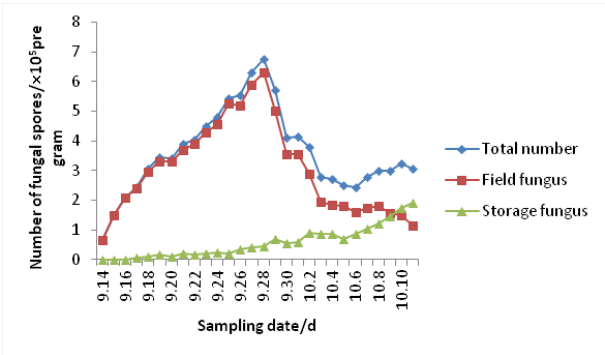
Fig. 5 The number curves of the of spores of rice fungal

Tab.3 The evaluation criteria of spoilage fungi spores in stored grains

Safty level	Fungal spores number	Safty
Level I	＜1.0×10^5	Security
Level II	（1.0～9.9）×10^5	Critical control criticality
Level III	（1.0～9.9）×10^6	Harm
Level	≥1.0×10^7	Serious harm

According to the ecological group, microorganisms can generally be divided into field fungi (infested by field growth, mainly parasitic and parasitism) and stored fungi (all kinds of saprophytic bacteria). Field fungi include *Alternaria*, *Fusarium*, etc., and storage fungi include *Aspergillus niger* and *Penicillium*[3]. Shown in Fig. 4 are the spores of the *Fusarium* spp. and *Aspergillus nigrum* under the microscope. During the experiment, samples were taken daily to detect field and stored fungal spores. A graph showing the change in the number of spores in rice cultivars is shown in Fig. 5. It can be seen from the figure that during the natural drying of rice, the total number of fungal spores first increased and then decreased. On September 28, the total number of fungal spores reached the maximum value of $6.75×10^5$/g. According to Table 3, the number of fungal spores that are hazardous to rice is at the critical control threshold, although this did not reach the level of harm. However, there is a great threat to the safe storage of rice. During the whole drying period, the

stored spores showed a gradual upward trend, and the number of spores in the field increased first and then decreased. Referring to Figure 3 and Figure 5, when the total number of fungal spores in the field reached the peak value, the rice water content was 15.55%. After that, the rice water reached a stable period with less than 1% change. Therefore, it is possible that the decrease in total moisture is the main reason for the decrease in the number of spores in the field.

3.2 Two methods of drying results analysis

Tab.4 Drying results in two ways

Dring method	Initial moisture (%)	Final moisture (%)	Drying amount (t)	Drying time (h)	Drying speed (1% moisture/h)
Natural drying	23.1	14.4	1.6	648	0.0134
Mechanical drying	23.1	11.8	10.0	5	2.26

Comparing Tab.4 with the drying results of two kinds of drying methods, it can be seen that natural drying has the characteristics of small treatment volume and slow drying speed, while mechanical drying has a large amount of processing and a high speed relative to rice batches.

3.3 Analysis of the impacts of two kinds of drying on rice quality

Tab.5 Rice crack ratio with two drying methods

Drying method	Natural drying	Mechanical drying
Crack ratio(%)	58.90±0.15a	57.90±0.36a

Note: Different letters represent significant differences, the same below.

The waist burst rate is an important indicator for assessing the drying process of rice. During the drying process, due to the different water loss rates of the inner and outer layers of the rice grains, a water gradient is generated, causing internal stress, and cracks appear when the stress exceeds the tensile strength of rice[4]. In the case of rice with a high waist rate, especially when the cracks are large and deep, it is not appropriate to process high-precision rice, otherwise it will increase broken rice and reduce the rice rate. Since the natural drying process does not assist the heating of rice, the waist rate is lower than that of the dryer. It can be clearly seen from Table 5 that the popping rate of natural dry rice is significantly lower than that of mechanical drying. [Table 5 shows no significant difference in crack rate between the two drying methods.] This is due to the high temperature environment in the mechanical dryer causing excessive water loss in the outer layer of rice grains.

Tab.6 Head rice yield with two drying methods

Drying method	Natural drying	Mechanical drying
Head rice ratio(%)	58.90±0.15a	57.90±0.36a

The head rice rate is the most important trait in the quality of rice milling, which affects the taste quality of rice to a large extent. Studies have shown that the higher the rate of whole polished rice, the greater the volume of rice swell and the better the quality of rice consumption[5]. According to Tab.6, it can be seen that the roughness of natural dry rice is significantly less than mechanical drying, and the percentage of whole rice is greater than that of mechanical dry rice, but the difference is not significant.

Tab.7 The germination rate of rice with two drying methods

Drying method	Natural drying	Mechanical drying
Germination rate (%)	87.5±5.5a	58.0±2.0b

The germination rate of rice is an important indicator for comprehensively measuring the new graininess of rice. The freshness of the rice and the quality of the food can be reflected by changes in the germination rate[6]. From Tab.7, it can be seen that compared with naturally-dried rice, the germination rate of mechanically dry rice is low, and the difference is significant. The results indicated that the vitality of natural air-dried rice embryos was well preserved, while the low-

temperature circulation dryer had a lower hot air temperature than the continuous dryer, but it still had a strong destructive effect on the internal physiological structure of rice, which seriously affected the growth of rice.

Tab.8 Fatty acid value of rice with two drying methods

Drying method	Natural drying	Mechanical drying
Fatty acid value (mgKOH· (100g)$^{-1}$)	25.11±0.57a	19.8±0.90b

Rice lipids are oxidized to produce free fatty acids, which are represented by fatty acid values. Fatty acid values are an important indicator of rice freshness, and the extent of rice quality changes can be judged based on changes in fatty acid values [7]. It can be seen from Tab.8 that the fatty acid values of rice obtained by natural drying are significantly greater than those of mechanical drying, and the differences are significant. The growth rate of free fatty acid content is significantly positively correlated with the relative activity of lipase[8]. Since natural drying has a lower ambient temperature, lipase maintains a higher relative activity during the drying process. In addition, the natural drying time is lower. Longer, higher accumulation of free fatty acids may result in relatively higher values of natural dry rice fatty acids.

Tab.9 The taste value of rice with two drying methods

Drying method	Natural drying	Mechanical drying
Taste value(%)	89.3±0.41a	83.7±0.41b

The main principle of the taste meter is to accurately determine the components of amylose, protein, water, and fatty acids that determine the taste of rice by the difference in absorbance generated by the near-infrared light at a specific wavelength, and then to compare different ingredients data with experimental rice taste. The data is combined and scored by simple numerical values to objectively reflect the purpose of eating rice[9]. As shown in Table 9, the taste value of natural dry rice was significantly greater than that of mechanical drying, indicating that rotary bins can better protect the food quality of rice, while mechanical dried rice has the poorest food quality, and is significantly different from the former two.

4. Discussions

In this study, the water loss characteristics of naturally dried rice field were studied. At a temperature of 19.3°C and a relative humidity of 58.8%, a total of 28 days of water reduction from 23.11 to 14.38% was observed. The water loss of rice showed a trend of rapid change and slow change. The analysis of the correlation between daily water loss and ambient temperature and humidity shows that the daily water loss is positively correlated with the ambient temperature, and the correlation is significant. It is negatively correlated with the relative humidity of the environment and the correlation is not significant. During the drying period, the number of fungal spores in the rice field gradually increased in the early stage, began to decline after reaching the highest value on September 28, and the number of stored fungal spores gradually increased, and exceeded the number of field spores in the late drying period. Mechanical drying was performed using a Guwang 5HXG-15B circulating dryer. The drying temperature was set at 42°C, and the water content was reduced from 23.1 to 11.8% after a total of 5 hours. The changes of spore count, fatty acid value, germination rate, waist burst rate, whole polished rice rate, and taste value of rice mold after drying were studied. The results showed that compared with the mechanical drying, the natural rice has a lower popping rate, a higher rate of whole milling, and a better processing quality. The germination rate of the dried rice is much lower than that of natural dry rice. In addition, the fatty acid value of natural dry rice is greater than mechanical drying, which may be caused by the natural drying time is too long. Using the taste meter to comprehensively score the rice obtained by the two drying methods, the natural dry rice score was 89.3, which was greater than the mechanical drying of 83.7, and it was found that the nature of natural drying and taste was better.

Comparing the drying results of the two methods of drying rice, it can be seen that natural dry rice has a small amount of processing and slow precipitation, but it has a good protection effect on rice processing, germination, and taste quality. While mechanical drying is relatively large and rapid with respect to rice batches, it has a great influence on rice quality. With the increasing living standards of the people, more and more importance is attached to the quality of rice. Therefore, in the future research process, it is necessary to further improve the drying machinery technology and improve the quality of rice.

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Germination rates of frozen grain legume seeds in Cameroon

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Abstract

A project on collection and conservation of genetic resources was carried out in Cameroon in 2014 in villages around Yaounde, Mbalmayo, and Ebolowa. Samples of all grain legume species cultivated by the farmers were collected from the 15th of March till early May 2015. Farmers in these zones cultivate mostly ground nuts, followed by soybean and cowpea. A total of 39, 13, and 45 samples were collected from Yaounde, Mbalmayo, and Ebolowa, respectively. After collection, samples were sun-dried, treated, labeled, plasticized, and stored in the freezer at -20°C in the Institute of Agricultural Research for Development (IRAD) store room at Nkolbisson, Yaounde. A trial was carried out at IRAD Kumba experimental farms in 2016 to purify and maintain 14 cowpea and 12 groundnut samples from the freezer, under the C2D project. There were highly significant differences ($P< 0.05$) amongst samples (treatments) for the germination rate. Cowpea samples had a germination rate ranging from 0.33 to 47.67%, while germination rates for groundnuts were between16.67 to 68.33%. Out of the 26 samples, only 5 (19%) had germination rates above 50%. Due to irregular power supply, freezing turned out to be an ineffective storage method for grain legume seeds. Seeds are now being maintained in vivo in small quantities and on seasonal basis which renders the job of plants breeders very difficult and ineffective. Alternatives storage methods and facilities for grains and seeds in developing countries like Cameroon remain an urgent need to boost research and ensure food security.

Keywords: conservation, grain legume, germination, seeds, developing countries.

Introduction

Biodiversity is the third component of biological diversity (the other two being the specific diversity (the individuals) and ecosystem diversity (populations and their habitats)). Biodiversity may refer to wild biodiversity and agricultural biodiversity. Since the Conference in Rio de Janeiro in 1992 organized by the Convention on Biological Diversity (CBD), to which Cameroon is party, other international legal instruments have been put in place in order to ensure the implementation of the relevant provisions of the CBD. Among these instruments signed and/or ratified by Cameroon, we may cite the International Treaty on Plant Genetic Resources for Food and Agriculture on the

facilitated access and benefit-sharing in the multilateral system of the Treaty, and the Protocol of Nagoya on access to genetic resources and the fair and equitable sharing of benefits arising from their exploitation.

Genetic resources are, in effect, the basis of variation. The development of plant varieties and animal breeds constitutes the main axis of research in order to increase agricultural production to ensure the food security of populations. This can be done either by conventional selection methods or by biotechnology which lead to the creation of genetically modified organisms (GMOs). Therefore, genetic resources, both exotic and landraces, constitute the essential raw material for researchers in selection and improvement of cultivated plants and animals.

The diversity of cultivated plants, animals, fisheries, and forest products are the 4 main components of agricultural biodiversity, and also are essential to the satisfaction of tastes and preferences of consumers that change constantly following culture and economic fluctuations.

The diversity of landraces cultivated by farmers is not sufficiently known to researchers, but nevertheless represents a reservoir for the search for genes important in the creation of varieties adapted to the unpredictable changes in the environment. These landraces, having been cultivated for many years, have acquired a stability of performance (Marchenay and Lagarde, 1986).

In the context of Cameroon, Nya Ngatchou Fondoun published a report of 385 pages in 1987 entitled "Inventories of plant genetic resources in the structures of the Institute of Agronomic Research (IRA)". This document, devoted to plant genetic resources, contained collections in the fields and in the cold rooms with a total of 9500 accessions. Of these, 5240 accessions were unimproved genetic material and 4260 accessions were improved. At that time, the Institute of Agronomic Research (IRA) still enjoyed a relative comfort in terms of preservation infrastructures and financial and human resources. The economic crisis of the mid-1980s led to financial hardship, and the samples were abandoned in the cold rooms. These lasted for 20 years, during which significant losses of genetic material were recorded in both *in situ* and *ex situ* collections. Recently, few studies have been carried out on the collection and characterization of grain legumes in Northern Cameroon (Gonne et al., 2013) and nation wide (Atemkeng and Yousseu, 2017). The results indicated that most of the landraces collected in the 1980s have become extinct. There was therefore the need to recollect and conserve local plant genetic resources for the development of new improved crop varieties. The objectives of this project was to collect and ensure the conservation of the major grain legume genetic resources cultivated by farmers in Southern Cameroon.

Materials and Methods

With funding from the public investment budget (PIB 2014), a project was carried out entitled: collection and conservation of genetic resources in Cameroon. A team made up of researchers, technicians, agricultural extension workers, and village facilitators worked as three groups in villages around Yaounde (Nkolfoulou, Minkoameyos, Elig Essomballa, Elumdem, Nkometou III, Mendong, Simbock), Mbalmayo (Nkolnguet, Melombo, Ekombitie) and Ebolowa (Nkoemvone, Biba, Assoo'seng, Ndengue). Samples of all grain legume species cultivated by the farmers were collected from the 15th of March till early May 2015. Farmers were contacted on the farm, market, and at times at home in the evening. In the process, the objective of the project was explained to farmers and some incentives in form of in-kind donations given to them to supply the samples. After collection, samples were sun-dried, treated, labeled, plasticized, and stored in the freezer at -20°C in the Institute of Agricultural Research for Development (IRAD) store room at Nkolbisson, Yaounde (Figure 1). A trial was carried out at IRAD Kumba experimental farms from September 2016 to December 2016 to purify and maintain 14 cowpea and 12 groundnut samples from the freezer, under the C2D project. The data collected were germination rate, days to first flowering, days to 50% flowering, number of seeds per pod, number of pods per plant, pest and disease scores, and grain yield per hectare. Here only data on germination rates is reported.

Data analysis

Analysis of variance was done using the R – package version 2016 and multiple mean separations were done using the Tukey test.

Fig. 1 Grain Legume Samples frozen at -20ºC.

Results

Farmers in these zones cultivate mostly ground nuts, followed by soybean and cowpea. Very few farmers cultivate common bean. The samples ranged from 20 to 50 grams per sample (Figure 2). A total of 39, 13, and 45 samples were collected from Yaounde, Mbalmayo, and Ebolowa, respectively. There were highly significant differences ($P < 0.05$) amongst samples for the germination rate in cowpea, while no significant differences existed among groundnut samples. Cowpea samples had a germination rate ranging from 0.33 to 47.67% (Table 1), while germination rates for groundnuts were between16.67 to 68.33% (Table 2). Out of the 26 samples, only 5 samples (19%) had germination rates above 50% (Tables 1 and 2).

Discussion

The results indicate that only 19% of the frozen samples had germination rates above 50%. This might have been caused by irregular power supply and low voltage. Consequently, freezing, which is very effective for storing grains for years, turned out to be an ineffective storage method for grain legume seeds in Cameroon. The efforts of the team went in vain, and the budget allocated for the project was sort of wasted as phase two of the project could not be implemented due to low germination rates. Seeds are now being maintained *in vivo* in small quantities and on a seasonal basis which renders the job of plant breeders very difficult and ineffective. Alternative storage methods and facilities for grains and seeds in developing countries like Cameroon remain an urgent need to boost research and ensure food security.

Fig. 2: Grain legume samples from farmers ranged between 20 to 50 grams per sample.

Tab. 1: Germination rates of cowpea seeds frozen for two years at -20ºC.

Cowpea gerplasm	Germination rate
Accession 1	0.33a
Accession 2	16.67ab
Accession 3	30.00ab
Accession 4	47.67bc
Accession 5	20.00ab
Accession 6	15.00ab
Accession 7	36.67ab
Accession 8	13.67ab
Accession 9	17.00ab
Accession 10	21.67ab
Accession 11	14.67ab
Accession 12	9.67ab
Accession 13	16.67ab
Accession 14	20.33ab
Accession 15	**76.67c**
DF	14
F	1.715
P	<0.001

Means followed by the same letters are not significantly different (*P*< 0.05). Highlighted samples had germination rates above 50%.

Tab. 2 Germination rates of Groundnut seeds frozen for two years at -20ºC

Variety	Germination rate (%)
Aboul Niveau	**68.33**
Afoumou	36.33
ICGV 86003	**59.67**
JL 24	50.67
Manipinta	**58.00**
Mfoumou	29.33
Minkonga	**54.00**
Ngomomou	20.00
Ngomomou Congo	25.67
Ngoxomou	34.00
Ossa owondo	45.33
Zebedee	16.67
DF	11
F	0.959
P	0.506

Means are not significantly different (P< 0.05). Highlighted samples had germination rates above 50%.

Acknowledgment

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Bioefficacy of Cameroonian Hemizygia welwitschii Rolfe-Ashby (Lamiaceae) leaf powder against Callosobruchus maculatus Fabricius in stored cowpeas seeds

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This work aims to evaluate the efficacy of Cameroonian Hemizygia welwitschii leaf powder against C. maculatus. The H. welwitschii leaf powder was applied at four different dosages 0.25, 0.5, 1 and 2 g/50g (corresponding to 5, 10, 20 and 40 g/kg) and SilicoSec (positive control) at 0.025, 0.05, 0.075 and 0.1 g/50g of cowpea (corresponding to 0.5, 1, 1.5 and 2 g/kg) and the untreated control (0 g/50g). 20 unsexed adults were introduced into the test jars to evaluate adult mortality and F1 progeny. To assess damage and seed viability, 30 unsexed insects were added to jars treated at the same concentration. Adult?s mortality was recorded at 1, 3, 5 and 7 days after treatment (DAT), damage and seed viability were evaluated after three months of storage. All the experiments were arranged in a completely randomized design with four replications. From the results obtained, the highest mortality rate (82.50%) was recorded in jar treated with H. welwitschii at 40 g/kg compared to 100% for SilicoSec (2 g/kg) at 7 DAT. Like SilicoSec, H. welwitschii significantly (P < 0.001) reduced the number of F1 progeny compared to the untreated control. Seed damage was found to decrease with increase in concentration of insecticide within the three months of storage. Germination rate of cowpea seeds treated with the highest dosage (40 g/kg) of H. welwitschii powder were 72.50% and for SilicoSec was 87.50% (1.5 g/kg). Our findings show that the leaf powder of H. welwitschii is very effective in protecting stored cowpea seeds against C. maculatus infestation and could be exploited by farmers.

Session 2
Biology, Ecology and Behavior

Insect infestation sources in stored maize grain; what is more important resident versus incoming infestation?

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Abstract

Most studies targeted pest control inside stores; incognisant of the population dynamics in the store vicinity; leading to product re-infestation. Distinction between storage insect pest source and sink grain patches is important for effective pest management strategies. We examined the role of resident versus incoming insect infestation in phosphine-fumigated closed or open and unfumigated closed or open maize farm stores. Grain quality measurements were recorded over 32 weeks for two storage seasons. Whether open or closed, fumigated grain had significantly lower ($p < 0.001$) grain damage and lower grain weight loss ($p < 0.05$) than unfumigated grain. Fumigated open stores had significantly higher ($p = 0.004$) grain damage and weight loss than closed ones. Grain damage was higher in unfumigated-closed than fumigated-open, evidence that resident infestation inflicted higher food loss than incoming infestation. *Prostephanus truncatus*, *Cryptolestes ferrugineus* and *Tribolium castaneum* had significantly higher populations ($p < 0.001$, $p = 0.018$ and $p = 0.001$; respectively) at bottom levels of unfumigated and fumigated grain (*T. castaneum*). *Sitotroga cerealella* and *Sitophilus zeamais* were significantly higher ($p < 0.001$) at the top of closed than open unfumigated compartments. Grain suffers less infestation and quality loss when it is a sink patch than when it is a source patch. Population build-up and 'settling' to inflict significant food loss takes longer for incoming compared to resident infestation. These results have ecological implications on postharvest IPM.

Key words: Grain sink-source patches, closed and open grain stores, fumigated and unfumigated grain, grain insect damage, grain weight loss, storage insect pests

1. Introduction

Stored product insect pests ecology has not been accorded the systematic scientific investigation it deserves, but effective stored product IPM from any perspective requires the understanding of insect pest behaviour and bionomics. Stored grain, compared to any other insect pest habitats resembles a unique and largely homogeneous habitat in which food availability for many storage pests is unlimited, making a perfect ecological system from which we can better understand the population dynamics, relationships and associations between storage pests (Athanassiou et al., 2005; Nansen et al., 2009). Studies on the ecology of most storage pests of maize have been done in laboratories giving results that are thus limited in scope of application to the farm situation. Farmer-managed stores have very diverse spectra of species, complex levels of inter-, and intra-specific competition, environmental conditions and the presence or absence of natural enemies that influence field ecological studies (Mvumi et al., 2003; Nansen et al., 2009). Therefore a study of the ecology of the maize pest complex on-station is meant to determine *in situ* activities of insect pests and associated trends in grain damage and weight loss. Many stored product pests are highly mobile and can freely move in and out of storage facilities (Campbell and Arbogast, 2004), however, it is often thought that grain insect pest infestation is largely facilitated by human activities during grain exchange, transportation and way of storage. Since insect pest status is often partly derived from their mobility to colonise unexploited grain patches, we set out to determine whether storage losses are higher when the grain patch acted as a sink (colonised only by incoming new infestation)

or as a source (colonised only by resident infestation). In the process, we determine insect succession in grain infestation, the abundance and length of storage period. We relate this to the damage and weight loss to get an indepth understanding of the stored grain ecosystem to enable development of postharvest IPM (Athanassiou et al., 2005; Carvalho et al., 2013). It has been reported, that the key to controlling stored product pests is to explore the potential connection between resident infestation (inside stored grain) and outdoor populations (Campbell and Toews, 2005). This is important to reduce the cost and risk associated with chemical pesticides (Campbell and Arbogast, 2004) in stored grain. The main objectives of the current study were to (1) determine which source of insect pest infestation between the resident (field infestation) and incoming (re-infestation) caused more grain damage and weight lossthan the other, (2) determine the trends in populations of different insect pest species over a storage season both on pest-free (fumigated) and on field-infested (unfumigated) grain; and (3)to investigate population dynamics, grain damage and weight loss and associated pest species in bulk grain in relation to granary depth as in (Athanassiou et al., 2005).

2. Materials and Methods

2.1 Granary preparation

The experiment was carried out at the Institute of Agricultural Engineering (IAE, Harare, Zimbabwe) in the granaries. Three granaries were selected, repaired, thoroughly cleaned and re-plastered using clay and small amounts of cow-dung (to prevent the clay from cracking), as per typical farmer practice.

2.2. Treatments

One tonne of shelled maize (SC 637 hybrid variety) was fumigated using phosphine tablets (Phostoxin®, Detia-Degesch GmbH, Aluminium phosphide 56% w/w + inert ingredients 44% w/w) at the recommended rate. Fumigation was done in a metal silo of volume 2.395 m3. The metal silo was placed on a strong iron bench and loaded with the grain. Ten tablets were applied to the grain at different levels (3 at the bottom, 4 at the middle and 3 at the top). This was achieved by driving a metal pipe to the desired level and then dropping the tablet through the metal pipe. The spouts of the metal silo were then immediately closed using custom-made tight fitting lids followed by extensively wrapped with packaging tape to make the silo air tight.

About 900 kg of the fumigated grain were weighed and separated into six portions of 150 kg each. These portions were loaded into granary compartments in three granaries (blocks). Each granary had two compartments loaded with the fumigated grain, immediately after loading one compartment was closed and sealed completely while the other was left open. The closed compartments (Fumigated Closed and Unfumigated Closed) were fitted with tightly closing doors whose surfaces were then plastered using clayey soil to make a continuous seal with the wall plastering. The same was repeated with un-fumigated grain. The grain treatments are shown in Table 1.

Tab. 1 Grain treatments.

Grain treatment	Entrance status	Treatment code
Fumigated	Open	FO
Fumigated	Closed	FC
Not fumigated (unfumigated)	Closed	UFC
Not fumigated (unfumigated)	Open	UFO

2.3 Grain sampling frequency

After every four weeks, grain samples were withdrawn collected using a multi-slotted double tube brass sampling spear (about 1.2 m long). The spear was dipped vertically inside the grain whilst it

was closed, it was then opened when its tip touched the bottom, before being shaken to enable grain to enter, then it was closed. The sampling pattern in each granary compartment was as shown in Figure 6. The depth of the grain (60 cm) in each compartment enabled sampling to be conducted from the top (50-60 cm), middle (20-30 cm) and bottom (0-10 cm) positions in the granary. Grain sampled from each level was packed and labelled separately. This was meant to enable observation of the differences in grain damage and pest densities and distribution between the top, middle and bottom layers of stored grain. Samples from each point per level were bulked to make a composite sample of size approximately 1 kg.

2.4. Data collection and analysis

For each 1kg grain sample, all insect pest species, were identified, counted and recorded. Insect-damaged and undamaged grains were separated, counted and weighed. This was achieved by dividing each sample into four equal sub-samples using a riffle sample divider. A sample was first poured out from the sample bag into the riffle divider to produce two equal sub-samples. These were each further divided in the same manner to produce a total of four equal sub-samples. Grain from three sub-samples were each poured out into white plastic trays and examined for insect damage. The fourth sub-sample was not considered. Data from the three sub-samples were averaged to give a sample average for damage and weight loss. Trash weight and insect counts were done for the entire sample. Data on grain damage (%) was arcsine square root–transformed before being analysed. Data on grain weight loss (%) were analysed without any transformation.

Data on insect numbers for each species were $\sqrt{(x+1)}$ -transformed (Fowler et al., 1998). All the data were then subjected to one way analysis of variance (ANOVA) in STATISTICA 13.3.Where the F-ratio was significant ($p < 0.05$), means were separated by Tukey-Kramer's HSD test.

3. Results

In season 1, grain damage started increase notably in the unfumigated grain (UFC and UFO) from week 12 - 32 (Fig 1A) and from week 8 – 32 in season 2 (Fig. 1B). Generally, unfumigated grain showed consistently significantly higher ($F_{(24, 288)} = 2.810$, $p = 0.0002$) grain damage than the fumigated grain regardless of being closed or open from week 12-28. In both seasons, at week 32, only the unfumigated open (UFO) had significantly higher grain damage ($p < 0.001$) than fumigated closed (FC). However, lack of significant differences between fumigated open (FO) (no resident infestation) and unfumigated closed (UFC) (with resident infestation) in both seasons signified that both sources of infestation were equally important over time (Fig 1 A and B). In both seasons, there was a significant interaction ($F_{(24, 288)} = 2.810$, $p = 0.0002$) (Season 1) and ($F_{(24, 288)} = 1.7711$, $p = 0.0161$) (Season 2), signifying that grain damage was significantly affected by the treatments over time (Fig 1A &B).

Grain weight loss was more pronounced in season 2 than in season 1 (Fig 2A & B). As observed in grain damage, significant increase was observed from week 12. Generally, unfumigated grain (UFC and UFO) specifically showed persistently significantly higher grain weight loss ($F_{(24, 288)} = 2.7946$, $p = 0.0003$ than fumigated grain (FC and FO) between 12-28 weeks in season 1. Again, in season 1, UFC showed consistently high grain weigh loss ($p < 0.001$) than FC between 12-28 weeks but was not significantly different from UFO. At week 32, although UFO had significantly higher grain weight loss than both FO and FC ($p < 0.001$), there were no significant differences between FO and UFC, again signifying that the visiting infestation (FO) and resident infestation (UFC) had equal similar impact on grain weight loss (Fig 2A). In season 2 however, there were no notable increases in grain weight loss from 0 – 24 weeks. Nevertheless, from week 28 – 32, unfumigated (UFC and UFO) grain started showing higher grain weight loss ($F_{(8, 297)} = 16.556$, $p = 0.0001$ than the fumigated grain (FC and FO). This showed that resident infestation had more negative impact than incoming infestation.
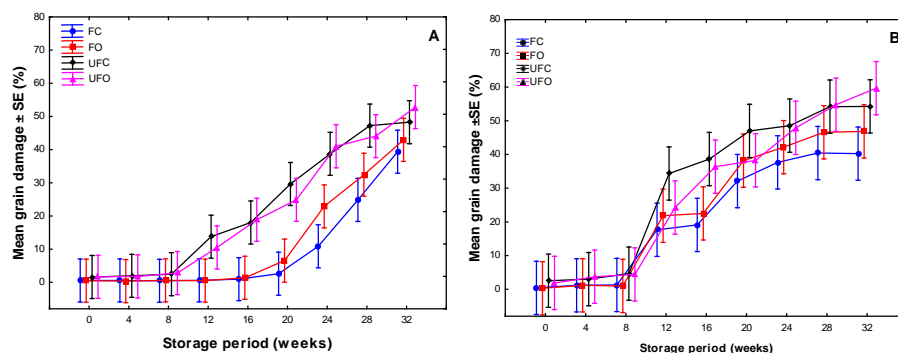
Fig. 1 Grain damage in (A) season 1 and (B) season 2 for different treatments: FC = Fumigated closed; FO = Fumigated open, UFC = Unfumigated closed and UFO = Unfumigated open
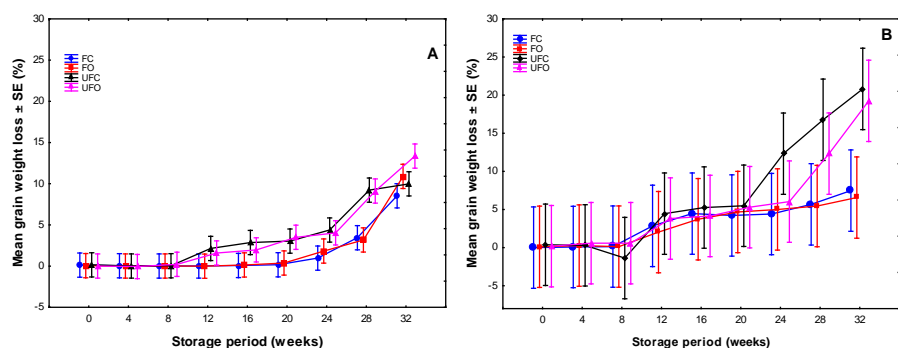
Fig. 2 Grain weight loss in (A) season 1 and (B) season 2 for different in different treatments: FC = Fumigated closed; FO = Fumigated open, UFC = Unfumigated closed and UFO = Unfumigated open.

In both seasons the opening or closing of the granary entrance did not show significant effect on grain weight loss compared to fumigation and non-fumigation. There was a significant interaction ($F_{24, 288}$ = 2.7946, p = 0.0003) between the length of storage period and treatments on grain weight loss for season 1, showing that the length of storage period affected grain weight loss for each treatment. However, this was not the case for some treatments in season 2 ($F_{(16, 297)}$ = 1.2473, p = 0.231).

We assessed the evolution of grain damage along the depth of the grain. In the granary in both seasons, grain damage was consistently low and constant for the first 8 weeks; significant increase changes at was observed from 12 weeks (Fig. 3A and B). Generally, the TOP layers of the grain had consistently higher grain damage in both season 1 ($F_{(16, 297)}$ = 2.3306, p = 0.00295) and season 2 ($F_{(16, 297)}$ = 2.8282, p = 0.00027) than the middle (MID) and the bottom (BOT) levels. The latter were not significantly different from each other in both seasons (Fig. 3A and B).
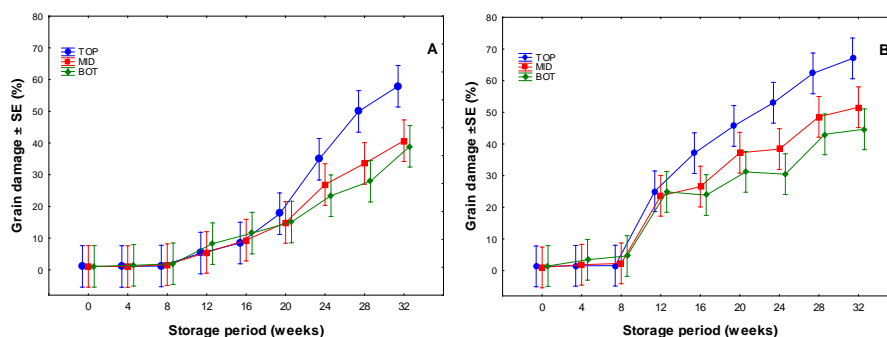
Fig. 3 Grain damage along the depth of grain at top (TOP), middle (MID) and bottom (BOT) in (A) season 1 and (B) season 2.
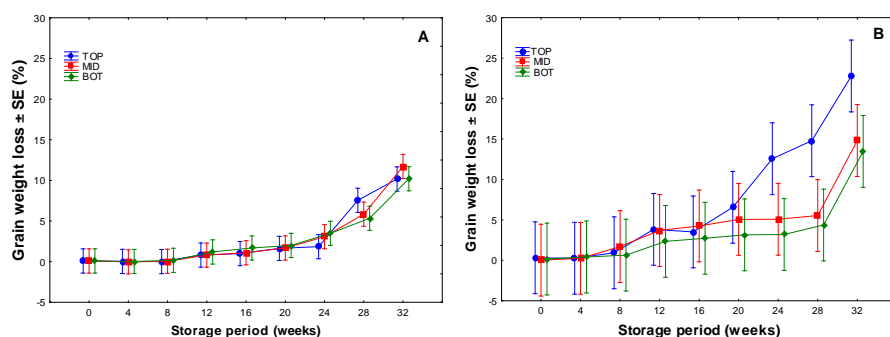
Fig. 4 Grain weight loss along the depth of grain at top (TOP), middle (MID) and bottom (BOT) in (A) season 1 and (B) season 2.

For grain weight loss however, notable increase was observed in week 28 through to week 32; with MID and BOT showing significant differences ($F_{(8, 297)} = 68.086$, $p < 0.0001$) between week 28 and 32. Nevertheless, the three levels did not show any significant differences ($F_{(16, 297)} = 0.66814$, $p = 0.82477$) among each other in season 1 (Fig 4A). This was inconsistent with season 2 which showed significantly higher ($F_{(2, 297)} = 16.555$, $p < 0.0001$) grain weight loss at the TOP level than the MID and BOT (28 weeks) and on the BOT only in week 32 (Fig 4B).

There was no significant interaction ($p = 0.82477$) (season 1) and ($p = 0.23100$) (season 2) between the level of grain and the length of the storage period on grain weight loss. This implies that in our results, length of storage period did not significantly influence grain weight loss for each grain level sampled.

For the sake of brevity, results reported for insect pest populations are for the second storage season only (2013/14). General increase in *S. zeamais* populations in grain was observed from 16 weeks of storage through to 32. Significantly higher ($p < 0.001$) was recorded in FO (922.3 insects/kg of grain) at 28 weeks.
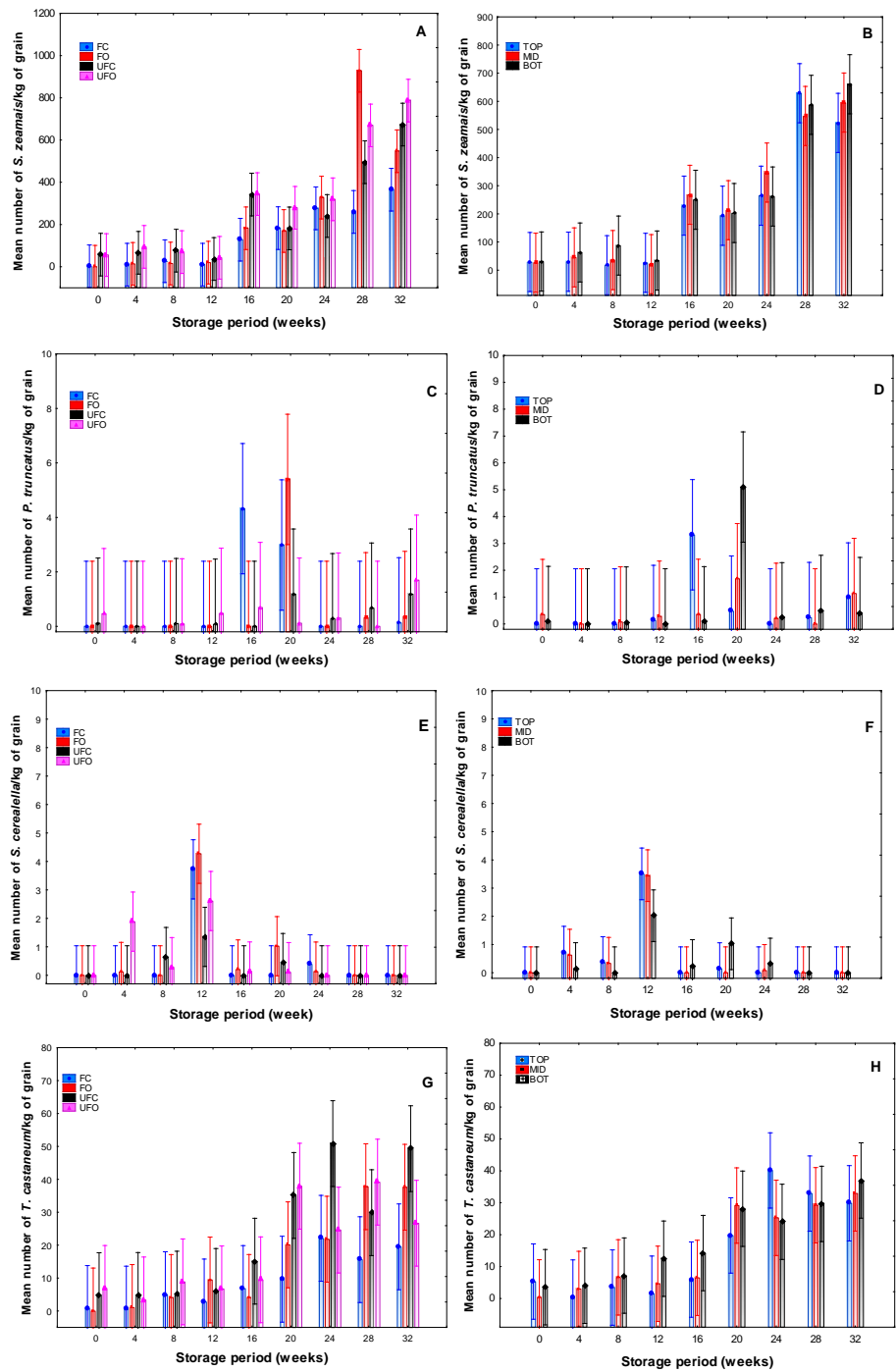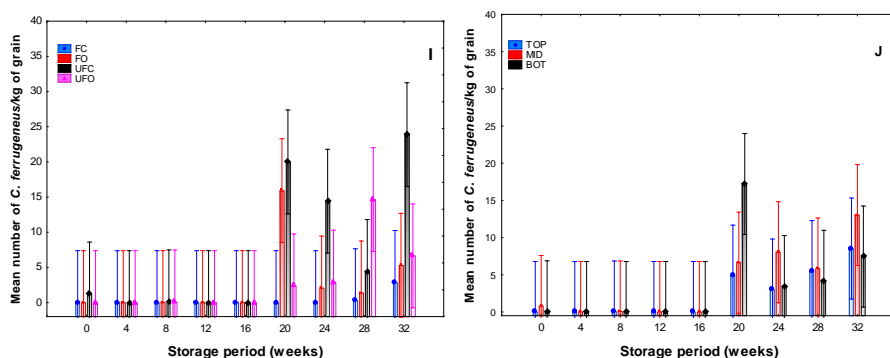
Fig. 5 Number of adult insects recorded over a 32 week storage period for each treatment and at different levels of grain depth (A &B) *S. zeamais*, (C & D) *P. truncatus*, (E & F) *S. cerealella*, (G & H) *T. castaneum*, and (I & J) *C. ferrugeneus*. (FC = fumigated closed, FO = fumigated open, UFC = unfumigated closed, UFO = unfumigated open; TOP, MID and BOT represent the top, middle and bottom level of grain depth in granary).

Unfumigated grain showed consistently high populations of *S. zeamais* up to 783.04 and 676.7 insects/kg of grain respectively at 32 weeks which did not significantly differ from each other. At termination, UFO (774.9 insects/kg) had significantly higher ($F_{(24, 288)} = 4.4915$, $p < 0.001$) *S. zeamais* populations than FC (357.9 insects/kg) (Fig 5A). Along the depth of the grain, although there was a general increase in populations from week 16-32, *S. zeamais* did not show significant ($F_{(16, 297)} = 0.40373$, $p = 0.9814$) preference for any specific level (Fig 5B). On the other hand, *P. truncatus* (Fig 5C and D) was detected in much lower (<10 insects/kg of grain) compared to *S. zeamais* and did not generally show significant differences between treatments ($F_{(24, 288)} = 0.84815$, $p = 0.67324$) and grain depth levels ($F_{(16, 297)} = 1.0419$, $p = 0.41205$ (Fig 5D). At peak populations (20 weeks) however, the bottom (BOT) level had significantly higher ($p < 0.001$) *P. truncatus* than the top (TOP) (Fig 5D), signifying *P. truncatus* tendency to concentrate at the bottom. *Sitotroga cerealella* increased quite earlier in storage (12 weeks)(Fig 5E) compared to other insect species. FO and FC had significantly higher ($F_{(8, 288)} = 13.175$, $p < 0.001$) populations than UFC, signifying that resident infestation had less impact in population build up compared to incoming infestation for this species. At peak populations, *S. cerealella* was significantly ($p < 0.001$) concentrated at the TOP and MID levels than the BOT (Fig 5F).

Tribolium castaneum and *Cryptolestes ferrugenius* were the major secondary pests recorded in this study. Significant increases in *T. castaneum* were observed from week 20 – 32, where it fluctuated in abundance between different treatments (Fig 5G and H). At week 20, *T castaneum* was more dominant in unfumigated grain (UFC and UFO), whereas at week 28, it was more dominant in both fumigated (FO) and unfumigated (UFO) open granaries (Fig 5G). This indicated that incoming infestation played a major role in population built up. On the contrary, at week 28 and 32, high *T. castaneum* populations were recorded in unfumigated closed (UFC) (Fig 5G) grain signifying the important role of resident infestation in population buildup. Although each of TOP, MID and BOT showed a significant increase in *T. castaneum* population over the storage period ($F_{(8, 297)} = 14.578$, $p < 0.001$), there were no significant differences between the different grain depths ($F_{(2, 297)} = 0.49571$, $p = 0.60964$) (Fig 5H). *Cryptolestes ferrugenius* was dominant in unfumigated closed (UFC) grain at weeks 20, 24 and 32 where it was significantly higher ($F_{(24, 288)}=1.8132$, $p = 0.001276$) than FC and UFO signifying the dominance of resident infestation(Fig 5I) There were no significant differences ($F_{(2, 297)}=.41696$, $p =.65943$) in the number of *C. ferrugenius* between different grain depths (Fig 5J).

4. Discussion

Regardless of being closed or open, the unfumigated grain recorded more damage and weight loss in both seasons, suggesting that resident infestation is very critical in food loss. However, open granaries generally recorded higher damage than closed ones especially at the top surfaces

signifying the importance of visiting infestation (re-infestation). Nevertheless, the significant differences observed in grain damage between the fumigated and unfumigated treatments attests to the fact that resident infestations play the major role in both grain damage and grain weight loss. This coupled with the low insect numbers in all fumigated closed and open compartments meant that incoming insects, although it should be carefully considered, does not play a key role in building up enough populations to ellicit significant grain damage and weight loss in initially pest-free grain especially in the short term.

The trend of *S. zeamais* populations remained fairly stable for the first 8 weeks, and began to show rapid increases from week 12, where higher numbers were observed at the middle and the bottom than the top layers of the granaries. In the unfumigated open environments, there were consistently higher *S. zeamais* populations at the top from around week 16. This agrees with reports by Mvumi et al. (2003) that *S. oryzae* (closely related to *S. zeamais*) in sorghum is consistently concentrated at the top levels. It is also interesting to note that there were very low populations in fumigated grain whether it was kept closed or open especially in the first 16 weeks. This shows that incoming infestations take time to build up as compared to resident ones. In open granaries, *S. zeamais* populations started to increase significantly at 16 weeks and were mainly concentrated at the top grain layers. In the closed granaries, the populations were higher at the bottom and middle layers. Campbell et al. (2006) explained that inside and outside grain storage structures, *S. zeamais* has patchy spatial and temporal distributions around the food source without a specifically apparent pattern (see also Throne and Cline, 1989). This is because of their high mobility on stored grain. Another possible explanation is that when the granary is open, insects are attracted to light and concentrate at the top layers, in addition, this within-store spatial distribution is also affected by temperature (seasons) (Athanassiou et al., 2005). Open granaries enabled insects to communicate with the outside environment by voluntary in and out movements.

Prostephanus truncatus did not occur in large numbers, but where it occurred, it was mainly found at the bottom layers, confirming reports by Vowotor et al. (2005) that the bostrychid favours bottom layers. It is postulated that bottom levels provides pressure from the grain above and *P. truncatus* manipulates this pressure to anchor its hind legs and bore into compacted maize kernels in straight lines (Vowotor et al., 2005). *Prostephanus truncatus* population trends showed that it began to appear at 16-20 weeks in fumigated open granaries at the bottom layers albeit at relatively lower populations compared to other species. This suggested that the population developed from incoming rather than resident infestation, from this standpoint, the low *P. truncatus* populations can also be explained by the fact that maize grain may not have as strong volatiles that attract *P. truncatus* compared to other comodities, e.g Cassava (Pike et al., 1994). Like *P. truncatus*, although there were fluctuations in the numbers, *S. cerealella*, was mainly detected in UFO and FO mainly at top and middle levels. This again resonates with Mvumi et al. (2002) who reported the same vertical gradient of *S. cerealella* along the depth of the grain. The invasion of fumigated grain by *S. cerealella* shows its ability to invade new territories as a primary moth and almost always appearing as the first pest on clean undamaged grain. The low numbers of *S. cerealella* observed in this study are attributable to the rapid movement and invasive nature of the moths as also reported for a similar moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Campbell and Arbogast, 2004) which could not be captured in significant populations due to the limitations of the sampling methods employed.

The source of infestation for all the fumigated closed compartments is not clear. Possibilities are that grain was infested in transit from the fumigation site to the granaries, or the re-plastering in granaries was not thorough enough to block resident insects in cracks and crevices inside the granary compartments. It is also possible that grain was infested during the short periods when these compartments were opened for sampling. Resistance of these species to the fumigant aluminium phosphide cannot also be ruled out (Daglish et al., 2004). Benhalima et al. (2004) reported detecting phosphine resistance by *S. oryzae* in Morocco and acknowledged receiving similar reports from many other countries due to the overuse of the fumigant.

We conclude that grain suffers more damage when it acts as the source patch than the sink patch. Resident infestation elicits more grain damage and weight loss than visiting infestation in the short term; but both elicit equal losses in the long term. *P. truncatus* and *C. ferrugenius* prefer the bottom levels of grain, whereas *S. cerealella* prefers top levels. *T castaneum* and *S. zeamais* did not show any specific grain depth preferences.

Acknowledgements

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Climate change and its implications on stored food grains

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Abstract

Safe food grain storages are considered as a measure to adapt to the changing global climates and as a channel to food security, particularly in periods when agriculture fails. However, grain storage themselves can be heavily affected by changing global climates. One main aspect of the 'climate change' is the rise of global temperature that may lead to an increase in atmospheric humidity. This climate change, warm and humid, are not suitable for grain storage. At such a scenario, stored grain is at a risk due to the favorable conditions developed for the growth of insect pests. Predicting the future ecological impact of climate change drivers requires understanding how these same drivers have acted in the past on the dynamics of insect's population. In the past ten years there has been a detailed documentation on the biotic and abiotic conditions of two storage sites in Israel. This historical ecological data can reveal long-term consequences of multiple drivers of climate change. The changes can be evident at the level of the species and at the level of the societies of insect-pest in the grain storage. The differences between two storages located at different climate regions in Israel further predict the direction current IPM practice may lead to. Following this understanding, we hope to develop feasible mitigation strategies that might overcome the changes ahead of us.

Keywords: Climate change, Historical ecology, grain storage insects

Introduction

The annual consumption of grain for human and animal consumption in Israel is about 5.1 million tons worth about 1.5 billion dollars. The insects living in grain storage can cause a great deal of economic damage: reduction in quantity and quality of the grains. In developing countries the damage can reach 30-50% and in developed countries the weight loss of grain after a prolonged storage period reaches up to 5-10%. In recent years, there is an increase in the population of grain storage insects [1]. There is a struggle to control the increase via raising the quantities and frequency of the provision of pesticides. This study examines the effect of climate change on the increase of insect populations in storage.

Much work has been done to examine the appropriate climatic and geographical position for grain storage. However, there is limited information to allow careful examination of the effect of climate change on the biotic factors, their interactions and their impact on the vulnerability of the grain storage. Insects infesting grain storages, among them: *Sitophilus oryzae* (Linnaeus), *Tribolium castaneum* (Herbst), *Rhizopertha dominica* (Fabricius), and *Oryzaephilus surinamensis* (Linnaeus), have a major effect on the quality and quantity of stored grains. They constitute a real threat that amounts to large economic losses [1]. The climatic changes can lead to changes in the geographical distribution of the pests, but also within the storage itself, such as changes in the rate of population development, an increase in the number of generations, an increase in the season of activity and changes in synchronization between the time of gathering the seeds from the field and the time of insect activity [2, 3, 4 ,5]. One of the most prominent effects on insects' success in grain storages is temperature [6]. The temperatures at which the insects can survive in a grain storage range from 8-41 ° C [1] with the optimum temperature ranging between 25 °C and 35 °C [1]. Environmental changes outside of this temperature range can directly and indirectly affect the storage insects. Climate changes can affect interactions and lead to the strengthening and / or extinction of species from the warehouse [7]. This work examines the possible significant interactions in the storage and their character (negative or positives) in light of future climate changes.

Materials and Methods

Storage facility

The study was conducted at two sites; northern site (NS) located in the Mediterranean climate zone in Israel (Jazreel Valley) and southern site (SS) located in a semi-arid zone, in the Negev desert. Each site has several storerooms (NS-10, SS-7). The storerooms are made from concrete and have solid roof. Grain introduction and fumigation are conducted yearly.

Sampling

In each storeroom temperature in-between flour grains, captured moisture of seeds and species presence were estimated monthly ranging between 1-34 locations on the grain mount, depending on the amount stored. Temperature in-between grains was estimated via a thermometer probe pushed 1 and 2 meters inside the grain mount. Grain moisture content was estimated in the lab. The presence of live insects was directly estimated via 1 kilo of seeds collected.

Results

In total, 147,328 insects were found during the entire sampling period. These can be characterized in 8 Coleoptera species and 2 Lepidoptera species (Table 1). The most abundant Coleoptera were *S. oryzae* and *O. surinamensis*. These two species correspond to >>50% of the total number of the individual collected. For Lepidoptera the most numerous Lepidoptera species were *P. interpunctata*, corresponding to 1% of the number of individuals found. Grain moisture content was in the range of 5-25%, temperature at the depth of 1 meter form the surface of the grain mount was 15-58 °C and 2 meter below surface 15-46 °C. Whereas the range of temperature in Israel during the sampling period 6-43 °C.

Site characteristics

Abiotic factors outside the storage- NS and SS annual temperature and humidity similarly deviate from the mean temperature and humidity 2008-2017. Whereas southern site is characterized in significantly higher temperature ($t_{(106)}$=5.276, P<0.0001, Fig. 1.1a,b) and lower humidity ($t_{(106)}$=3.142, P<0.0001).

Abiotic factors inside the storage- grain moisture content, temperature at 1 meter below grain surface and temperature 2 meters below surface were significantly higher at the SS (Table 2).

Insects' dynamics

SS had in total more insects than the NS (Table 2). At both sites there is a significant linear regression between the years since the commodity started to function and the level of infestation (NS; R^2=0.870, P<0.001, SS; R^2=0.737, P<0.003). In 2010 *S. oryzae* is the dominant pest both NS and SS. After 2010, in the north the dominancy alternates between *S. oryzae* and *O. surinamensis* and in the southern storage *O. surinamensis* uniquely dominates the storage. There is a significant negative correlation between *S. oryzae* and *O. surinamensis* in both sites (NS; Pearson -0.956, P<0.0001 and SS; Pearson -0.983, P<0.0001, Fig. 1). There is no evident correlation between these species and the other species in both sites (Pearson ranges between -0.204 to -0.083, P value between 0.848 to 0.628).
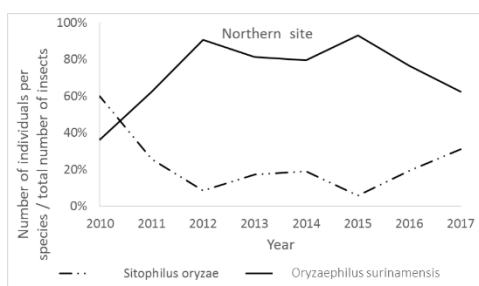
Fig. 1a Proportion of two main species found in the grain storage at the Northern Site.
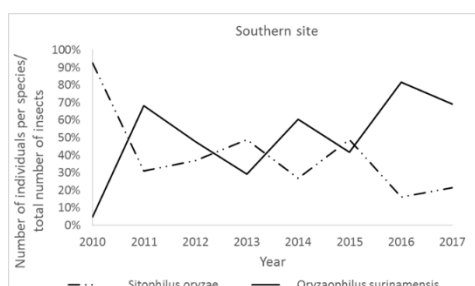
Fig. 1b Proportion of two main species found in the grain storage at the southern site.

Table 1. Insects found during the entire sampling period.

Species/taxa	%Southern Site	%Northern Site
Coleoptera		
Silvanidae		
Oryzaephilus surinamensis	55.542	66.941
Curcullonidae		
Sitophilus oryzae	24.133	14.987
Tenebrionidae		
Tribolium castaneum	12.859	11.072
Tenebrio molitor	0.000	0.014
Tenebrio mauritanicus	0.078	0.011
Bostrichidae		
Rhyzopertha dominica	0.311	0.909
Mycetophagidae		
Typhae stercorea	0.041	0.032
Cucujidae		
Cryptolestes ferrugineus	6.549	1.539
Lepidoptera		
Gelechiidae		
Sitotroga cerealella	0.063	1.384
Phycitidae		
Plodia interpunctella	0.424	3.111

Table 2. In-between grain temperature at 1 and 2 meters below grain mount surface, grain moisture content and total number of insects (both latter collected from the surface of the grain mount).

	Site	n	Mean	± std	F	P
Temperature (°C)-1 meter	NS	449	28.07	5.193	8.622	0.003
	SS	1300	28.23	4.547		
Temperature (°C)-2 meter	NS	796	30.67	4.273	5.390	0.020
	SS	1681	31.08	4.032		
Grain moisture content (R.h)	NS	1946	11.70	0.969	33.178	<0.0001
	SS	3734	12.09	2.897		
Total number of insects	NS	1974	18.46	66.02	16.589	<0.0001
	SS	3782	25.25	46.69		

Discussion

Our understanding of climate change and its implications on stored food grains, is still limited. The historical ecological data collected in the north and south of Israel can unravel long-term consequences of multiple drivers of climate change. The most prominent result of this study is that infestation levels are higher at the southern site of Israel. Such an observation stands in accordance with the faster developmental rate at higher temperature and humidity (so long it does not exceed the maximum temperature of survival). The main insect species that dominant the northern and southern grain storage are *S. oryzae* and *O. surinamensis*. The results of this study reveal significant negative correlation between the species. There are two non-mutually exclusive explanations; 1- dynamics between primary, *S. oryzae*, and secondary pests *O. surinamensis*. Grain damage caused by primary pest are known to facilitate colonization by secondary pests and reduce the infestation level of the primary pests [8]. 2- Each species has a unique range of optimal temperature, humidity and time of activity. This explanation stands in accordance with previous studies indicating that they have different spatial distribution [9] and temporal distribution (personal observation, Gottlieb Daphna). Although both explanation can explain the phenomenon the first is less likely as it assumes that there is a limited amount of grains in the storages. We are currently conducting a detailed analysis of monthly data to reveal the possible interaction between these species.

In both sites there is a significant linear regression between the years since the commodity started to function and the level of infestation. This can suggest that the facility itself, during the course of the years, accumulates increased amount of insects and treatment in-between storage is not sufficient or the insects developed resistance to pesticides (e.g. previous studies [10]). We are currently studying populations' dynamics within the year to reveal if a new harvest of grains initiates with high infection or if the infection level is equal in all years at the beginning of the storages but reacts differently to insecticides.

Acknowledgement

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Innovative stored plant products in Germany and the potential threat by native and invasive pest insects

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Abstract

Climate change, economic-political developments as well as new trends in diet and in bio-economy considerably influence the assortment of cultivated plants in Germany and thereby, determine the plant products which have to be stored after harvest. In the light of the International Year of Pulses 2016 and also, as a result of the European Soya Declaration, the acreage cultivated with new plants such as pulses, stress tolerant wheat varieties and also oil seed rape expanded worldwide. Due to increasing stocks of novel commodities, the emergence of economically important insects infesting stored products and the possible risk caused by native and invasive pest species have to be generally considered during storage. In this overall context, we studied the capacity of various stored-product pest insects to infest two important pulses. In laboratory tests different varieties of soy and lupine have been offered as whole seeds, grist and flour to selected moth and beetle species common in Germany. Over 14 weeks we examined the developmental time from egg to eclosion as well as the number of adults in the F1 generation compared to control insects reared on their standard feeding substrate. First findings under laboratory conditions (20-25 °C, 65-70 % RH) indicate that these innovative stored products, and in particular its simply processed plant products are highly susceptible to moths (i.a. *Ephestia elutella, Plodia interpunctella*) and to a much lesser extent also to some beetle species (i.a. *Callosobruchus chinensis, Tribolium confusum*), but the usally recommended optimal storage conditions (T ≤ 16 °C, RH ≤ 65%) can prevent a loss of volume and quality.

Keywords: pulses, soy, lupine, stored-product pests, risk of infestation

Introduction

Following the goals of the United Nations Agenda 2030 adopted in 2015 (United Nations, 2016) and the European Union's Sustainable Development Strategy adopted in 2001 (Commission of the European Communities, 2001), the German Federal Ministry of Food and Agriculture (BMEL) has started to prioritise food security and improve nutrition. In addition, the BMEL has stepped up his support for more sustainable and resilient agricultural systems, responding to advancing climate change, social-political developments and new consumer trends in diet and bio-economy.

As part of the German engagement, promoting the production of legume crops destinated for animal and human consumption, a so-called "protein crop strategy" has been initiated to improve cultivation of field beans and peas as well as that of more alternative protein consisting crops such as soy bean and various lupine species ("Eiweißpflanzenstrategie", BLE announcement, No. 20/17/31, 2017). Moreover, in July 2017 fourteen European ministers have signed the European Soy Declaration (Council of the European Union, 2017), an initiative launched by the German and the Hungarian Minister of Agriculture. Both political strategies underline that legume crops are vital to the agricultural system in Europe. As only 3% of the arable land in Europe is used for legume crops, the political guidelines aim to encourage the cultivation of pulses on the one hand and the scientific research related to the primary production of protein plants on the other. In this way, the participating countries will gain greater independence from non-European food and feed imports and also, support a diversification of crop rotation.

The domestic lupine (*Lupinus* spp.) and soybean (*Glycine max*), which have been introduced to Europa 150 years ago, represent excellent alternative protein sources and a protein-rich feedstuff. Both pulses have a protein content of up to 45% and thus, provide valuable plant-based protein for the production of food, feed and nutritional supplements (Bader et al., 2009; Hartman et al., 2011).

The yields of these legume crops per ha in Europe are comparatively high (e.g. to date in Germany the yield of soy is 34.4 dt/ha and the yield of lupine 18.2 dt/ha; Federal Statistical Office, 2017) and for soybean similar to those recorded in the USA and Brazil (www.usda.com; www.soystats.com). Nevertheless, Germany has a protein gap of approx. 2.5 million tons, which represents 65% of the 3.66 million tons of protein consumed in 2015 and therefore, still needs to import ca. 3.5 million tons of soy per year (http://www.fao.org/faostat/en/#home; www.ovid-verband.de). For this reason, it is a first success of the European and national politics that the area of soybean, cultivated mainly in southern parts of Germany, has been increased from estimated 5,000 ha in 2011 to 19,000 ha in 2017 (Donausoja, 2017). The harvested amounts for their part have increased from 13,000 to 66,000 t which represents a fourfold increase of the amount of inland harvested soybeans (Federal Statistical Office, 2017).

Currently, three agricultural lupine species are cultivated in Germany, the blue lupine (*Lupinus angustifolius*), the yellow lupine (*L. luteus*) and the white lupine (*L. albus*) (Bader et al., 2009; Bremer, 1999). The blue sweet lupine species, for example, are cultivated on about 30,000 hectares in the North German region (Ruge-Wehling et al., 2016) and about 53,000 t were harvested in 2017 (Federal Statistical Office, 2017).

In total, the arable acreage of pulses in Germany reached nearly 197,000 ha in 2017 (Federal Statistical Office, 2017). With increasing yields the stored amounts of these pulses will grow as well and aspects of stored-product protection have to take into consideration. Nevertheless, in case of infestation some main pesticides could be used for chemical protection of pulses, oilseeds and expeller but no pesticide especially approved for the use in stored soy and lupine is available in Germany.

Moreover, very little is known about stored-product pests associated with soy and/or lupine and their potential to develop on these plant products and consequently the damage they could provoke in middle latitudes. More than 60 insect species are described to occur in soy storages

(Hagstrum and Subramanyam, 2009) but central European storekeepers state that up to date pest insects do not represent an economic risk during storage of soy. Only the Mediterranean flour moth has been observed in greater numbers during storage in big bags (Ghosh and Jayas, 2010).

To get recommendations for good storage practice, the present study should clarify the following questions:

1) Can local stored-product pests infest stored soy and lupine and develop successfully?

2) How do they develop compared to those reared on standard (control) feeding substrate?

3) Does the processing level/degree of plant products have any influence on the development of pest insects?

4) How is the respective damage pattern provoked by the tested pest species?

Materials and Methods

To study the potential of common (native and invasive) stored-product pests to develop on soy and lupine adults or eggs of the respective beetle and moth species were placed together with 200 g of whole beans as well as of the simply processed plant products (grist and flour) of these pulses in rearing glass jars (3 L) covered with cotton cloth (Tab. 1). For experiments with soy the differently processed substrates were infested by adding 10 adult moths or 30 adult beetles. Whole beans of lupine were infested by adding 100 moth eggs or 50 adult beetles. Test beetles were all of the same age without taking into account sexes. Additionally, grist and flour of a mix of 4 blue sweet varieties (*Boregine, Boruta, Mitrabor, Probor*), one white sweet variety (*Energy*) and one blue bitter variety (*Karo ZS*) were infested by adding 100 moth eggs of *Plodia interpunctella* and *Ephestia elutella* at the beginning of the experiment. As control the indicated amounts of the respective standard laboratory feeding substrates (equivalent to the test substrates) for each pest species were used (Tab. 1). Each treatment was replicated 6 times.

After one week the beforehand added adults were removed and glass jars were maintained at 22 or 25 ± 1 °C and 65-70 % RH in climate chambers (Tab. 1). The glass jars were checked every two days and newly hatched adults were removed. We measured the time until first hatchings of new (F1) adults and counted the number of fully developed individuals. The mean development time from egg to adult and the number of hatched adults on soy and lupine were compared with those reared on control feeding substrate (Tab. 1). Experiments were stopped when in control jars no further hatching was observed during 5 days.

Results & Discussion

In the present study we showed that the stored-product moth species *E. elutella* and *P. interpunctella* have the potential to multiply on stored pulses, especially on its simply processed forms, grist and flour (Tab. 2 - 4). Progeny of both moth species tested here developed well on soy beans and the corresponding processed substrates (Tab. 2). Mean number of hatched adult *P. interpunctella* on soy grist and flour was comparable to those on the control standard feeding substrate. But the development time was significantly longer on soy and hatching start of F1 adults was shifted for 3 weeks in *E. elutella* and for 2 weeks in *P. interpunctella* (Tab. 2).

Moreover, *P. interpunctella* showed the potential to develop on lupine beans and new F1 adults hatched on all varieties tested but also, significantly later and to a much lesser extent than on the control feeding substrate (Tab. 3). Most adults were counted on the white sweet lupine variety *Energy* followed by the blue bitter one *Karo ZS* and the blue sweet variety *Boruta*. On the processed plant material of lupine, *P. interpunctella* and *E. elutella*, developed better than on whole beans and in comparable numbers to the standard feeding substrate but hatching start was shifted for 2-3 weeks as well (Tab. 4). Highest numbers of hatched larvae of *P. interpunctella* were found on grist and flour of the sweet lupine mix (100% compared to control). In the two experiments, live larvae and adult individuals of moths were found and damage was displayed by feeding traces, feces and webs (Tab. 4). However, under laboratory conditions the tested stored-product pest beetles did not

develop or not at all on the different substrates and thus, demonstrated a very low risk of infestation. This is possibly due to the biology of these species which are most probably specialized on other feeding substrates than soy and lupine. Even bruchids that might start to colonize these seeds in the field directly on plant could not develop properly. In this context, no live adults of *Sitophilus granarius* were found and the substrate was totally undamaged. Some *Callosobruchus chinensis* individuals developed at 22°C on soy beans and at 25°C on soy grist as well but significantly fewer adults were found compared to the control substrate. *Tribolium confusum* developed better on the processed grist and flour than on whole soy beans but hatching start of F1 adults was shifted for more than 5 weeks (Tab. 2). Observed damage patterns were live individuals and the typical smell associated with *Tribolium*-infestation. On the six varieties of sweet and bitter lupine beans none of the three tested beetle species developed and only some *Rhyzoperta dominica* adults were found (Tab. 3). The significant longer development time until hatching of new *P. interpunctella* and *T. confusum* adults on soy and flour at 25°C and 65% RH compared to the standard feeding substrate (Tab. 2) is comparable to data from literature (Cox and Simms 1978).

Tab. 1 Experimental design to test the development time from egg to adult (F1) of different stored-product pest species (moth and beetle) on 200 g of whole beans, grist or flour of one soy variety (*Sultana*) and six lupine varieties (blue sweet (*bs*): Boregine, Boruta, Mitrabor, Probor; white sweet *(ws): Energy;* blue bitter *(bb): Karo ZS*), as well as on the corresponding standard fedding substrates as control (N=6).

PESTS	CONTROL	Variety	SOY beans, grist, flour — Sultana	LUPINE beans (*I-VII*), grist+flour (*V-VII*) — I) Boregine (bs)	II) Boruta (bs)	III) Mirabor (bs)	IV) Probor (bs)	V) Sweet mix† (bs)	VI) Energy (ws)	VII) Karo ZS (bb)
Moths	**Standard feeding substrate**		**Infested with**							
Ephestia elutella	Wheat bran (200g)		10 adults	--	--	--	--	100 eggs*	100 eggs*	100 eggs*
Plodia interpunctella	Wheat bran/almond grist (185/15g)		10 adults	100 eggs	100 eggs	100 eggs	100 eggs	100 eggs*	100 eggs*	100 eggs*
Beetles										
Acanthocelides obtecus	Black-eyed beans (100g)		--	50 adults	50 adults	50 adults	50 adults	--	50 adults	50 adults
Callosobruchus chinensis	Peas (200g)		30 adults	--	--	--	--	--	--	--
Callosobruchus maculatus	Mung beans (100g)		--	50 adults	50 adults	50 adults	50 adults	--	50 adults	50 adults
Rhyzopertha dominica	Wheat grain (200g)		--	50 adults	50 adults	50 adults	50 adults	--	50 adults	50 adults
Sitophilus granarius	Wheat grain (200g)		30 adults	--	--	--	--	--	--	--
Tribolium confusum	Wheat grist/yeast (191/9g)		30 adults	--	--	--	--	--	--	--

† Mix of 4 blue sweet lupine varieties: *Boregine, Boruta, Mirabor, Probor*.
* Additional experiment by infesting grist and flour of lupine sweet mix (*V*) and varieties *VI* and *VII* with 100 moth eggs each.

Since development not only depends on the feeding substrate but also on factors such as temperature and humidity (Dettner and Peters, 2011), the experiments presented here (under specified temperature, product moisture and relative humidity) only give a first indication of whether the tested species represent a real risk to stored soy and lupine. In fact, higher temperatures seem to favor the potential of beetles and pests of tropical origins to develop on the tested feeding substrates. Therefore, during cold, dry and well-ventilated storage these pest insects probably do not represent a high risk for the stored pulses. Here, the temperature effect has been observed on *C. chinensis* (Tab. 2) which may be an indication of the potential for increased reproduction on soy at higher temperatures. This in turn implies that the potential to develop on innovative stored products (pulses) in Germany may rise with increasing global warming.

In any case, a thorough cleaning before storage of soy and lupine is to be recommended, in order to prevent the spreading of harmful insects, even after a nonmonitored infestation. Consequently, the most important preventive measures against pest insects and future infestations in practice are

well-cleaned storage facilities, cool storage temperatures (10-16 ° C) and for long-term storage kernels with no more than 11% residual moisture (Landwirtschaftliches Zentrum für Sojaanbau und Entwicklung, 2015).

Tab. 2 *The potential risk of stored soy beans, grist and flour to get infested by common stored-product pests.* Summary of experiments analyzing the capability of different moth and beetle species to develop on whole beans, grist and flour of the soy variety (*Sultana*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

PESTS ON SOY (Sultana)	Development time compared to control (weeks)			Mean n° of hatched adults compared to control (%)			Damage pattern	Risk of infestation
	beans	grist	flour	beans	grist	flour		green levels: low risk / red levels: potential risk
Moths								
P. interpunctella (at 25°C)	>	>	>	22.1	75.7	80.1	Feces Webbing Larvae	High potential to infest soy, especially the processed forms, grist and flour. Loss of quality due to moth webs and larvae. Moth develop well.
E. elutella (at 25°C)	>>	>>	>>	20.2	57.1	54.8	Living individuals Feces Webbing Larvae	High potential to infest soy, especially the processed forms, grist and flour. Moth develop well. Loss of quality due to moth webs and larvae.
Beetles								
T. confusum (at 24°C)	>>>	>>>	>>>	0.3	8.2	6.3	Living individuals Typical smell	Higher risk of infestation on soy grist and flour at warmer temperatures.
C. chinensis (at 25°C)	>>	>>	X	3.1	5.0	X	Living individuals Laid eggs No drill holes	Risk of infestation on soy beans and grist increases with increasing temperatres .
C. chinensis (at 22°C)	>>	X	X	0.5	X	X	Living individuals Laid eggs No drill holes	Very little risk of infestation and only on soy beans
S. granarius (at 20°C)	X	X	X	X	X	X	None	No expected infestation since beans are too big and without the necessary endosperm.

>:   Development time slightly longer than on control substrate (shift ca. 2 weeks)
>>:   Development time longer than on control substrate (shift ca. 3 weeks)
>>>:   Development time much longer than on control substrate (shift > 5 weeks)
X: No development (no adult individuals hatched)

Tab.3 *The potential risk of stored lupine beans to get infested by common stored-product pests.* Summary of experiments analyzing the capability of different moth and beetle species to develop on whole beans of six lupine varieties (*Boregine, Boruta, Energy, Mitrabor, Probor, Karo ZS*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

PESTS ON LUPINE whole beans	Development time compared to control (weeks) / Mean n° of hatched adults compared to control (%)						Damage pattern	Risk of infestation
	Boregine (bs)	Boruta (bs)	Mirabor (bs)	Probor (bs)	Energy (ws)	Karo ZS (bb)		green levels: low risk / red levels: potential risk
Moths								
P. interpunctella (at 25°C)	>> / 3.0	>> / 5.1	>> / 2.5	>> / 3.8	>> / 8.5	>> / 5.0	Living individuals Feces Webbing Larvae	Some potential to infest all varieties of sweet and bitter lupine whole beans. Loss of quality due to moth webs and larvae.
Beetles								
R. dominica (at 25°C)	>>> / 0.2	>>> / 0.4	>>> / 0.2	>>> / 0.3	>>> / 0.4	>>> / 0.1	Very low	Almost no risk of infestation on all varieties of lupine whole beans.
C. maculatus (at 25°C)	X	X	X	X	X	X	None	No expected infestation.
A. obtecus (at 25°C)	X	X	X	X	X	X	None	No expected infestation.

>>:   Development time longer than on control substrate (shift ca. 3 weeks)
>>>:   Development time much longer than on control substrate (shift > 5 weeks)
X: No development (no adult individuals hatched)

Tab.4 *The potential risk of lupine grist and flour to get infested by common stored-product pests.* Summary of experiments analyzing the capability of *P. interpunctella* and *E. elutella* (100 eggs initially) to develop on grist and flour of a mix of 4 blue sweet varieties (*Boregine, Boruta, Mitrabor, Probor*), one white sweet variety (*Energy*) and one blue bitter variety (*Karo ZS*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

PESTS ON LUPINE grist and flour	Development time compared to control (weeks) / Mean n° of hatched adults compared to control (%)						Damage pattern	Risk of infestation
	Sweet mix (bs)		Energy (ws)		Karo ZS (bb)			green levels: low risk red levels: potential risk
	grist	flour	grist	flour	grist	flour		
P. interpunctella (at 25°C)	>/ 100	>/ 100	>/ 89	>/ 76	>/ 98	>/ 97	Living individuals Feces Webbing Larvae	High potential to infest processed lupine (grist and flour). Moth develop well. Loss of quality due to moth webs and larvae.
E. elutella (at 25°C)	>/ 83	>/ 92	>/ 90	>/ 81	>/ 98	>/ 97	Living individuals Feces Webbing Larvae	High potential to infest processed lupine (grist and flour). Moth develop well. Loss of quality due to moth webs and larvae.

>: Development time slightly longer than on control substrate (shift ca. 2 weeks)

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Biological abilities of storage pests required for the successful penetration of food packages or seeds

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Abstract

Storage pests cause enormous damage to stored seed commodities and packaged food. Most of the work published on pest risk assessment concentrates mainly on the effects of "pest –package" or "pest-seed" interactions: i.e. if some species is able (or not able) to penetrate in a sound kernel or package. Based on such "YES-NO outcomes", the particular stored product pest species is then categorized to either as a "primary" or "secondary" seed feeder; or "penetrator" or "invader" of packages. However, less research attention is paid to the functional explanations of the observed interaction-outcomes. This work therefore deals with comparison of morphological adaptation in various species storage insects with regards to their penetration abilities. For this analysis our original data as well as data from literature were used. As the most important morphological (pre-) adaptations, modulating penetrative/invasive success of storage insect pests, have been recognized: (i) shape and hardness of mandibles, (ii) size and strength of mandibular muscles, (iii) morphology of tarsi enabling climbing and/or firm stance on smooth surfaces. In addition to the morphological adaptations the specific genetically pre-programmed behavioural patterns and abilities may also play a significant role. It will be demonstrated that the above morphological abilities must be taken into account while establishing standard methods of testing of various packages in terms of their sensitivity to penetration/invasion by various species s of storage pests.

Keywords: food packages, morphology, madibulae, tarsi, claws, *Sitophilus granarius* and *Rhyzoperta dominica*

Introduction

Storage pests cause profound injury and damage to stored seed commodities (Stejskal et al., 2014) and packaged food products (Essig et al.1943; Hubert et al., 2011; Stejskal e al., 2015). In order to reach protected food resources, pests must be able to overcome physical and chemical defences present on the surface of seeds and food packages. As a natural defence, many types of plant parts (seeds, fruits, and leaves) have very smooth and/ or waxy surfaces (Al Bitar et al., 2009). In addition, seeds are equipped with hard and smooth protective layers (e.g. Fig. 1) that are impenetrable for many morphologically maladapted stored product pests. Unlike undamaged seeds, the processed food (i.e. cereal products, energy fruit bars, and cornflakes) is usually served without any protective hard surfaces. In order to protect food from pest infestation and/or contamination, early civilisations came up with an idea of "artificial- peel" centuries ago that is nowadays known as protective food packaging. During the course of human history, many types of packaging materials have been developed (Athanassiou et al., 2011). However, their protective properties still differ profoundly: chemical composition and number of layers of the film were recognized among the most important factors affecting film resistance against pest penetration (e.g. Lee et al., 2017; Trematerra and Savoldelli, 2014, Stejskal et al., 2017). It has been also shown that various pest species differ in their ability to penetrate or invade protective food-packaging films (Cline, 1978). Riudavets, et al., (2017), based on SEM microscopy, described various types of physical injuries and damages caused by particular species of stored product pests.

Most of the work published on pest risk assessment concentrates mainly on the effects of "pest – package" or "pest-seed" interactions: i.e. if some species is able (or not able) to penetrate in a sound kernel or package. Based on such "YES-NO outcomes", the particular stored product pest species is then categorized to either as a "primary" or "secondary" seed feeder; or "penetrator" or "invader" of packages. However, less research attention is paid to the functional explanations of the observed interaction-outcomes. This work therefore deals with comparison of morphological adaptation in various species storage insects with regards to their penetration abilities. For this analysis our original data as well as data from literature were used. As the most important morphological (pre-) adaptations, modulating penetrative/invasive success of storage insect pests, have been recognized: (i) shape and hardness of mandibles, (ii) size and strength of mandibular muscles, (iii) morphology of tarsi enabling climbing and/or firm stance on smooth surfaces.

Shape and hardness of mandibles

Protective surface of various seeds (such as seeds of bean; pea, barley; wheat; corn and pearl millet - Fig.1) and packages are usually hard. Storage pests have differential morphological ability and hardness of mandibles to penetrate seed surface. Based on biological abilities, the particular stored

product pest species is then categorized to either as a "primary" or "secondary" seed feeder. The relationship between mandible morphology and diet has been studied on different insect taxa, e.g. on grasshoppers (Patterson, 1984; Smith and Capinera, 2005), carabid beetles (Acorn and Ball, 1990) or ladybirds (Samways et al., 1997). Generally, there coud be differences in relative molar and incisor length, in mandible apex (multidentate/unidentate), or in general mandible shape (width/length ratio) according to type of food (i.e. herbivorous *vs* carnivorous, graminivorous *vs* forbivorous etc.). Nevertheless, there is no research on relationship between morphological characters and ability to penetrate food packages in stored pests. Besides the mandible shape, hardness (which is caused manily by presence of metals in cutting edge) of mandibles can also play a significant role in ability of infest packed food. For example, high contrentations of zinc and manganese were detected in mandibles of stored pest larvae that bore into the seed, whilst in species that feed on already damaged seed there was no metal in the mandibles (Morgan et al., 2003).

Size and strength of mandibular muscles

Even very hard and sharp mandibular tools cannot efficiently serve their purpose without being equipped an adequate muscle system. However, the size and strength of mandibular muscles has not been studied in stored pests so far. In reality, there exists little information about biting forces in insects at all. In carabid beetles, it seems that mandibular force is not dependent on size of the species (Wheater and Evans, 1989), so the species size is probably not a good predictor of the species penetration ability. On the other hand, there are indices that size of mandibular (adductor) muscle is related to the mandibular and head size (Li et al., 2011). Weihmann et al. (2015) found that there is relationship between mandibular adductor size and diet in different insect taxa.

Morphology of tarsi enabling climbing and/or firm stance on smooth surfaces

Various seeds (Fig.1) or food packages show diverse structure of their surfaces: from rough, to smooth. To be evolutionary successful, phytophagous pests have developed differential climbing and surface attachment morphological devices and adaptations. Tarsal claws are adapted for movement on rough surfaces, while various adhesive tarsal devices (i.e. pads, arolium, pulvilli, etc.) enable to attach to smooth surfaces. Although there are studies on movement and adhesive abilities of insects (mainly in context of plant vs plant pest/pest predator; e.g. Al Bitar, et al., 2009, Gorb and Gorb, 2002; Eigenbrode, 2004) and other organisms (spiders, geckos, etc.; e.g. Bhushan, 2012; Wolff and Gorb, 2012), studies dealing with tarsal morphology and its relation to the climbing performance in stored product pests are surprisingly lacking. One of the very few work on this topic showed high variability in climbing abilities of stored product pests on several packaging materials (Cline and Highland, 1996). For example, whilst some species (e.g. Sitophilus oryzae, Lasioderma serricorne, Oryzaephilus surinamensis) had no problem to climb in angle 90°, several species (Rhyzopertha dominica, Attagenus megatoma) were almost unable to move on the materials. This work thus raises a question which morphological features stand behind the variability in the ability of climbing on artificial smooth surfaces.

Previous studies showed morphological adapatations on attachment ability on smooth (e.g. arolium in Blattodea, Lepidoptera and Hymenoptera, pulvilli in Diptera or setal tarsal pads in Coleoptera) and rough (claws – Fig.2, different types of setae in adhesive pads) surfaces. Hence, thanks to their variability in attachment ability, stored product pests may serve as an additional organism group for study of morphological (pre-) adaptations of climbing abilities.

Conclusions

The article summarized the selected morphological abilities that must be taken into account while establishing standard methods of testing of various packages/ seeds in terms of their sensitivity to penetration by various species of storage pests. In addition to the morphological adaptations the specific genetically pre-programmed behavioural patterns and abilities of phytophagous stored product insects may also play a significant role.
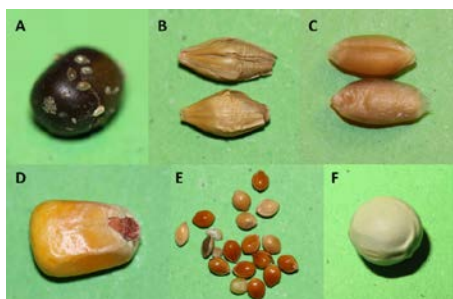
Fig. 1 Protective surface of various seeds are usually hard and smooth: A- beans; B- barley; C – wheat; D- corn; E- pearl millet; F- pea. Storage pests have differential climbing and attachment morphological ability (shape of tarsal claws or adhesive pads) to smooth surface of seeds as well as different ("primary" or "secondary" seed feeder) morphology and hardiness of mandibles to penetrate seed surface.

Fig. 2 Comparison of tarsal claws of two primary pests *Sitophilus granarius* and *Rhyzoperta dominica*. The relative length of claws is considerably larger in *R. dominica* (cca 25% of tarsal length) than in S. granarius (cca 12% of tarsal legth).

Acknowledgement

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Constraints in Grain quality management: A warehouse journey

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Abstract

India produces about 150 million tons of food grains per year. The major components of production are 47 million tonnes of wheat, 64 million tonnes of rice, and 13 million tonnes of pulses. Seasonal fluctuations in harvesting of grains impose efficient design for long term storage. Quality of grains will be retained by proper storage. Post harvest processing and storage conditions such as temperature, humidity, aeration, insect infestation, rodents, fungus, etc., at a particular geographical location influence the qualitative and quantitative losses of grains. Approximately about 10% of produce wasted during post production such as harvesting, threshing, and storage which means that about 15 million tons of grains are being washed out per year. Main intention of any government in warehousing is to offer a safe buffer stock during off-season. Knowledge about existing storage criteria creates a vision to develop new strategies. Based on this concept, a compartment in a godown of dimension 37.2m x 24.2m x 8m made of concrete and asbestos roof, with six doors and thirty-four windows was selected for the research. The stacks of dimension 6.5m x 3.9m x 6.1m with two hundred and sixty-four numbers of gunny bags filled with grains arranged above the wooden dunnage were selected for insect and chemical analysis. Temperature, humidity and aeration rate were recorded at four corners and at center of the stack and also at 26 different spots in whole godown. The influence of various factors on insect infestation in grains during storage was studied. The results will help to design an advanced scientific grain storage godown for safe storage of grains in gunny bags for longer duration.

Keywords: Godown, Dunnage, Insect infestation, Temperature, Humidity.

Introduction

Agricultural products such as grains, cereals are stored for facing shortage of commodities during off-season, droughts and natural calamities. They are usually stored for 3–12 months by farmers, traders and by the public sector agencies like Food Corporation of India, the Central Warehousing Corporation, State Warehousing Corporations and State Civil Supplies Corporations which handle

about 30% of the production (TIFAC, 1996). In many developing world, post harvest losses of cereals accounts to 10-15% (Lucia and Assennato, 1994). Post harvest losses are mainly due to insect infestation which found their food and shelter and also contaminate the grains by their by-products and making them unfit for consumption resulting in qualitative as well as quantitative losses.

Tropical and humid areas are mostly prone to pest infestation on stored foods. The tropical climate of India is highly favourable for continuous survival of storage insect pests throughout the year. Insects gain access to storage area at various stages of processing of grains; during the development seeds/grains, processing in threshing yards, during transport or during storage. Major sources of infestations are old bags, storage structure, old containers, and cross over infestation (Pruthi and Singh, 1950).

Infestation of whole storage area is facilitated by movement of grains from one area to another or by active flight of insect pests as some of the adult insects are strong fliers. Monitoring the stored grain pest, by finding the insect population or infestation level in a period of time helps to understand the behaviour of insects with respect to environmental conditions. These will further help to determine the time for pesticide application and effectiveness of pest management actions. The emphasis of tropical storage pest management is thus on constraining the increase and spread of such infestations. Thus the following study was undertaken to determine the population pattern of insects with respect to tempertature and humidity in a godown.

Materials and Methods

A compartment in a godown of dimension 37.2m x 24.2m x 8m made of concrete and asbestos roof, located in Thanjavur, Tamil Nadu, India was selected for the research. The compartment was ventilated with six doors and thirty four windows. Paddy was stored in the gunny bags. About with two hundred and sixty four numbers of gunny bags filled with grains were arranged above the wooden dunnage. Individual stack has the dimension of 6.5m x 3.9m x 6.1m. Among them one stack was selected for studying the population behavior of insects. The temperature and humidity were recorded using HOBO data logger at points near the ventilation and far from ventilation from 10.00 am to 4.00 pm at an interval of 2 hours. Simultaneously, insect population at the top, middle and bottom of the stack was also counted. The study was conducted during the post monsson season (December) with an average outdoor temperature of 25º C. Based on the readings, the population pattern of insects with respect to temperature, humidity and time was investigated.

Results

The results of the study showed that temperature was found maximum in the interior part of compartment while ventilated areas near door and windows recoreded minimum temperature. The humidity was found to be higher in ventilated areas than interior part. It was observed that five fold increase in insect population at the top of stack during day time and about seven fold increase in insect population after 4.00 pm.

During storage, the paddy was attacked by many insects including the *Sitotroga cerealella,, Rhyzopertha dominica, Tribolium castaneum, Sitophilus oryzae,* etc., But the major pest was identified as *Tribolium* sp., which feeds on broken grains resulted in dust formation. Similarly Rajan *et al.* (2018) explained that the most abundant species caught was *T. castaneum* across all of the localities sampled. Infested grains emitted sour and pungent smell, which was due to some secretions of beetles.

Discussion

The observation on the insect population or infestation level in a period of time along with temperature and humidity helps to understand the behaviour of insects with respect to environmental conditions. In general, the minimum temperature threshold for *T. castaneum* flight initiation in the laboratory being 25º C (Cox *et al.,* 2007). The results of the present study showed that temperature was found maximum in the interior part of compartment while ventilated areas

near door and windows recoreded minimum temperature. The humidity was found to be higher in ventilated areas than interior part.

Tab. 1 Population strength of insects with respect to temperature, humidity and time

Time	Temperature (ºC)		Relative Humidity (%)		Insect Count at T_1			Insect count at T_{avg}		
	T_1	T_{avg}	T_1	T_{avg}	B	M	U	B	M	U
10.00 am	25.8±0.5	26.8±0.7	73.4±3.3	73.2±2.2	1±0	2±1	2±1	1±0	1±1	4±1
12.00 noon	26.7±0.2	27.8±2.0	72.5±3.3	69.9±2.3	1±1	0±1	4±2	1±1	0±1	6±3
2.00 pm	26.7±0.3	28.9±1.0	70.9±3.8	68.0±5.0	2±1	2±2	9±3	1±2	3±3	13±7
4.00 pm	27.0±0.5	28.5±0.9	70.0±3.9	68.5±5.0	5±3	6±3	14±4	2±2	4±1	17±11

T_1 – Near the ventilation area; T_{avg} – Far from ventilation; B – Bottom of stack; M – Middle of stack; U – Upper portion of stock

It was observed that five fold increase in insect population at the top of stack during day time and about seven fold increase in insect population after 4.00 pm. The results are in line with the report of Rajan *et al.* (2018) who reported that vast numbers of *T. castaneum* take flight inside godowns in the late afternoon. The resutls of the present study will help to design an advanced scientific grain storage godown for safe storage of grains in gunny bags for longer duration. It will also help develop effective management tactics to reduce the severity of infestations caused by stored product insects.

5. Future Progress

Effect of temperature, humidity and ventilation on insect population was studied for short duration. To establish the efficient pest management practices, the influence of all the above said factors on microbial growth and chemical analysis has to be studied. Further pest management by integrating the different methods based on the results of the study in large scale godown has to be studied.

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Modelling of population dynamics of insects in any ecosystem with several distributions of insect development: A Review

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Abstract

Predicting the occurrence of insects with a high accuracy requires the estimation of insect development time and the variation among individuals for each life stage and species under different environmental conditions such as fluctuating temperature, variation of relative humidity, different body sizes and stages of the insects, levels of crowding, and food supply. This review summarized the modeling methods of population dynamics of

insects with several distributions of insect development, assumption and prediction accuracy of these developed models, and disadvantages and advantages of these modelling methods. These modeling methods include degree day model, nonlinear model, and distribution delay models. The structure of most common models are cohort, Leslie matrix, simulation, and individual based. The relationships among the modeling assumptions, effects of temperature, and other environmental factors, and structures of the developed models were examined. A new modelling approach such as physiological-biological time scale and chaos theory was suggested.

Key words: Degree day, nonlinear model, distribution delay, Leslie model, chaos.

Introduction

Predicting the occurrence of insects in an ecosystem with a high accuracy is essential for conducting integrated pest management. These predictions require the estimation of insect development time and the variation among individuals for each life stage and species under different environmental conditions such as fluctuating temperature, variation of relative humidity, different body sizes and stages of the insects, food sources, and levels of crowding. This adds another level of complexity to models already complicated by accounting for the variable time of development (Stinner et al. 1974, Wagner et al. 1984 a and b, Briere et al. 1999, Gramig et al. 2015), variations under different temperatures (Anderson et al. 1982, Worner 1992, Regniere et al. 2012, Damos and Savopoulou-Soultani 2012, Moore and Remais 2014), variations among different growth stages and ages, and discrepancies under constant and fluctuating temperatures (Hagstrum and Milliken 1991, Nachman and Gotoh 2015). However, complexity does not assure more accuracy in all cases. Therefore, the right mathematical approach and theoretical assumptions should be developed to model population dynamics of insects with several distributions of development time. This review outlines the basic modelling methods, the disadvantages and advantages of the methods, reasons for the low accuracy of these developed models from their applications, and suggestions for future model development.

Temperature effect and modelling

Temperature is the only considered factor in almost all, if not all, of these published models in the literature (Damos and Savopoulou-Soultani 2012) because temperature is the most critical factor affecting insect development. Damos and Savopoulou-Soultani (2012) reviewed the temperature-driven models for insect development. Empirical and semi-theoretical mathematical models have been developed. Even though none of these models is based on accepted biophysical laws such as Eyring's absolute reaction rate theory, temperature effect on enzymes has been recognized by the empirical equations of Van't Hoff's law and Arrhenius (Schoolfield et al. 1981). The basic assumption of the enzymes was used for most model development such as degree day and nonlinear models. To calculate the temperature effect, three general types of models are developed: degree-day summation, non-linear temperature inhibition, and distribution delay. These empirical or semi-theoretical equations predicting average development rates are in exponential or logistic format and explain the thermodynamics of complex biological processes by the laws of chemical reactions. This provides biologists with a greater understanding of the temperature-dependent and developmental responses for a given insect species. With this approach, developed models could be extended beyond the range of temperatures specified by model theory or beyond the range of temperatures measured. However, these empirical and semi-theoretical models are not valid for most practical cases because exponential or logistic increase is observable on a limited range and not throughout all temperature regimes. Therefore, different researchers modified these empirical equations (Sharp and DeMichele 1977). However, these modified equations have limited application because of their limited fit to laboratory data, and usually these developed models have not been validated with field data because it is difficult to collect field data; there are inherent variations in the field data; and predictions from models are uncertain.

There are three issues with the modelling temperature response of insects: development rates, development times at temperatures near thresholds (extremes) where excessive mortality or

developmental abnormalities can occur, and individual variation from the average developmental rates and reproductive responses. These three issues are handled in the models of degree day, nonlinear, and distribution delay differently.

Degree day models

To model the effect of temperature, the development of an organism is viewed as a biological clock that measures time thermal units, and it is often referred to as thermal time. Although physiological time accelerates or slows under different temperature fluctuations, the thermal time unit to complete a particular developmental event under both field and laboratory conditions is assumed to be the same by modellers because thermal requirement is the basis for insect development. This is the basic assumption of the degree day model. Degree-day models were initially developed for agricultural applications and have been widely used since the 1730s in many research areas related to farming (Moore and Remais 2014). The degree-day model, without considering the individual variation from the average developmental times or rates and reproductive responses, is a simplistic representation of a potentially complex developmental process (Moore et al. 2012).

Degree day model is the most widely used mathematical format to estimate insect development time because it requires minimal data for formulation, calculations are easy and applications are simple, and often yields approximately correct values. Most of degree day models only estimate the average development time, and do not consider the individual variation from the average developmental times or rates and reproductive responses. This simple format of degree day model is widely used in many agricultural areas. Recently, Nachman and Gotoh (2015) developed a biological age model with the consideration of the individual variation from the average developmental rates and reproductive responses. This newly developed model used probability distribution to estimate the individual variation and this probability distribution was related to temperature effect and development time mathematically. This mathematical relationship among development probability distribution and temperature effect and development time is the modification of the degree day model. Therefore, this new developed model combined the degree day model with distribution delay model. This modeling framework was successfully used to model several insect species (Skovgard and Nachman 2017).

Even though different researchers used different mathematical equations to calculate the degree days (Moore and Remais 2014), and used different assumptions to quantify the relationship between the sum of degree days and the insect development, the developed degree day models have a low prediction accuracy. A degree day model developed under constant temperature cannot be used to predict insect population under fluctuating temperature (Hagstrum and Milliken 1991, Jian et al. 2017). Developmental times of many species under constant temperature differ from these under fluctuating temperatures with the same mean (Hagstrum and Milliken 1991) because short periods of colder or warmer temperatures under fluctuating temperatures may have an overriding influence on development rate when compared to the mean temperature over a longer period of time (Hagstrum and Milliken 1991). Low prediction accuracy of the degree day model under fluctuating temperature will have a large drawback because most life tables studied are conducted at constant temperature under laboratory conditions. The development differences between constant and fluctuating temperatures could not only increase the complexity of the degree day model, but also make the degree day model unsuitable. Researchers have shown that these differences could be resolved by integration of temperature developmental times over the fluctuating temperature cycle to predict development times at fluctuating temperatures (Dallwitz 1984, Hagstrum and Milliken 1991). Therefore, combination of the degree day model with other mathematical tools might be one of the choices to improve prediction accuracy. However, deciding the integration interval is part of arts and science, and different researchers used different assumptions

The main reason causing this low prediction accuracy of degree day models might be the basic assumption of the degree day model. The basic assumption of the degree day model is that: the

completion of a given stage in development requires an accumulation of a definite amount of heat energy, thus, degree day models apply the accumulated temperatures as the heat energy to establish the relationship between the development and the environmental conditions without considering the additive effect of the accumulated energy and morphological change of the organisms. Insects might have a mixture of liner and non-linear development. It is difficult to find the right equation because both linear and non-linear models generally cannot represent the complete effects of temperature on an organism. Nonlinear indicates that the whole becomes something greater than the mere sum of its individual parts or linear parts due to the interaction effects between factors. Moore and Remais (2014) found the difference between linear and non-linear predictions of *Nephus bisignatus* (Bohrman) (Coleoptera: Coccinellidae) emergence can be up to a week, which is not trivial and have important implications for the use of degree-day models in ecological applications. It is common that temperature not only influences the rate of chemical reactions, but also induces conformational changes in biological systems. For any degree day model, the most challenging task is to find the base temperature. Insects under fluctuating temperature may not have a distinct low temperature development threshold, and development rates become asymptotically smaller as temperature decreases (Eubank et al. 1973). The organism's response to high and low temperatures, as well as to the specific methods used to estimate accumulated degree-days, can lead to markedly divergent model predictions. Therefore, prediction of the degree day model is very sensitive to the tailored system, region, and time scale. This requires development of models that are tailored to the specific system, region, and time scale under a good fit. Therefore, a new modeling framework to cater these effects on insect development should be developed.

Nonlinear models

The initial objective for most of the nonlinear regression models is to describe developmental rate of insects over the full range of temperatures. This modeling procedure can be easily generated using several different software if the developed models are only used to predict the average developmental time or rate (reverse of the average development time, lifespan, or LT50). Most of these developed models considered the maximum and minimum development temperatures. These maximum and minimum development temperatures correspond to the assumption that there is no growth below the minimum temperature threshold, while developmental rate increases to reach a maximum at optimal temperature, and then declines rapidly approaching zero at the maximum temperature threshold that is often considered as the lethal temperature. To include the prediction of the distribution of the development time delay, this developed nonlinear model becomes complex because probability and/or likelihood estimation must be used.

One type of nonlinear temperature inhibition models is the biophysical model. Biophysical models are developed based on Van't Hoff's law which states that the rate of chemical reactions increases between two- and three-fold for each 10°C rise. The Arrhenius equation relates the chemical reaction rate to temperature and the activation energy of the reaction in an exponential equation. However, these models usually have a large prediction error because exponential increase is observable in a limited temperature range and not throughout all temperature regimes, and temperature affects not only the rate of chemical reactions, but also induces morphological changes in biological systems (Schoolfield et al. 1981, Sharpe and DeMichele 1977, Briere et al. 1999). Nonlinear models predicting average development rate with considering minimum and maximum development temperatures are usually complex (most have more than four thermodynamic parameters) and can only be used for the insects for which the model was initially developed (Schoolfield et al. 1981, Wagner et al. 1984 a and b, Wang and Engel 1998, Briere et al. 1999, Hansen et al. 2011, Regniere et al. 2012). These thermodynamic parameters were found to be highly correlated (Schoolfield et al. 1981, Briere et al. 1999). To eliminate this correlation, different researchers (SchoolÞeld et al. 1981, Briere et al. 1999) re-parameterized these models, and some researchers just used different mathematical equations to best fit the data which cover the entire

insect development temperature (Jian et al. 2007). This parameterization in turn results in a nonlinear model with no or few biophysical assumptions.

Distribution delay models

Life history studies usually require to determine recruitment (actual number), duration, and survivorship for each life stage. The most used methods to model this life history are Leslie matrix format, distribution delay model (referred to as distributed maturation models or variable development rate models), and combination of both. In a Leslie matrix format, the organism's life cycle is divided into sub-stages with a length equal to the length of the shortest stage. At each time step, all individuals in the population are advanced to the next sub-stage and the time step is usually set as the sub-stage length. All individuals in a cohort can advance in age at the same rate (development index model) or change from one stage to the next at the same age (sojourn time models). Mathematically, the development index model is a special case of the sojourn time model (Schaalje and Vaart 1989). The Leslie matrix model has been of limited use in ecology because it models exponential population growth. One format of the modified Leslie matrix is the distribution delay. In the distribution delay format of the Leslie matrix model, advance of an individual from one stage to the next is not only based on the mean length of development time, but also the variability among individuals. The advance of an individual can be calculated from a probability distribution based on the mean and standard deviation or is assigned a predetermined probability (Schneider and Ferris 1986). Survivorship of an individual can also use the same method as that used for the advance of an individual or use mathematical equations describing the survivorship pattern. Weibull function is mostly used to describe this probability distribution (Schneider and Ferris 1986). Erlang probability distribution can be used to describe the asymmetric and positively skewed development rates within the population (Wegner et al. 1984 a and b, Schneider and Ferris 1986). These asymmetric and positively skewed development rates are assumed as the effect of temperature and enzyme concentration (Curry et al. 1978, Sharpe and DeMichele 1977). The stochastic treatment of the Leslie matrix model can include the insect density effect and stochastic process on the survivorship and development rate by making the elements of the projection matrix vary with the age distribution or density (Leslie 1959, Vansickle 1977, Desharnais and Cohen 1986, Desharnais and Liu 1987, Liu and Cohen 1987). More complex models could be formulated to allow for time delays, but the above are commonly used. During the model development of a Leslie matrix, the temperature effect is usually implicit because it is difficult to combine the effect of fluctuating temperatures in each time step when the Leslie matrix is advanced to the next time step. For example, if temperature is changed every hour and this fluctuating temperature effect will influence the Leslie matrix, then the Leslie matrix should be calculated every hour. This will increase the difficulty of parameter estimation for the Leslie model calculation. There is no model developed in this way because the use of the Leslie matrix formulation allows the overall model to be stated concisely and this small time step will downgrade this advantage. Because the fecundity and death rate may change abruptly as an individual matures from one stage to the next, the Leslie, Von Foerster (Longstaff 1988) and related models are implicitly formulated in terms of growth stages. Cuff and Hardman (1980) calculated the fecundity and survival rate by considering the effect of temperature, moisture content, weight of free water, weevil density, and oxygen concentration. These calculated fecundity and survival rates were the basic components of the Leslie matrix in each time step. Other environmental factors such as respiration of insects, feeding, and egestion activities were also considered by modifying the Leslie matrix in each time step. Because these environmental factors influence the insect development rate, Cuff and Hardman (1980) used both physiological and chronological time scale to track insect age in each sub-stage and advance the sub-stage, respectively. This increases the complexity of the Leslie matrix model. This might be one of the reason few Leslie matrix models with distribution delay were developed after the 1990s.

Models that predict this stochastic development distribution usually involve application of probability distributions and likelihood estimation. These developed models are similar except they use different 1) variables, such as mean or median development time (Wagner et al. 1984 a and b,

Gramig et al. 2015) or development rate; 2) forms of frequency distribution, such as probability or cumulative density function; 3) types of probability distribution such as normal quadratic and beta; and 4) equations, such as Erlang probability distribution function, Weibull function (Wagner et al. 1984b), nonlinear functions with different assumptions (Gramin et al. 2015, Nachman and Gotoh 2015). The most commonly used model strategies are distributed delays (Nisbet and Gurney 1982, Wagner et al. 1984 a and b, Schneider and Ferris 1986), cohort-based (Sharpe and DeMichele 1977, Nachman and Gotoh 2015, Skovgard and Nachman 2017), Leslie model based (Longstaff and Cuff 1984, Henson 1999), simulation based (Longstaff 1988, Maggi et al. 2013), and individual-based (Regniere et al. 2012). The parameter values estimated from these developed models are usually not comparable even for the same stage of an insect species under different environmental conditions because the chronological time is used as the time scale and these factors are changing with time. The distribution of development time and the variation in the chronological time scale is different under different temperatures.

Other environmental factors

The major constraint of most developed models is directly related to temperature and do not take into account other climatic variables such as photoperiod, humidity, nutrition, as well as crowding and competition at different density levels and in different patch sizes. Incorporating more factors in the equations, temperature-driven models have the potential to describe the general ecological behaviour, abundance, distribution, and outbreaks of insects on a regional or even global scale, with important practical applications. Nachman and Gotoh (2015) claimed their developed model framework could simulate the growth of an insect population in a variable environment by modifying the response variable y in the equation (y was a product of limiting factors) with the assumption of a multiplicative relationship between the environmental factors. Nisbet and Gurney (1982) considered the quantity of food. Cuff and Hardman (1980) considered other environmental factors. However, this modelling approach has not been verified. This modelling approach increases the complexity and the y in Nachman and Gotoh's model has no basic biological meaning. Therefore, a new modeling approach should be developed to effectively predict the effect of these environmental factors with sound biological meaning.

Future model development

Physiological or biological time is intuitively obvious to some extent, but has been explicated in various ways in the literature. It is referred to as heat units and is measured in degree-days and development accumulation as the basis of physiological time scale in model development. Physiological age as a life-history event are sometime related to cyclic event such as biological rhythmicity. Nachman and Gotoh (2015) used biological age as a measure of the cumulated day-degrees an individual has achieved while in a given stage. From the view of an insect body, ageing might be the result of physiological and biological advance in the chronological time scale. Therefore, a physiological-biological time scale which can normalize the distribution of the development time and the variation should be developed.

The time delays, cyclical patterns of insect populations (periodic forcing), and nonlinearities in population models are the typical characteristics that lead to chaos in the natural ecological world (Logan and Allen 1992, Boeing 2016). Even though whether the insect populations have the chaos is still in debate, this debate has largely been carried out on theoretical grounds, and chaos occurring in the time series of forest insect pests have been proven (Turchin 1990, Turchin and Tylor 1992). Analysis of insect-population data collected inside lab or controlled field conditions for the signature of chaos presents significant limitations because: 1) chaos analysis is unrealistically data intensive; 2) data collected under lab and/or controlled field conditions usually reduce the dimensionality such as that interactions between species or among species and food sources are usually simplified and completely sampling of the entire population is usually difficult or impossible, and this simplification will rarely occur in nature; 3) chaos characteristics usually show after a few

generations of insect populations (Turchin and Tylor 1992), and collation of the lab or field data are usually interrupted before chaos shows; and 4) analysis of a complex ecological system in reduced dimensionality will tend to obscure complex dynamics and ignore the chaos (Logan and Allen 1992). Therefore, study of chaos in a real system by using mathematical modelling is critical. To analyze the chaos of population dynamics, this developed model should be a reasonable representation of the natural system and parameter values should be in a realistic range. This requires complex models which represent the reality in nature and the developed model should also be validated.

Acknowledgement

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High Quality Genomic Resources for Stored Product Insects

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Abstract

The expansion of genomic resources for stored product insects has largely been hampered by cost, time required for inbreeding, and technical issues that can arise during genome assembly from pooling multiple individuals together for DNA isolation and library preparation. However, newer library methods, such as 10X Chromium libraries, largely overcome these issues in that sufficient DNA can be recovered from a single individual for library prep and allelic variants are assembled as separate phase blocks, eliminating the need for inbreeding. Using 10X Chromium libraries coupled with 150 x 150 bp HiSeqX sequencing to a depth of at least 60X coverage, we are developing high quality draft genome assemblies for eight different stored product insect species, including Dermestidae (*Trogoderma variabile*, *Trogoderma granarium*, and *Dermestes maculatus*), Tenebrionidae (*Tribolium confusum*), Anobiidae (*Lasioderma serricorne* and *Stegobium paniceum*), Bostrichidae (*Prostephanus truncatus*), and Pyralidae (*Plodia interpunctella*). Overall, BUSCO (Benchmarking Using Single Copy Orthologs) scores exceeded 95% in all assemblies with few fragmented or duplicated genes, suggesting a high quality assembly of the gene space. Further, scaffold N50s exceeded 1 Mb in many cases and further

improvements to these scaffolding metrics will be made using linkage maps and Hi-C libraries. Overall, this approach will yield high quality assemblies for eight different insects and could be used to quickly and efficiently generate draft assemblies of invasive or emerging stored product pests.

Keywords: khapra beetle, Bostrichidae, Dermestidae, Anobiidae, Pyralidae.

Introduction

Genome sequences have provided tremendous insight into the physiological and metabolic capabilities of various insect species, led to the identification of causative mutations associated with pesticide and fumigant resistance (Schlipalius et al., 2012), facilitated the identification of taxonomically informative loci for DNA barcoding (Chesters et al., 2015), and identified copy number expansions that may allow insects to exploit new ecological niches (McKenna et al., 2016). Despite these utilities, only one stored product insect genome is publicly available (*Tribolium castaneum*) (Tribolium sequencing consortium, 2008) while a small, but growing number of transcriptome assemblies, are available for other stored product species. Since its initial assembly, the *T. castaneum* reference assembly has been used to identify mutations in a gene coding for dihydrolipoamide dehydrogenase (DLD) associated with phosphine resistance (Schlipalius et al., 2012), biorational gene targets for pest control via RNAi (Dönitz et al., 2014), and causative mutations associated with sensory system defects (Angelini et al., 2009). This assembly has even facilitated the discovery of a mutation associated with phosphine resistance in lesser grain borer (*Rhyzopertha dominica*), which happens to occur in a DLD ortholog (Schlipalius et al., 2012). However, the biologies of stored product insects vary tremendously across various taxonomic groups. Further, different taxonomic groups may evolve different strageies for overcoming biotic and abiotic stresses. In this case, having genome references available would greatly facilitate genome wide association analyses to identifiy causative mutations associated with tolerance to stress. In other cases, some species are more inherently tolerant to certain biotic and abiotic stresses and genome sequences could lead to the identification of genetic factors associated with tolerance.

Historically, genome sequencing for insects has been cost prohibitive; however, new library approaches coupled with the reduced cost of sequencing is making genome assembly more affordable and accessible than ever. One major challenge faced by those working with insects is obtaining sufficient quantities of DNA for library preparation and assembly. For Illumina mate-pair and PacBio long-read libraries, over 10 ug of DNA must be provided. This requires pooling multiple individuals for sequencing. This practice leads to multiple allelic variants derived from the same locus in the DNA pool, which can introduce bubbles and breaks into the assembly graph, reducing overall contiguity. Although sequence variations among individuals can be reduced through inbreeding for several generations, this often involves a significant time investment and the number of backcrosses required to obtain homogeneity varies by species and their recombination rates. One new library approach (10X Chromium) largely overcomes these limitations in that sufficient quantities of DNA can be recovered from a single insect for library construction and haplotypes are assembled as separate phase blocks, reducing the number of bubbles in the assembly graph and improving contiguity. In addition, during library construction, HMW DNA is separated into microfluidic chambers designed to hold exactly one molecule of DNA per chamber. Within each chamber, DNA is fragmented and DNA derived from the same molecule is tagged with the same barcode. In this manner, sequencing reads with the same barcode can be linked together during the assembly stage to form long scaffolds and contigs. This approach greatly improves assembly contiguity compared to other short read assembly methods.

In order to improve genomic resources for stored product insects, we sequenced 10X Chromium libraries derived from eight different species of stored product insects from the families Dermestidae (*Trogoderma variabile*, *Trogoderma granarium*, and *Dermestes maculatus*), Tenebrionidae (*Tribolium confusum*), Anobiidae (*Lasioderma serricorne* and *Stegobium paniceum*), Bostrichidae (*Prostephanus truncatus*), and Pyralidae (*Plodia interpunctella*). Genomes were assembled using the program Supernova and assemblies will be superscaffolded to chromosome

scale using linkage maps and/or chromatin contact maps. Overall, these assemblies exceeded the quality of many publicly beetle genome assemblies and thus, represent a viable strategy for generating genome sequences for underrepreted groups of insects. Not only will these assemblies be useful for mapping traits, conducting population genetics studies, and understanding genetic similarities and differences between various stored product species, but they will also allow for broader evolutionary analyses regarding gene order, gene duplications, and the evolution of different gene families across different taxonomic groups. Such broad scale analyses can lead to the identification of convergent strategies for overcoming stress, such as mutations in orthologous genes associated with stress response shared across species, and can also shed light on family-, genus-, or species-specific adaptations.

Materials and Methods

High molecular weight (HMW) DNA was isolated from single individuals using several different approaches. For *S. paniceum*, *T. confusum*, *T. granarium*, *D. maculatus*, and *P. interpunctella*, DNA was isolated using the Qiagen MagAttract HMW DNA Kit (Gaithersburg, MD) following the manufacturer's directions. Unfortunately, insufficient quantites of HMW DNA were recovered from *L. serricorne*, *P. truncatus*, and *T. variabile* for 10X Chromium library preparation using this approach, so other approaches were attempted. For *L. serricorne* and *T. variabile*, an agarose isolation method was used. In brief, single insects were macerated with a pestle in a nuclei isolation buffer containing 2% Triton X-100 (w/v), 10 mM EDTA, 100 mM KCl, 4mM spermidine, 1 mM spermine, and 17.1% sucrose. 50 μL of the supernatant was transferred to 75 μL molten InCert agarose (Lonza, Basel, Switzerland). The solution was placed in a gel mold and allowed to set for 10 mins at 4℃. The sample was lysed overnight at 50℃ in a solution containing proteinase K and 1% sarkosyl. The gel plugs were rinsed in TE buffer and proteinase K was deactivated for 1 hour using phenylmethane sulfonyl fluoride (PMSF). Four washes in TE buffer were used to remove PMSF and DNA was recovered from the gel plug using an agarase treatment (Wieslander, 1979). For *P. truncatus*, a salting out approach was employed. In brief, insects were macerated in a lysis buffer containing 10 mM Tris-HCl, 400 mM NaCl, 2 mM EDTA, and 0.5% SDS, and incubated overnight at 37℃ with mixing. 1.2 mL of 5 mM NaCl solution was added to 'salt out' the DNA and the sample was centrifuged for 15 mins at 4℃ under low speed (1,000 x g). The supernatant containing the HMW DNA was washed with ethanol and centrifuged at medium speed (6250 x g) for 5 mins. Ethanol was removed and the pellet was resuspended in TE buffer. The full protocol can be found at https://support.10xgenomics.com/genome-exome/sample-prep/doc/demonstrated-protocol-salting-out-method-for-dna-extraction-from-cells.

In all cases, the final concentration of the DNA was validated using the dsDNA High Sensitvity Assay on the Qubit Fluorimeter (Thermo Fisher Scientific, Waltham, MA) and, when sufficient DNA quantities were available, the quality of the DNA was validated using Pulsed-Field Gel Electrophoresis (PFGE). 1-5 ng of HMW DNA were used to make 10X Chromium libraries at Hudson Alpha Biotechnology Institute (Huntsville, AL) and libraries were sequencing using 150 x 150 bp reads on the Illumina HiSeq X-ten instrument to a depth of at least 60X. All genomes were assembled using the Supernova assembler with barcode subsampling in order to normalize coverage across the barcodes and improve the contiguity of the assemblies. Subsampling was performed from 30 to 70% to determine how much subsampling was needed to produce the most contiguous assembly, which was gauged using the programs QUAST (Gurevich et al., 2013) and BBTools (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/). Criteria that were used to select the most optimal assembly included scaffold N50, maximum scaffold length, recovery of conserved single copy orthologs (BUSCOs) (Simao et al., 2015), and scaffold length accumulation curves.

Once the Supernova assemblies were finalized, either linkage maps or HiC chromatin contact maps were prepared in order to obtain chromosome scale assemblies. Linkage maps were pursued for non-quarantine and non-invasive species with short generation times that were relatively easy to

rear, including *L. serricorne*, *T. variable*, *D. maculatus*, and *T. confusum*. In brief, crosses were set up between males and females collected from different and isolated populations of each insect species. F_1 individuals were collected and sibling crosses were performed from the F_1 through the F_3 generations to facilitate recombination. At the F_4 generation, four sets of F_3 parents and 40-50 offspring derived from each set of parents were collected for genotyping using ddRAD-Seq. Samples were barcoded and sequenced on a single lane on the Illumina HiSeq 2500 platform using 100 x 100 bp paired end libraries. Prior to mapping, the genome was masked for repeats using RepeatModeler for *de novo* repeat analysis and RepeatMasker. The *T. castaneum* repeat library was used for masking in addition to the predicted repeats from RepeatModeler. The Stacks pipeline is currently being used for genotyping and variant calling and LepMap-3 will be used to identify and order linkage groups (Rastas et al., 2015). For HiC contact maps of *T. granarium*, *P. interpunctella*, *P. truncatus*, and *S. paniceum*, pools of insects were macerated and treated with formaldehyde to cross-link DNA and chromatin complexes (Belton et al., 2012). Endonucleases and restriction enzymes were used to digest the uncrosslinked regions, cross linking was reversed, and DNA was sequenced to a depth of at least 50 million 100 x 100 paired end reads on a HiSeq 2500 platform. Reads will be eventually mapped to the 10X assemblies using BWA (Li and Durbin, 2009) and LACHESIS (Burton et al., 2013) will be used to identify regions of DNA that shared chromatin contacts.

Results

HMW DNA was successfully acquired from single insects in sufficient quantities to generate 10X Chromium libraries for all eight stored product species. No major differences in DNA quality were noted across species. It was more difficult to obtain HMW DNA from *P. truncatus* using either the gel plug extraction method or the Qiagen MagAttract kit; however, HMW DNA was obtained using a salting out approach that had been previously used for prepare HMW DNA for 10X Chromium libraries for other insect species. Additionally, HiSeqX yields across all eight species were relatively consistent and ranged from 700-875 million reads. All reads were initially used for Supernova assemblies; however, barcode subsampling was employed to normalize read coverage across barcodes, which can vary significantly for genomes smaller than 1 Gb. The amount of barcode subsampling required to produce the most contiguous assembly varied by predicted genome size, with larger genomes requiring less subsampling compared to smaller genomes. Genome size estimates ranged from 150 Mb (*L. serricorne*) to 500 Mb (*D. maculatus*). Examples of assembly improvements with subsampling are shown for *T. variabile* and *L. serricorne* in Tables 1 and 2. For *T. variabile*, subsampling 160 million reads generated the best assembly metrics, including longest contig and scaffold N50s, longest maximum contig and scaffold lengths and the highest percentage of the assembly in scaffolds > 50 Kb (Table 1). Additionally, a significantly higher percentage of the genome was present in long scaffolds when 160 million reads were subsampled (Figure 1a). Similarly, subsampling 350 million reads led to the best assembly metrics for *L. serricorne* (Table 2) while subsampling either 300 or 350 million reads led to the highest percentages of the assembly in long scaffolds (Figure 1b).

Tab. 1 Assembly improvement with barcode subsampling for *Trogoderma variabile*. Assemblies were performed using Supernova with various levels of subsampling to generate the most contiguous assembly. Subsampling 160 million reads of a total of 812 million reads led to the assembly with the highest contig and scaffold N50s, the highest maximum contig and scaffolds lengths, and the highest percentage of the genome in scaffolds > 50 kb. Thus, this assembly was selected as the most optimal.

	140M	150M	160M	170M	All
Number contigs	12,688	12,643	12,903	13,622	32,292
Contig N50	250 Kb	277 Kb	294 Kb	274 Kb	33 Kb
Max Contig Length	748 Kb	605 Kb	650 Kb	619 Kb	185 Kb
Number of Scaffolds	7,003	6,894	7,122	7,687	21,917
Scaffold N50	3.8 Mb	4.9 Mb	7.0 Mb	6.3 Mb	1.0 Mb
Max Scaffold Length	17 Mb	14 Mb	22 Mb	17 Mb	8.7 Mb

Number of Scaffolds > 10 Kb	640	679	586	722	1,727
% of Genome in Scaffolds > 50 Kb	89.3%	89.2%	89.7%	88.1%	71.4%
Total Contig Assembly Length	264 Mb	265 Mb	265 Mb	269 Mb	272 Mb
Total Scaffold Assembly Length	271 Mb	274 Mb	273 Mb	278 Mb	310 Mb
% Gap	2.6%	2.8%	2.8%	3.1%	12.4%

Tab. 2 Assembly improvement with barcode subsampling for *Lasioderma serricorne*. Assemblies were performed using Supernova with various levels of subsampling to generate the most contiguous assembly. Subsampling 350 million reads of a total of 870 million reads led to the assembly with the highest contig and scaffold N50s, highest max contig and scaffold lengths, and the largest percentage of the assembly present in scaffolds > 10 Kb. Thus, this assembly was selected as the most optimal.

	160M	270M	300M	350M	400M	All
Number contigs	9,705	10,270	10,288	10,057	10,405	17,892
Contig N50	52 Kb	61 Kb	69 Kb	79 Kb	72 Kb	27 Kb
Max Contig Length	776 Kb	3.8 Mb	2.1 Mb	3.8 Mb	2.0 Mb	1.6 Mb
Number of Scaffolds	9,266	9.653	9,594	9.315	9,540	16,588
Scaffold N50	55 kb	75 Kb	87 Kb	119 Kb	118 Kb	38 Kb
Max Scaffold Length	851 Kb	3.8 Mb	3.8 Mb	3.8 Mb	2.9 Mb	2.8 Mb
Number of Scaffolds > 10 Kb	2,562	2,313	2,105	1,963	2,125	3,061
% of Genome in Scaffolds > 50 Kb	56.7%	61.1%	64.4%	66.4%	64.2%	42.0%
Total Contig Assembly Length	143 Mb	154 Mb	154 Mb	156 Mb	161 Mb	160 Mb
Total Scaffold Assembly Length	144 Mb	155 Mb	155 Mb	154 Mb	159 Mb	164 Mb
% Gap	0.1%	0.2%	0.2%	0.2%	0.3%	0.4%
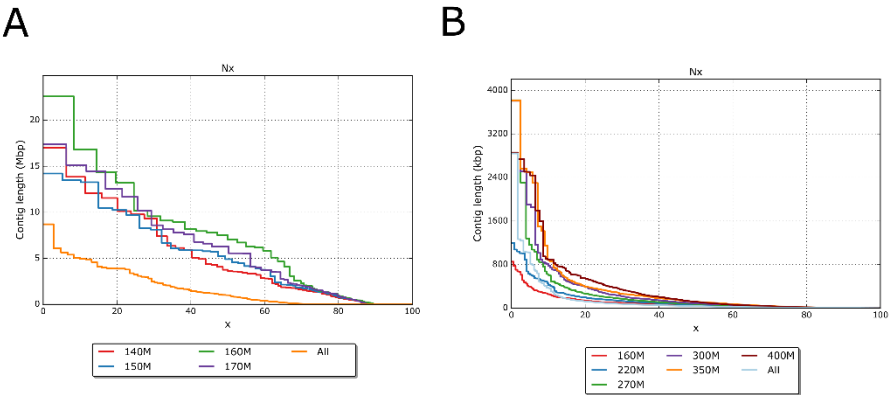
Fig. 1 Scaffold size distribution for a) *Trogoderma variabile* and b) *Lasioderma serricorne*. Y-axis represents scaffold length and X-axis represents number of scaffolds at each length. For *T. variabile*, subsampling 160 million reads led to the highest representation of long scaffolds in the assembly and for *L. serricorne*, subsampling either 300 or 350 million reads led to the highest representation of long scaffolds.

Assemblies for *T. granarium T. confusum*, and *D. maculatus* were similarly optimized. Although the assembly contiguity varied among these three insects, with contig N50s ranging from X to Y, scaffold N50s ranging from Z to Q, maximum contig lengths ranging from Y to Z, and maximum scaffold lengths ranging from blah to blah, all three assemblies had over 80% of their total assembly lengths in scaffolds > 50 kb and over 94% of conserved single copy orthologs were detected (Tables 3 and 4). While we are still awaiting sequencing data for *P. interpunctella, P. truncatus, and S. paniceum*, libraries have been prepared. We are also in the process of improving our scaffolding metrics using linkage maps and HiC contact maps.

Tab. 3 Assembly metrics for Trogoderma granarium, Tribolium confusum, and Dermestes maculatus optimized with barcode subsampling.

	T. granarium	*T. confusum*	*D. maculatus*

Number contigs	25,392	16,079	32,623
Contig N50	50 Kb	129 Kb	102 Kb
Max Contig Length	862 Kb	734 Kb	930 Kb
Number of Scaffolds	18,835	10,607	23,540
Scaffold N50	570 Kb	1.2 Mb	876 Kb
Max Scaffold Length	3.6 Mb	11 Mb	6.4 Mb
Number of Scaffolds > 10 Kb	1,596	702	1,747
% of Genome in Scaffolds > 50 Kb	80.1%	90.1%	83.1%
Total Contig Assembly Length	297 Mb	294 Mb	456 Mb
Total Scaffold Assembly Length	329 Mb	306 Mb	460 Mb
% Gap	9.5%	3.9%	3.8%

Tab. 4 BUSCO (Benchmarking Using Single Copy Orthologs) metrics for stored product assemblies completed to date.

	L. serricorne	*T. variabile*	*T. granarium*	*T. confusum*	*D. maculatus*
Complete/Single Copy	94.3%	98.3%	94.9%	96.9%	96.6%
Duplicate/Single Copy	0.8%	0.7%	0.9%	0.8%	0.5%
Missing	2.8%	0.5%	0.5%	0.5%	2.0%
Fragmented	2.9%	0.5%	1.9%	1.8%	2.7%

Discussion

Overall, the 10X Chromium libraries alone produced high quality assemblies that recovered significant percentages of the predicted gene space as the recovery of BUSCOs ranged from 92 to 98%. Assembly qualities were also relatively consistent regardless of genome size or taxonomic group and in all cases, over 80% of the assembly was present in less than 1,000 scaffolds, suggesting that these libraries can be a good first approach for assembling high quality draft genomes from insect species from many underrepresented taxonomic groups. In addition to the assemblies presented here, 10X Chromium libraries have been also used to produce high quality assemblies of aphid, butterfly, and dipteran genomes, providing further support for the use of this technique (Talla et al., 2017). Using linkage maps or HiC analyses, higher order assemblies will be obtained and will be almost to chromosome scale. Assemblies of this caliber can lead to the identification of syntenic orthologs across species. The identification of syntenic orthologs is important because orthologous genes present in the same chromosomal locations across species often have conserved functions, which can greatly facilitate functional annotation for non-model species (Zheng et al., 2004). In addition, the identification of syntenic orthologs may also expedite the identification of genetic targets for pest control (Futahashi, et al., 2011). For example, if knocking down a syntenic ortholog in one species reduces fitness or causes lethality, knocking down the same gene in other species that share synteny will likely also cause similar phenotypes. Although long-read sequencing approaches, such as PacBio, can generate assemblies of similar contiguity, the cost of the 10X libraries and the accompanying seuqencing is substantially less, potentially facilitating larger-scale comparative genomics studies that can be used to address broader evolutionary questions. In addition, because inbreeding is not necessary, 10X libraries can be generated much more rapidly relative to long-read sequencing approaches, which may greatly expedite genome assemblies for emerging or invasive pests.

Acknowledgements

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DNA barcode of stored-product Pests based on Mitochondrial Cytochrome Oxidase I Gene

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Abstract

The stored-product pests are economically important that can be spread through grain trade. Most stored-product pests, including eggs, nymphs, and adults, are very small and difficult to identify morphologically. Also the classification and identification of them have always been hindered by the overwhelming number of species, widely distribution. Here, we collected 43 stored-product pests from 46 geographical locations in China and other countries. The mtDNA COI gene sequences were sequenced. Software MEGA 5 was used to analyze the sequence comploition and genetic distances. Three molecular phylogenetic trees of Platypodidae were recomstructed using PAUP4.0 according to distance/ the neighbour-joining (NJ) and maximum parsimony (MP). The molecular results were compared with the morphological taxonomy. The interspecific genetic distance of the stored-product pests was significantly higher than the intraspecific genetic distance according to the barcoding gap analysis. This work provides a practical approach for the precise and rapid diagnosis of stored-product pests.

Keywords: DNA Barconding, stored-product pests, mtDNA COI gene, phylogeny

Introduction

Stored-grain insects and mites are great economic significance for grains and other products during storage. They are closely related with human life. The rapid identification is the precondition and basis for the comprehensive prevention and control of the pests. The modern identification of stored-grain insects by molecular biology techniques is able to get rid of the influence of the growth situation of individual specimen and the environment, and get accurate and reliable result from their DNA.

Traditionally, the species have been identified based on morphological characteristics of the adult. However, the identification of the species based on immature stages (i.e., egg, larva or pupa) or adult body parts, which lack distinct diagnostic characteristics, is very difficult and somethimes not reliable (Li et al., 2011). Traditional morphological identification is also time-consuming, requires specialized taxonomic knowledge and microscopy techniques (Yang et al., 2013; Jiang et al., 2014)

Recently, molecular identification based on nucleotide sequence analysis has become an effective method used to complement traditional taxonomic identification. For some important insect pests of stored products, AFLP has been used to diagnostic *Liposcelis* (Qin et al., 2008; Li et al., 2011), *Sitophilus oryzae* and *Sitophilus zeamais* (Hidayat et al., 1996), DNA barcode technology for *Liposcelis entomophila* (Yang et al., 2012). Some recent studies have implemented by PCR with species-specific primer pairs (Zhao et al., 2016). Species-specific primer identification is a PCR-based procedure that yields a unique band of known size and allows a species to be identified directly after gel electrophoresis (Wu et al., 2016). In animals, mitochondrial cytochrome c oxidase I (COI) gene, has been shown to be a reliable, quick and cost-effective tool for the identification of organisms of various taxa in all life stages. More and more important insect pests have been identified by this way (Namikoshi et al., 2011; Zhang et al., 2012; Jiang et al., 2014). It is a great advantage especially for the identification of small size pests.

DNA barcoding is a DNA-based species identification system which offers a promising supplemental technique with standardized portions of the genome (Hebert et al., 2003). The most commonly used barcode gene, mitochondrial (mt) DNA cytochrome c oxidase I (COI), has been shown to be a reliable, quick and cost-effective tool for the identification of organisms of various taxa in all life stages (Augot et al., 2010; Cywinska et al., 2010). A threshold of 2-3% mtDNA COI sequence divergence was recommended to define separate species for insects and mammals (Hebert et al., 2003). In studies of butterflies and ants, DNA barcoding has been successful in defining species boundaries by genetic distance thresholds (Hebert et al., 2004; Smith et al., 2005); however, there is no established universal distance threshold value to distinguish between taxonomic groups.

In the present study, we describe a reliable and efficient method based on conventional PCR with mtDNA COI gene, and we set up a DNA barcode data bace for stored-product Pests, which we hope will prove useful for the rapid diagnosis.

Materials and Methods

Specimens used in this study are collected from different provinces in China, including 46 geographical locations as fig. 1, some mites form Czech Republic, and except *Liposcelis bostrychophila*, *L. entomophila*, *L. decolor*, *and L.paeta*, the samples of *Liposcelis* from the Plant quarantine laboratory of China Agricultural University (CAUPQL). There are 43 species of stored-grain insects/ mites in total, 415 individuals, every species at least 5 specimens.

Total genomic DNA was extracted from the entire body of individual adults using the TIANamp Genomic DNA kit (DP304, TIANGEN, China) following the manufacturer's protocol for animal tissue. Five individuals from each species were used. PCR was performed with a pair of universal primers, LCO1490 (fw) 50 GGT CAA CAA ATC ATA AAG ATA TTG G 30 and HCO2198 (rev) 50 TAA ACT TCA GGG TGA CCA AAA AAT CA 30, amplifying an approximately 710 bp fragment of the standard mtDNA COI-5 barcode (Folmer et al., 1994). PCR products were separated on a 1.0% (w/v) agarose gel (1×TAE), stained with ethidium bromide, and visualised under UV light. The agarose gel slice

containing the PCR amplicon of interest was excised and placed in a centrifuge tube. The agarose gel slice containing the PCR amplicon of interest was excised and the DNA was gel extracted. Bidirectional sequencing reactions were carried out from a single individual of each geographical isolate (Beijing Aoke Biotechnology Co., Ltd.).

DNAMAN software (Lynnon Biosoft, Vaudreuil, Quebec, Canada) was used for DNA multiple sequence alignment using an optimal alignment method. Genetic diversity was estimated for haplotype diversity (Hd) and nucleotide diversity inDnaSP version 4.10.1 (Librado & Rozas, 2009). Pairwise genetic distances for COI were calculated using the Kimura-2-Parameter (K2P) distance model implemented in the software Molecular Evolutionary Genetics Analysis 5 (MEGA 5; Tamura et al., 2011). All phylogenetic analyseswere carried out using the program PAUP 4.0 (Swofford, 2002). Two different types of phylogenetic trees, neighbour-joining (NJ) andmaximumparsimony (MP), were graphically displayed and compared. A heuristic search was employed using tree bisection and reconnection (TBR) branch swapping and randomaddition for 100 replicates, and bootstrapping was performed using 1000 replications.

Results

In this study, we tested and evaluated the general genes of COI, which is an appropriate gene for identifying the DNA barcode of stored-grain insects/mites – a section of 650bp COI gene in mitochondria (primer pair LCO490/HCO2198). The mtDNA COI sequences of 415 obtained in this study. The sequences were all trimmed to a 650 bp core region that could be unambiguously aligned to one another. No sequences contained indels or nonsense codons, allowing for easy alignment and supporting their origin in the mitochondrial gene.

Fig. 1 Distribution of sampling sites for stored grain insects and mites in China

A rapid identification DNA barcode sequence database of stored-grain insect/mite is established, including 43 species of stored-grain insects/ mites. Every species has at least 5 specimens, we get the COI barcode sequence of 415 specimens. 98 haplotypes, among which only 1 haplotype is found in 20 species of insects/mites, showing the diversity of stored-grain insect is relatively low. Also most showed low intra-species divergence. Based on DNA barcode sequence and distance method, the neighbour-joining (NJ) and maximum parsimony (MP), the phylogenetic tree of stored-grain insect/mite is built. Both the NJ and MP phylogenetic analysis of the COI gene generated the same tree topology. The resulting trees showed a clear clade and every species has individual branch.

DNA barcode in the mitochondria COI sequence can be used to identify the species of stored-grain insects/mites rapidly and accurately.
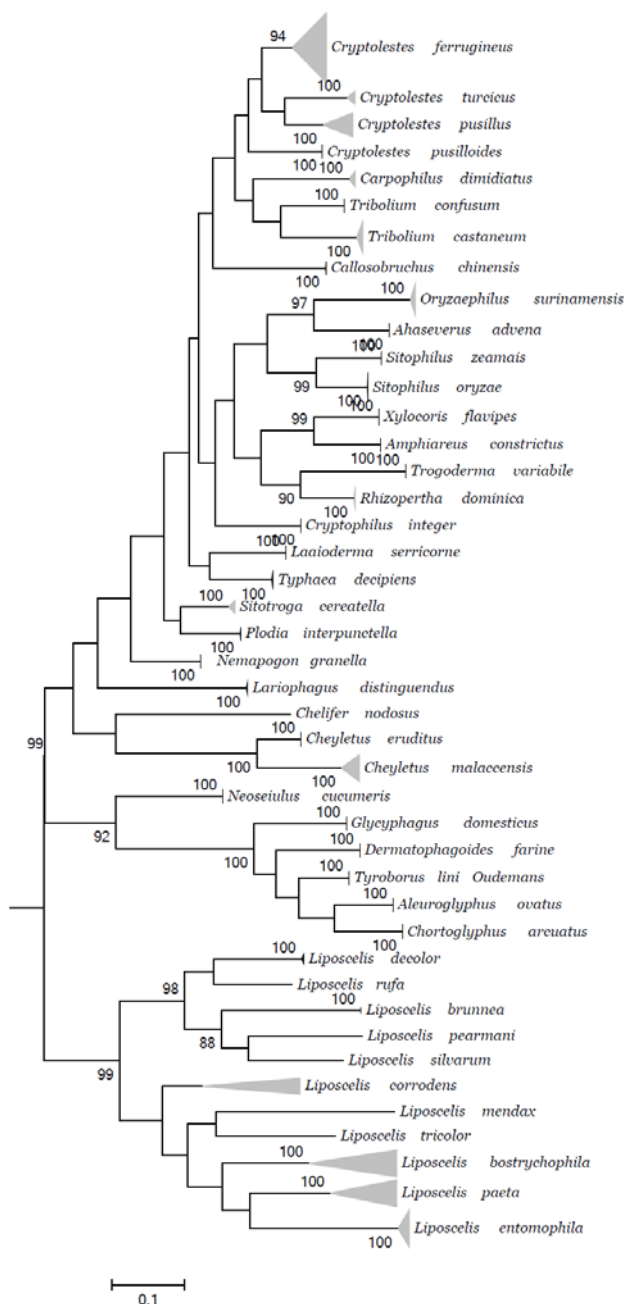
Fig. 2 Neighbour-joining tree of stored product insects and mites

Acknowledgement

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Effect of delayed mating on reproductive performance of *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae)

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Abstract

With the ban of methyl bromide and the many problems associated with the use of other synthetic chemicals, current research have focused on non-chemical alternatives and integrated pest management approach for the control of stored product insect pests. Mating disruption is one technique being investigated for its effect on stored product insects. In this study, we determined the effect of age at mating on the reproductive rate and longevity of the cigarette beetle, *Lasioderma serricorne* (Coleoptera: Anobiidae). We disrupted the mating approach by delaying the insects from mating for different time periods in days. Same age virgin male and female cigarette beetles were paired to mate soon after emergence (0 d old), or delayed from mating for 1–14 d. In another experiment, we maintained the age of the male at 0 d old and varied the age of the female from 0–14 d old and vice versa. Insects were observed daily for longevity and F_1 progeny was recorded 7–10 weeks after mating pairs were put together. Progeny production generally decreased with age of adults at mating. The number of F_1 progeny produced by same age adults varied from 10 per female to 59 per female. Similarly, the number of progeny decreased the longer one sex was delayed from mating. Findings from this study may provide information for the development of mating disruption techniques that can delay mating and may be effective in keeping populations of *L. serricorne* below levels that would warrant a control action.

Keywords: cigarette beetle, stored products, mating disruption, progeny production, methyl bromide alternatives

1. Introduction

Lasioderma serricorne (F.) (Coleoptera: Anobiidae), commonly known as the cigarette beetle or the tropical warehouse beetle, is a common stored product insect pest of feed mills and retail stores. *L. serricorne* causes significant damage to grain-based products, tobacco products, and other commodities of animal or vegetable origin (Arbogast, 1991; Dimetry et al., 2004; Mahroof and Phillips, 2008). The damage caused by this pest can account for millions of dollars in the Food and Feed Industries (Arbogast, 1991).

The ban of methyl bromide and the development of resistance to phosphine by the cigarette beetle (e.g. Savvidou et al., 2003; Sağlam et al., 2015; Fukazawa and Takahashi, 2017), has resulted in the search for potential non-chemical alternatives for the control of this pest (including Adler, 2003; Roesli et al., 2003; Conyers and Collins, 2006; Yu, 2008; Mahroof and Phillips, 2014).

Delayed mating techniques have been widely studied and used successfully in the control of many insect pests (including Ellis and Steele, 1982; Lingren et al., 1988; Fadamiro and Baker, 1999). Mating disruption involves the use of synthetic pheromones that mimic the natural sex pheromone normally released by the female. The release of high concentrations of the synthetic chemical 'confuses' the male which expends energy in finding the source of the pheromone and ends up delaying mating or not mating all together. To our knowledge, however, limited studies have been carried out on stored product beetles on mating disruption and mating delays. Few studies have been carried out on lepidopteran insects including the Indian meal moth, *Plodia interpunctella* (Hübner) (including Mbata, 1985; Huang and Subramanyam, 2003), with only one published studies on *L. serricorne* (Mahroof and Phillips, 2014). Mbata (1985) reported that a delay in mating resulted in a significant reduction of the number of eggs laid by *P. interpunctella* female as mature eggs were retained in the ovaries. Huang and Subramanyam reported that fecundity in female *P. interpunctella* significantly decreased by about 25 eggs for each day mating was delayed. The authors also reported that delaying mating in both sexes for 5 d resulted in the production of non-viable eggs by the female. Mahroof and Phillips (2014) studied the effect of the synthetic form of the predominant sex pheromone, serricornin, on the mating disruption of *L. serricorne*. The inhibition of proper orientation behavior of the males to females disrupted mating, resulted in delay in mating, and reduced the mating success. As a result, a significant reduction in the population size of subsequent generations was reported. From this study it was not clear why males fail to locate females in an environment purged with high concentration of synthetic pheromone. False trail following, masking of natural female pheromone or habituation of olfactory receptors may delay the age of mating (Mahroof and Phillips, 2014). The objective of this study was therefore to

investigate the effect of adult age at mating on the fecundity of females and the longevity of adult *L. serricorne*.

2. Materials and Methods

2.1. Insects

L. serricorne used for this study were from colonies which had been maintained at the Stored Products Entomology Research Laboratory at South Carolina State University since 2010. Prior to the bioassays, new colonies were established by transferring newly emerged adults to 473 ml rearing jars (Ball Corporation, Broomfield, CO, USA) with food made of 95% whole wheat flour and 5% yeast. The adults were allowed to lay eggs for 48 h and the rearing jars were then incubated for 31–35 days to attain the pupal stage of the insect. Cigarette beetle pupae were sexed using differences in the genital papillae (Halstead, 1963) and kept separately in jars containing some food. The jars were checked daily for adult development. Adults that developed in each jar were collected daily and kept in separate jars to be used when required. Adults of 0–14 d old were used in this study.

2.2. Mating of insects

One male and one female adult cigarette beetles of the same age were paired in a 5 cm high, 2 cm diameter plastic vial that contained 2 g of the diet mix. Same age insects (0-14 d) were paired up in a 5 cm high, 2 cm diameter plastic vial that contained 2 g of the diet mix. For each of the 15 ages, ten vials were set up. The vials were kept in an incubator at approximately $27.6 \pm 0.1°C$ and $60.8 \pm 0.8\%$ RH. We determined the longevity of mated adult insects. The vials were checked daily until all adults died.

In another set of experiments, newly emerged (0-d old) virgin males were paired up with newly emerged virgin females or with 1–14 d old virgin females. Also, newly emerged virgin females were paired up with newly emerged virgin males or with 1–14 d old virgin males. Each mating treatment was done in a 10 cm high, 2 cm diameter plastic vial that contained 5 g of the diet mix. Each mating treatment was replicated 10 times.

2.3. Data analyses

The number of adults that developed in each vial was recorded weekly beginning 7 weeks after set up until 10 weeks. Data on the number of F_1 progeny produced in each mating treatment were subjected to one-way ANOVA and means were separated using Tukey's Honest Significant Difference (HSD) test when the ANOVA was significant at $P \le 0.05$ (PROC GLM, SAS Institute, 2013).

We also determined the relationships between the longevity of the adults and their age at mating using regression analysis in TableCurve 2D software (Systat Software Inc., 2002).

3. Results

3.1. Effect of delayed mating on progeny production

The number of F_1 progeny produced by same age adults was significantly different as delay in mating increased (F = 33.36; df = 14, 135; $P < 0.0001$). Fewer progeny were produced by newly emerged adults (0-d old), with the highest progeny production by adults delayed from mating for 1- or 2-d. Delaying mating for two days significantly reduced the number of progeny produced (Fig. 1). The number of progeny produced by 6–11 d old adults did not differ significantly among each other.

The number of F_1 progeny produced when newly emerged males (0-d old) were paired with newly emerged females or with 1–14 d old females was significantly different (F = 39.47; df = 14, 135; P < 0.0001). The highest number of progeny were produced when both parents were 0 d old but it was

not significantly different from when the female was 1 d old. The number of offspring produced by 1–4 d old females were similar. The longer mating was delayed, the fewer the offspring produced (Fig. 2).
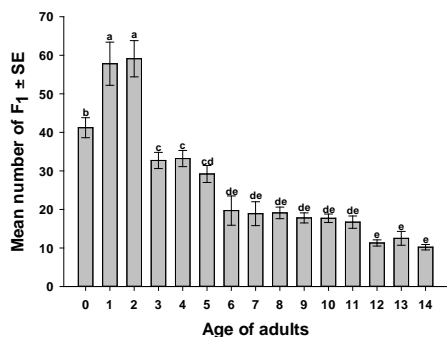
Fig. 1 Mean progeny production $\pm$ SE in same-age adult *Lasioderma serricorne* delayed from mating for different days. Bars with different letters represent means that are significantly different (Tukey's Honest Significant Difference test, $P < 0.05$).
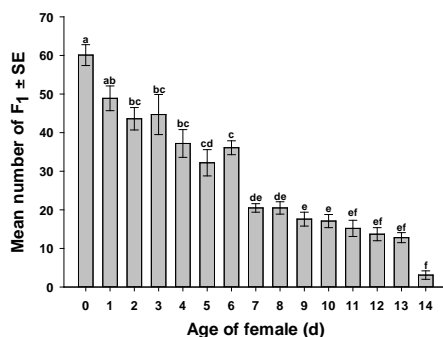
Fig. 2 Mean progeny production $\pm$ SE in adult *Lasioderma serricorne* delayed from mating for different days. Newly emerged (0-d old) males were mated with 0–14 d old females. Bars with different letters represent means that are significantly different (Tukey's Honest Significant Difference test, $P < 0.05$).

When 0-d old females were mated with 0–14 day old males, the number of F_1 progeny produced varied significantly (F = 10.31; df = 14, 135; $P < 0.001$) (Fig. 3). The trend was similar to that of the mating treatments where females were delayed from mating with newly emerged males. Similarly, newly emerged adults produced the highest number of progeny, however, not significantly different from progeny produced as a result of mating 0 d females with 1 d old males. Generally, the older the male, the fewer the number of progeny produced.
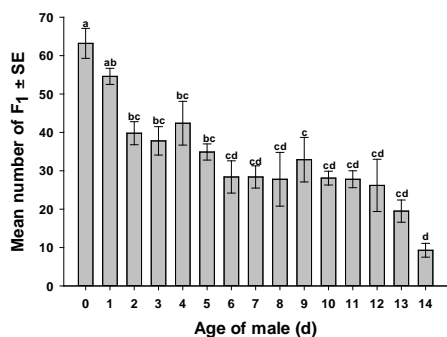
Fig. 3 Progeny production in adult *Lasioderma serricorne* delayed from mating for different days. Newly emerged (0 d old) females were mated with 0–14 d old males. Means followed by different letters are significantly different (Tukey's Honest Significant Difference test, $P < 0.05$).
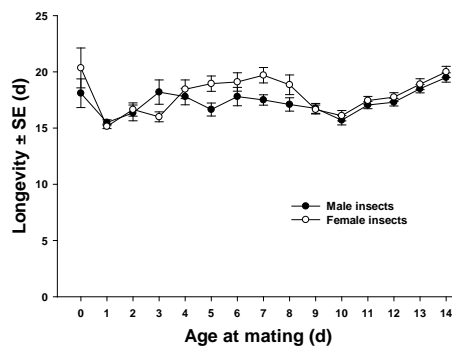
Fig. 4 Longevity of same-age adult *Lasioderma serricorne* mated at different ages. Longevity data accounts for their age at mating and the number of days they lived post-mating.

3.2. Effect of delayed mating on adult longevity

Average longevity ranged from 15.5 $\pm$ 0.2 to 19.5 $\pm$ 0.4 d in mated males and 15.2 $\pm$ 0.2 to 20.4 $\pm$ 1.8 d in mated females. Longevity was reported as the total lifespan of the insects, accounting for their

age at mating and the number of days they lived post-mating. There was a weak relationship between female longevity and age at mating ($y = 17.77 + 0.0000019^x$; $n = 20$; $r^2 = 0.14$) but a moderate relationship between male longevity and age at mating ($y = 17.05 + 0.0000022^x$; $n = 20$; $r^2 = 0.42$) (Fig. 4).

4. Discussion

The effect of delayed mating on progeny production in *L. serricorne* was investigated in this study. Progeny production was highest when there was no mating delay. Mating without delay may encourage multiple mating, probably because the adults are able to start mating early, subsequently resulting in an increase in the number of progeny produced (Huang and Subramanyam, 2003). As either sexes (Fig. 1) or one of the sexes (Figs. 2 and 3) age, fewer progeny was produced. Our findings were similar to those of other authors that reported the significance of multiple mating in progeny production in insect pests (including Huang and Subramanyam, 2003; Jiao et al. 2006; Yu, 2008). Huang and Subramanyam (2003) reported a significant reduction in fecundity of *P. interpunctella* for each day mating was delayed. The authors also reported that the majority of eggs laid by the female were laid within 4 d of mating. Jiao et al. (2006) reported a significant decrease in fecundity with increasing age at mating in the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae). Yu (2008) reported a significant decline in daily egg production in *L. serricorne* females 7 d after being paired with males. In our study, for each day that mating was delayed in any of the two sexes, 8–57 less progeny were produced. Delaying male or female mating for 2 d or more may have a significant impact on fecundity of *L. serricorne* and this could lead to a significant suppression in the population size of subsequent generations.

Studies have shown that increased fecundity in some multiple-mated females, and therefore increase in progeny production, may be due to the repeated transfer of some compounds including nutrient secretions and other hormones from the male to the female during copulation (Benz, 1969; Henneberry and Clayton, 1984; Park et al., 1998).

Many factors including mating status and diet have been reported to affect the longevity of stored product insect pests. Huang and Subramanyam (2003) reported that mated *P. interpunctella* moths lived for approximately 4–7 d. Yu (2008) reported that mated *L. serricorne* adults lived for 17–23 d, while unmated adults lived for 29–35 d. Findings in our study are similar to those of Yu (2008). In our study, we reported longevity of approximately 15–20 d in mated males and 15–22 d in mated females. Although not presented here, we observed that unmated males lived for approximately 28 d while unmated females lived approximately 3 d longer. The mating status of *L. serricorne* therefore seems to have an effect on the longevity of the insect. Adult longevity in *L. serricorne* has also been reported to be influenced by the diet on which the insect is raised (Mahroof and Phillips, 2008). The authors reported that adult longevity varied from 10–20 d depending on the food source.

Although the age of insects at mating has been established to be important in determining the fecundity (Mbata, 1985; Makee and Saour, 2001; Huang and Subramanyam, 2003), other factors such as diet, temperature, light have been shown to be equally important as well (Mbata, 1985; Shinoda and Fujisaki, 2001; Mahroof and Phillips, 2008; Vukajlović and Pešić, 2012). These factors may also be investigated to help develop pest management techniques. Findings in this study may be useful in the development of mating disruption techniques as an alternative control method that may be essential in managing *L. serricorne*.

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Larvae of Trogoderma respond behaviorally to whole body extracts

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Abstract

Behavioral responses to semiochemicals by *Trogoderma* (Coleoptera: Dermestidae) stored product pests were assayed in a small arena. Hexane extracts were obtained from Khapra beetle, *Trogoderma granarium*, warehouse beetle, *Trogoderma variable*, and the larger cabinet beetle *Trogoderma inclusum* that were killed by being frozen for 48 hours at -20° C. These extracts were analyzed using gas chromatography coupled with mass spectrometry (GC/MS), and it was confirmed that they contain several cuticular hydrocarbons, fatty acids and sterols. Two choice experiments were performed inside Petri dish arenas, with filter paper fully covering the bottom surfaces. Two smaller 3cm filter papers were placed on opposite ends within each arena. Each of the smaller papers were folded three times in parallel to present a corrugated surface that the insects could move underneath if they chose. In each case, one paper had a 100µl aliquot of one of the extracts, and the other 100µl of hexane as a control. 10 late instar larvae of the same species as the treatment extract were placed in the arena and allowed to acclimate overnight in a dark room. For all three species, it was found that larvae were more likely to be found on the side of the Petri dish with the hexane control rather than the conspecific larval extract. They were also more likely to be on or near the smaller corrugated filter paper treated with the control as opposed to the filter paper treated with the larval extract. Thus repellency of the conspecific extract was demonstrated at that particular dose. Further assays using different doses of the raw extracts and their individual chemical components are planned. The use of these semiochemicals in novel management strategies will be considered.

Keywords: Aggregation, behavior, khapra beetle, management, pheromone

Introduction

The khapra beetle (KB), *Trogoderma granarium* is a serious pest of stored products and is the only stored products pest that is currently quarantined in the United States. KB larvae feed on a wide range of dry food products of plant and animal origin including cereal grains, dried fish and museum specimens (Hagstrum et al. 2013). It currently has an extensive distribution throughout warm and arid regions of Eurasia and Africa. It is a quarantine pest in the United States, which has a history of interception at ports and successful eradications of various scales, at specific locations (Armitage, 1958, Myers and Hagstrum 2012). Preventing establishment of the khapra beetle in the US is crucial to maintaining access of products to export markets. Various kairomone attractants have been developed for monitoring the species. Additionally a pheromone produced by adult females is used in current APHIS-PPQ monitoring efforts (Barak, 1989).
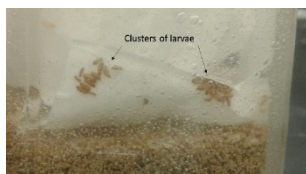
Fig. 1. KB larvae assembling in clusters on paper placed in a laboratory colony jar.
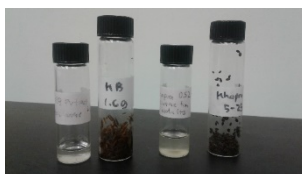
Fig. 2. Hexane extracted from KB larvae. The extract is contained in the hexane in vial on the left, which was removed from the one on the right containing the dead larvae.

Fig. 3. Bioassay showing that in this instance, KB larvae preferred to remain on the left side of the arena, but without substantial clustering on the smaller treatment paper.

The possibility that pheromones affect the movement of larvae has not been investigated. Any such chemicals potentially could be incorporated into novel management strategies. For larvae, it was shown some time ago that carbon dioxide and certain food odors can be attractive, while many short chain (3-7 carbon) alcohols and organic acids can be repellant (Spangler 1965). Currently the adult pheromone and host associated kairomones are used in traps. However, it is readily observed that KB larvae, as well as those of other Trogoderma species will assemble in clusters, even on non-food sources such as papers placed in laboratory colony jars (Figure 1). The question of whether there may be semiochemicals that mediate this particular behavior has not been investigated. In this study, this possibility was researched in KB, as well as two related species, the warehouse beetle (WB), *Trogoderma variable*, and larger cabinet beetle (LCB), *Trogoderma inclusum*. We included the additional species because it is of interest to what degree such behaviors are common in the genus, particularly since several species share the same adult pheromone.

Materials and Methods

Hexane extracts were obtained from late instar larvae and adult that were killed by being frozen for 48 hours at -20° C (Figure 2). Extracts were made at a ratio of 4ml of hexane/ 1 g of larvae of each species. These extracts have been analyzed using gas chromatography coupled with mass spectrometry (GC/MS), indicating the presence of a number of compounds with fragmentation patterns that are consistent with those of cuticular hydrocarbons. Previously published research confirms that similarly prepared extracts of khapra beetle larvae using dichloromethane rather than hexane contained a number of cuticular hydrocarbons (Maliński et al., 1986). Furthermore adults also have a similar hydrocarbon profile (Dubis et al., 1987). In our newly prepared extracts, there were no noticeable differences among the traces for any of the different species. Furthermore each contained a region of several compounds indicative of hydrocarbons, all at similar retention times. The identities of such compounds in our extracts have not been confirmed, but there is little reason to believe they are different from the chemicals described in the literature. There were also a number of other compounds in the extracts, including particular fatty acids and sterols. These compounds also may potentially affect behavior.

For assessing whether the extracts can influence the behavior of the larvae, two choice experiments were performed in static air enclosures. Inside of 15cm glass petri dish arenas, a filter paper was placed that fully covered the bottom surface (Figure 3). Within each of these arenas, two smaller 3cm filter papers were placed, which were used for presenting chemical treatments and providing a possible clustering surface. To encourage clustering, each of the papers were folded three times in parallel to present a corrugated surface. For each experimental replicate that was performed in an arena, one of the smaller corrugated papers received a 100µl aliquot of one of the extracts, and the other smaller paper 100 µl of hexane as a control. This was a dose of roughly five larval equivalents. Ten late instar larvae were placed in the arena and allowed to acclimate overnight in a dark room.

Long-chain hydrocarbons have very low volatility, and if behaviorally significant, generally will function as close range pheromone attractants. Thus the static air environment of the Petri dishes is not different from the context within which the larvae are likely to respond to such signals. The dose of five larval equivalents was selected because it would represent the chemical equivalent of an existing cluster of larvae.

Results

For all three species, the larvae were more likely to be found on the side of the Petri dish with the hexane control, while less likely to be on the side with the conspecific larval extract. A total of 36 replicates were performed for each species and the percentages of larvae on the control side of the arenas were 63% for KB, 72% for WB, and 71% for LCB. Most of these larvae were not found on or under the folded control or treated filter papers. Thus 66% of KB, 65% of WB, and 69% of LCB, were found in other parts of the arena. When not clustered on the folded papers, several were often

clustered in other locations. Among those that were on the clustered papers, 80% of KB, 76% of WB, and 75% of LCB, were on the control paper, versus the treated one. Thus the conspecific extract was repelling the assembly of the larvae of all three species. The attraction that was expected given the observations of clustering behavior did not occur.

Discussion

In considering these results, it should be noted that the dose applied was greater than the equivalent of a single larvae, and thus the concentration of the chemicals may indicate a biologically unrealistic situation. Furthermore, it is not clear yet whether the cuticular hydrocarbons are producing this reaction or if some of the other compounds in the extracts, such as the fatty acids and sterols may be causing repulsion. It is possible that production of these same compounds, or others were elicited by the stress of the insects being frozen to kill them before the extraction. Any such a compound would thus function as an alarm pheromone that repels other insects from joining with and aggregating near other larvae that are distressed.

Whatever the causal factor of the repellency may be, understanding the mechanism may provide a product useful for the management of KB. For example it may be possible to incorporate such a compound into treatments of products or their packaging in a way that repels the larvae to protect the products. There is also the possibility that a repellent compound could perhaps be used in push-pull trapping strategies.

Additionally, it may also be worth revisiting the idea of whether clustering can occur if perhaps only the cuticular hydrocarbon portions of the extracts are used. If there is another compound causing repellency, it could be masking the effects of attractive compounds. There are also potential dosage issues with any behaviorally active compound. It could be that repellent compounds are attractive at other doses. Thus much additional work will be needed to fully utilize the capabilities of the behaviorally active components of such extracts. However, it is promising that at this stage in our investigations, behavioral activity has been clearly demonstrated.

Another final consideration may be whether adults have similar responses to such compounds. We did attempt to assess the response of adults to such extracts in the assay described above. However, it became clear very quickly that the adult insects were highly mobile and did not settle in clusters like the larva. Many were actively crossing between the sides of the Petri dish as they were being evaluated at the end of the overnight period. Thus, the evaluation of the adult behavior with respect to these extracts may require a different assay. For example, a small scale wind tunnel, or two-choice olfactometer with moving air, may be more applicable.

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Necrobia rufipes (De Geer): an emerging pest associated with pet store chain in Europe

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Abstract

Necrobia rufipes is a cosmopolitan pest, causing considerable damage to stored commodities such as copra (dried coconut), cheese, dried fish, ham. The present study was undertaken to investigate the occurrence of these insects on pet store chain in Europe. In the last year *N. rufipes* was found associated with pet food, especially in Mediterranean countries, causing considerable economic damage and loss of product. The causes of such sudden diffusion are not known but some considerations are reported. Future studies will be needed to collect data on development on pet food and on the possibility to monitor *N. rufipes* in wharehouses and pet stores.?

Keywords: pet food, pest infestation, red-legged ham beetle, Cleridae.

Necrobia rufipes (De Geer) is a beetle, belonging to family Cleridae. Riley (1874) gave it the common name of red-legged ham beetle, while in the Pacific Island is known as copra beetle (Froggatt, 1911). Riley made the first economic investigation, citing cases of extensive injury to hams in St. Louis and Boston (USA). It is a cosmopolitan pest, associated to copra (dried coconut), cheese, dried fish, cured ham and bacon. However it is reported to feed on other pests that infest products or decaying animal matter (Simmons and Ellington, 1925; Ashman, 1963; Peck and Thomas, 1998). *N. rufipes* was also found on mummies (Panagiotakopulu, 2001), and it is considered in forensic entomology since it is usually found on carrion after most of the flesh has been remove, presumably feeding other insects rather than on the carrion itself (Kulshresthaa and Satpathy, 2001).

In 2003 the red-legged ham beetle was found in retail pet store and in 2007 it was reported infesting pet food in Brazil, but the origin of infestation was unknown (Roesli et al., 2003; Gredilha and Lima, 2007).

In the present note we report the *N. rufipes* presence in Mediterranean countries, especially associated with pet food warehouses and retail pet stores. The first report dates back 2015 in Israel, in 2016 it was found in Southern Italy (Puglia region), in 2017 in Greece, Turkey, Montenegro, Germany and Czech Republic, Southern France, Spain, Northern Italy, all Southern regions and island.

The causes of such a wide spread of this pest are, for now, unknown. Pet food products are rich in animal protein content, particularly suitable for *N. rufipes* development, and pet food packaging are often not resistant to insect infestation. Different Authors suggest that the presence of *N. rufipes* is mainly linked to the predation of other pests (Kulshresthaa and Satpathy, 2001; Roesli et al., 2003). The red-legged ham beetle is reported as a facultative predator on larvae of *Lasioderma serricorne* (F.), *Oryzaephilus mercator* (Fauvel), *Carpophilus dimidiatus* (F.) (Simmons and Ellington, 1925; Ashman, 1963). In effect, in three cases of pet food infestation we verified the simultaneous presence of *N. rufipes* and the sawthoothed grain beetle, *Oryzaephilus surinamensis* (L.).

An important way of diffusion of the pests is represented by pallets and packaging materials. Several *N. rufipes* larvae were found on crevices of pallet wood and packaging like carton, protected in a white pupal chamber produced by themselves. Furthermore adults are able to fly and are attracted by food odors from long distance. We observed in several retail pet stores an incorrect management of waste material; broken pet food bags, pallets, cartons were stored near the entrance doors. We also verified that pallets and packaging are preferred by *N. rufipes* larvae for pupation and this is the way to spread infestation.

A critical point to manage *N. rufipes* infestation is that the monitoring is difficult, as there are no

commercial lures specifically available for baiting traps. A sexual attractant was identified and described in a Chinese patent, but not commercially available (Lei and Guangwei, 2016). *N. rufipes* larvae and adults were captured with commercial pitfall traps baited with food oil and pheromone lures (not specific for *N. rufipes*) from Trécé, in pet food retail stores (Roesli et al., 2003), but currently there is not a trap specifically set up for *N. rufipes*. This is a weak point for the management of infestations as it is not possible to constantly monitor the presence of the insect in industries, warehouses, pet stores.

Another aspect to take in account is the lack of specific information on control strategies. Only Roesli and Subramanyam (2002) reported that precision targeting using sanitation alone did not have an impact on *N. rufipes* adults, but reduced larval presence. Precision targeting with sanitation followed by cyfluthrin spray greatly reduced both larvae and adults.

We can conclude that the diffusion of *N. rufipes* is actually favored by a lack of specific monitoring traps, by incorrect application of pest prevention tecniques, combined with the incorrect management of storage warehouses and retail stores.

Further researches are needed to better understand food preference and behavior of this emerging pest associated with pet store chain.

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The orientation of *Tribolium castaneum* adults in the presence of aggregation pheromone 4,8-Dimethyldecanal and food oils

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Abstract

Monitoring of *Tribolium castaneum*, the red flour beetle, involves the use of aggregation pheromone 4,8-dimethyldecanal (4,8 DMD) and kairomones such as cereal oils. Despite their present use, certain information which maximizes the efficacy of these compounds is still lacking. These experiments tested the effects of distance from the pheromone and edible oils on the orientation of *T. castaneum* adults. The movement of adults toward the aggregation pheromone was determined by changing the distance from the pheromone or the air flow. The adults released inside a glass apparatus tested their orientation either toward the food oils or the empty vial. The maximum trap catch was recorded at distances up to 60 cm from the pheromone and with the presence of air flow. The oils having botanical origin successfully attracted adults than those of animal origin. It is concluded that the orientation of *T. castaneum* adults varies with the distance from pheromone, air flow and the nature of food oil.

Keywords: Aggregation pheromone, distance, Kairomone, air flow, Tribolium castaneum

1. Introduction

Tribolium castaneum (Herbst), the red flour beetle, is a serious pest of stored agricultural products (Rees, 2004; Trematerra and Sciarretta, 2004). The most popular control measures for stored-product insects include the use of contact insecticides (Ghimire *et al.*, 2016) and fumigants (Hill, 1990). Due to the negative impact of the residual effect of insecticides on human and the environment, control methods using compounds other than neurotoxic chemicals are emphasized for stored-product protection.

Male adults of *T. castaneum* release the aggregation pheromone 4, 8-dimethyldecanal (4,8 DMD) that attracts both sexes (Suzuki *et al.*, 1984). Commercial pheromone lures use a combination of pheromone and kairomone (Campbell, 2012). However, certain information that maximizes the efficiency of this aggregation pheromone is still lacking. Also limited research has been conducted on the response of *T. castaneum* adults to food volatiles (Campbell, 2012). Therefore, the objectives of this research were to determine the effect of distance from the aggregation pheromone, air movement and food oils on the orientation of *T. castaneum* adults.

2. Materials and methods

One-month-old *T. castaneum* adults (50) were released at distances 30-120 cm from the trap having pheromone 4,8 DMD. From each distance, three replicates were tested. The control experiments were done using only the plastic trap (without pheromone or kairomone). The beetles trapped following releasing was counted. The effect of airflow was tested by using an exhaust fan.

A glass chamber having two holes on the bottom plate and vials underneath was used to test the effect of edible oils on *T. castaneum* movement. The food oils, egg albumin, the commercial kairomone solution (Trece Inc., USA) or two pheromone septa (Trece Inc., USA) was placed inside one vial. Fifty *T. castaneum* adults were released at the center of the chamber, and the number of beetles in each vial was counted.

3. Results and discussion

The attraction of *T. castaneum* adults to pheromone decreased when the distance at which the beetles released was increased (Tables 1 and 2).

Tab.1 *Tribolium castaneum* adults trapped when released at different distances from the trap- presence of air flow.

Distance (cm)	Trapping in control (%)[*]	Trapping in treatment (%)[*]
30	0d	21.33a
60	0d	18.66a
120	0d	2.66c

* Percentage trapped followed by the same letter in a column are not significantly different at p=0.05 according to Tukey's test.

Tab. 2 *Tribolium castaneum* adults trapped when released at different distances from the trap- absence of air flow.

Distance (cm)	Trapping in control (%)[*]	Trapping in treatment (%)[*]
30	0c	13.33a
60	0c	10a
120	0c	1.33cb

* Percentage trapped followed by the same letter in a column are not significantly different at p=0.05 according to Tukey's test.

Olive oil, kairomone solution (Trece), pheromone solution and Mee oil attracted significantly higher number of adults than their controls. The adults attracted to rice bran oil, corn oil, cod-liver oil, ghee, coconut oil or sunflower oil were not significantly different from their controls. Lowest attraction was shown by the egg albumin and the mustard oil.

Tab. 3 *Tribolium castaneum adults* attracted by different food oils, pheromone or kairomone.

Source	Trapping in Treatment (%)*	Trapping in Control (%)
Olive oil	29edc**	15**
Rice bran oil	21ed	25
Coconut oil	52ab**	8**
Kairomone	44abc**	5**
Pheromone	53ab**	17**
Sunflower oil	36bdc	17
Mee oil	59a**	14**
Mustard oil	29ecd**	26**
Gingelly oil	22ed	21

* Percentage trapped followed by the same letter in a column are not significantly different at p=0.05 according to Tukey's test.

**denotes significant difference from the control.

The aggregation pheromone 4, 8 DMD released from *T. castaneum* adults is dispersed effectively up to 60 cm from the trap. Air flow increases the beetle orientation towards the source. Coconut oil and Mee oil equally attract adult beetles as the synthetic pheromone and kairomone.

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The responses of *Tribolium castaneum* to wheat germ oil and fungal produced volatiles

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Abstract

The red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is a significant pest affecting a wide variety of different stored products around the Globe. Despite its economic impact, there is evidence that the lures currently used in traps to monitor for this species are largely ineffective. Based on the evolutionary history of *T. castaneum*, and the ecological niche it occupies, the volatiles of wheat germ oil and volatiles produced by grain-associated fungi have the potential to act as attractants for this species. We used electroantennography (EAG) to measure the electrophysiological response elicited by sixty-eight volatile compounds found in wheat germ oil and/or grain-associated fungi in two *T. castaneum* strains; an established lab population (CTC12 strain) and a recently caught wild population. Many volatile compounds from both sources elicited strong antennal depolarisations, and the responses of both strains were highly correlated. We then tested whether the compounds that triggered the strongest antennal depolarisations also elicited behavioural responses by using Y-tube olfactometer bioassays and identified several compounds attractive to both strains. The discovery of novel compounds that elicit strong EAG signals and behavioural responses could prove useful in the design of

improved lures for *T. castaneum* and other stored product pests. Our future research will identify how effective these attractive volatiles might be when used in combination, and when used under conditions that more closely replicate a stored product environment.

Key words: *Tribolium castaneum*, electroantennography, Y-tube olfactometer, fungal volatiles, wheat germ oil

Introduction

Tribolium castaneum and its sister species *T. confusum* are both economically significant pests of the stored product industry. They are particularly damaging owing to their global distribution and the wide variety of food products that they can infest, including nuts, milled grains and dried cereal products (Bell, 2014). Severe *Tribolium* infestations can also produce a "conditioning" effect in the medium they infest, which is characterised by depletion of the nutrient value of the medium, the accumulation of toxic benzoquinones secreted by the beetles and a build-up of debris such as larval casts and dead adults (Ghent, 1963). *Tribolium* infestations are a particular problem in stored product warehouses where damage by insects, mites and other microorganisms accounts for an estimated global annual post-harvest loss of 10-15% (Neethirajan *et al.*, 2007). This problem is especially severe in developing countries, where post-harvest losses can be as high as 20% (Phillips and Throne, 2010).

One of the main ways that infestations of *Tribolium* species and other stored product pests can be detected and monitored is using lure baited traps. These lures typically contain insect pheromones in a slow-release formula and are commercially available for over 20 species of stored product insect, including *T. castaneum* and *T. confusum* (Phillips and Throne, 2010). Multispecies lures, containing the pheromones of different insects, are also available and are a common feature of integrated pest management strategies as they overcome the need to have multiple different lures for the different pest species encountered (Cox and Collins, 2002). Many of these lures combine insect pheromones with a food based kairomone such as wheat germ oil (Campbell, 2012), and this can make up 90% of the concentration by weight of these products. Wheat germ oil appears to be attractive to *T. castaneum* (Phillips *et al.*, 1993), but the specific compounds in this mixture that elicit this attraction have not yet been identified. Despite the widespread use of these lures, there are reports from users in the stored product industry that they are not very effective (Semeao *et al.*, 2011). This is supported by experimental data showing that the responses of *T. castaneum* to these pheromone baited traps is limited in ideal conditions and can be minimal in an environment with no air-flow (Campbell, 2012). In a simulated warehouse experiment, less than 2% of *T. confusum* released within a 60 cm distance from a pheromone trap were caught (Hawkin *et al.*, 2011). As a result of this there has been a focused effort towards improving the efficacy of lures for *T. castaneum* and other stored product pests (Cox and Collins, 2002).

One major barrier in improving the ability of these lures to attract *T. castaneum* and other stored product insects is the lack of knowledge about the specific odours that attract these insects to stored product environments. This is particularly true for *T. castaneum* where little is known about its attraction to specific food related volatiles (Campbell, 2012). As the common name of *T. castaneum,* the red flour beetle, implies, flour and other milled grains are a major food source for this species. However, experimental data show that the odours of flour are only marginally attractive to this species (Campbell, 2013). *Tribolium castaneum* appears to be more attracted to cereal grains that exhibit signs of damage from decay or pest infestation (Trematerra *et al.*, 2000). They are also more attracted to wheat kernels with other insects present, and to those that have been damaged by other pest species (Trematerra *et al.*, 2000). *Tribolium castaneum* also exhibits attraction to fungal odours, specifically the volatiles of fungi associated with cotton seed lint (Ahmad *et al.*, 2012). Indeed, they were shown to be more attracted to these odours than to the odours of conventional food sources such as wheat (Ahmad *et al.,* 2012). These preferences could be explained by the fact that *T. castaneum* is a 'secondary pest' which primarily feeds on grains that are rotten or have been damaged by the infestation of other insects, or mechanically processed by humans, i.e. milled

(Trematerra and Sciarretta, 2004). The presence of wheat germ and fungal volatiles would therefore be an indicator that the grains are in a suitable condition for *T. castaneum* to feed on.

Despite the knowledge that *Tribolium* species are generally attracted to the odours of both wheat germ oil and fungi the specific volatiles underpinning this attraction have not been clearly identified. As many current lures contain wheat germ oil as a food based attractant there is the potential to improve the efficiency of these traps by only including the specific volatiles in wheat germ oil that attract *T. castaneum*. The incorporation of attractive fungal volatile compounds also has the potential to improve the attractiveness of lures. To identify specific volatiles of wheat germ oil and grain-associated fungi that are attractive to *T. castaneum* we have used a combination of electroantennography (EAG) and behavioural bioassays. We have used EAG as an efficient method of identifying which compounds can be detected by the antennae of *T. castaneum* and have determined whether *T. castaneum* are attracted to the volatiles that they can detect using Y-tube bioassays. A variety of different compounds have already been tested for *T. castaneum* using EAG (Balakrishnan *et al.*, 2017; Verheggen *et al.*, 2007), but this is the first screen focusing specifically on the volatiles found in wheat germ oil and produced by grain associated fungal species.

Methods

Tribolium husbandry

Two *T. castaneum* strains were used in the experiments; the CTC12 strain and a wild captured Zimbabwean population. The CTC12 strain originates from an organophosphate resistant strain from Australia (Champ and Campbell, 1970) that has since been cultured in the laboratory. This strain was used to represent an established laboratory population. The wild captured population were cultured from a population found inside a shipment of infested grain from Zimbabwe in 2017. Cultures for both strains were maintained at 30°C in containers of 200 g of whole grain flour with the addition of 10 g yeast powder (as an additional protein source) and 1 g of the antimicrobial agent Fumagillin (to inhibit fungal growth in the cultures). All beetles used in the experiments were aged between 4 and 8 weeks post-emergence.

Volatile compounds

Sixty-eight volatile organic compounds that are either present in wheat germ oil or produced by grain-associated fungi were used (Table 1). The 34 wheat germ oil volatiles used in these experiments had been previously identified through headspace-solid phase microextraction of a sample of wheat germ oil (Niu *et al.*, 2013). Fungal compounds (28) were identified from a review listing the volatiles produced by common fungi grown on cereal and grain substrates (Magan and Evans, 2000). Six compounds were identified as being found in both wheat germ oil and produced by grain-associated fungi. Synthetic DMD (4,8-Dimethyldecanal), the *Tribolium* spp. aggregation pheromone, was used as a positive control as it is known to be behaviourally attractive and elicit strong antennal depolarizations in *T. castaneum* (Levinson and Mori, 1983). All odorants were diluted to working concentration using hexane, an established solvent for use in insect olfactory behavioural experiments that has previously been shown to not elicit significant EAG depolarisations or behavioural attraction in *T. castaneum* (Verheggen *et al.,* 2007). All compounds were obtained from commercial suppliers.

Table 1. The environmental volatiles used in our experiments and whether they were identified as being found in wheat germ oil, produced by grain-associated fungi or as both.

Compound	Source	Compound	Source	Compound	Source
5-methyl-3-heptanone	Wheat germ oil	3-octanone	Fungal	3-methyl-1-butanol	Both
trans-2-heptenal	Wheat germ oil	butyl acetate	Fungal	hexanal	Both
ethyl hexanoate	Wheat germ oil	benzaldehyde	Fungal	1-octen-3-ol	Both
Limonene	Wheat germ oil	3-methylanisol	Fungal	1-hexanol	Both
trans-trans-2,4,-heptandinal	Wheat germ oil	2-methylacetophone	Fungal	nonanal	Both

2-heptanone	Wheat germ oil	1-pentanol	Fungal	ethanol	Both
trans-2-pentenal	Wheat germ oil	trans-2-hexen-1-al	Fungal		
iovaleraldehyde	Wheat germ oil	2-methyl-2-butanol	Fungal		
Octanal	Wheat germ oil	damascenone	Fungal		
amyl acetate	Wheat germ oil	3-octanol	Fungal		
trans-2-octene	Wheat germ oil	dimethyl benzene	Fungal		
1-penten-3-one	Wheat germ oil	styrene	Fungal		
ethyl benzene	Wheat germ oil	2-butanol	Fungal		
trans-2-pentanal	Wheat germ oil	naphthalene	Fungal		
1-octene	Wheat germ oil	1-butanol	Fungal		
trans-cinnamaldehyde	Wheat germ oil	2-methyl-1-propanol	Fungal		
ethyl octanoate	Wheat germ oil	2,2,4-trimethylhexane	Fungal		
Pentane	Wheat germ oil	2-nonanone	Fungal		
2-pentylfuran	Wheat germ oil	acetone	Fungal		
trans-2-octenal	Wheat germ oil	butyl acetate	Fungal		
Undecane	Wheat germ oil	2-methylfuran	Fungal		
1-heptene	Wheat germ oil	octyl acetate	Fungal		
Nonane	Wheat germ oil	2-pentanone	Fungal		
trans-5-decene	Wheat germ oil	1-phenylethanol	Fungal		
Toluene	Wheat germ oil				
trans-3-octene	Wheat germ oil				
2-methyl-2-butene	Wheat germ oil				
p-anisaldehyde	Wheat germ oil				
trans,trans-2,4-decadienal	Wheat germ oil				
trans-2-decenal	Wheat germ oil				
4-allylanisol	Wheat germ oil				
octanoic acid	Wheat germ oil				
Tridecane	Wheat germ oil				
Hexane	Wheat germ oil				

Electroantennography

The electroantennography protocol was adapted from the Syntech Electroantennography manual (Syntech, 2004). Only female beetles were used, as in preliminary experiments (not reported) we found there were no significant differences between the responses of male and female beetles. The same finding has recently been reported by Balakrishnan *et al.* (2017). For each strain, a live female beetle was carefully positioned on a glass slide with adhesive tape to restrict movement and allow EAG recordings to be taken (N=8). A thin strip of double-sided adhesive tape was placed under the head of the beetle. This was sufficient to prevent movement of the antenna with the addition a small drop of cyanoacrylate glue to stick down the head of the beetle. Care was taken to not get any glue on the antenna of the beetles. Small holes were pierced into the tip of the antenna and through the eye of the beetle with an electrolytically sharpened tungsten wire to allow glass capillary electrodes filled with Ringer solution, in contact with silver wire, to be inserted. Filtered air continuously flowed over the restrained beetle and the test odorants were delivered by an air-puff from a Syntech stimulus controller. When triggered the stimulus controller delivered a one second puff of air to the end of a Pasteur pipette pointed at the head of the restrained beetle. Strips of Whatman filter paper with 5 µl of 20% vol/vol dilution in hexane of each volatile compound were inserted into this pipette to present the beetles with the different volatiles used in the experiments. Every 10 volatiles the responses of the beetles were tested against DMD (positive control) and hexane (negative control) and the responses of the preceding 10 volatiles were normalised against the DMD response. The EAG potential was recorded on a computer using a signal amplifier, IDAC convertor and EAG 2000 software.

Y-tube bioassay

The Y-tube olfactometer apparatus consisted of a 20 cm long, 6 cm in diameter, glass cylinder that branches in the middle to form a two-armed (Y-shaped) glass tube. The Y-tube was connected by PTFE tubing to a vacuum pump, which drew an air-flow through each of the two Y-arms at a rate of 0.2 L/min. Each arm was in turn connected by PTFE tubing to three sealed vials, the first containing Whatman paper disks to which 5 µl of a 200 ng/µl dilution of the test volatile was added, the second

containing activated charcoal and the third containing water. The Y-tube olfactometer had a sealable hole on the main stem that allowed for insects to be inserted while the vacuum pump was running. A single beetle was inserted into the Y-tube through this hole for each trial and its movements were observed for five minutes. Once a beetle had walked 2 cm down one of the two branches of the Y-tube it was recorded as having chosen that arm of the olfactometer. If no choice was made within five minutes the beetle was deemed to be non-responsive and was discarded. The odorants connected to each arm of the olfactometer were switched over every 10 trials to prevent the direction of the arms from biasing the choices of the beetles. Only females were used as other researchers have suggested that aggregation pheromone could be produced by males within the olfactometer, which could influence the behaviour of beetles used in subsequent trails (Ahmad et al. 2012). All trials were conducted in a 20°C controlled temperature room.

Statistical analysis

Differences between the EAG responses of the two strains to the different volatiles tested were analysed with a two-way mixed ANOVA. Where significant differences were found they were followed up with pairwise paired t-tests (for within-strain differences) or unpaired t-tests (for between-strain differences). To correct for the error associated with number of statistical tests the significances were adjusted using a false discovery rate method. All statistics were performed using IBM SPSS Statistics 24.

Results

Electroantennography

The average antennal depolarisations elicited by the volatiles presented to female *T. castaneum* after being normalised against the response to the DMD positive control are shown in Figure 1. It was noted that the absolute depolarisations of the CTC12 strain were much larger than that of the wild strain for all volatiles tested with the compounds on average eliciting depolarizations of twice the response from the CTC12 strain as from the wild strain. However, after normalisation of the data, the overall trend in the responses of both strains to the different volatiles was remarkably similar. A two-way mixed ANOVA revealed a highly significant effect of the volatiles ($F_{1,66}$= 10.59, p< 0.001), and a significant effect of the interaction between strain and volatile ($F_{1,66}$= 1.362, p= 0.032). This indicates that the size of the antennal response changed depending on the volatile tested and that there were some differences between responses of the two strains to the same volatile. However, no significant effect of strain alone was found ($F_{1,66}$= 1.384, p= 0.259Post hoc paired-t tests with a false discovery rate applied were performed to identify volatiles that were significantly different from the hexane control. The relationship between the responses of the two strains is also shown by a bivariate plot of the normalised responses of the two strains to the different volatile compounds (Fig. 2), which revealed a strong correlation between the responses of both strains to the different volatiles (r = .809, n = 68, p < .001).
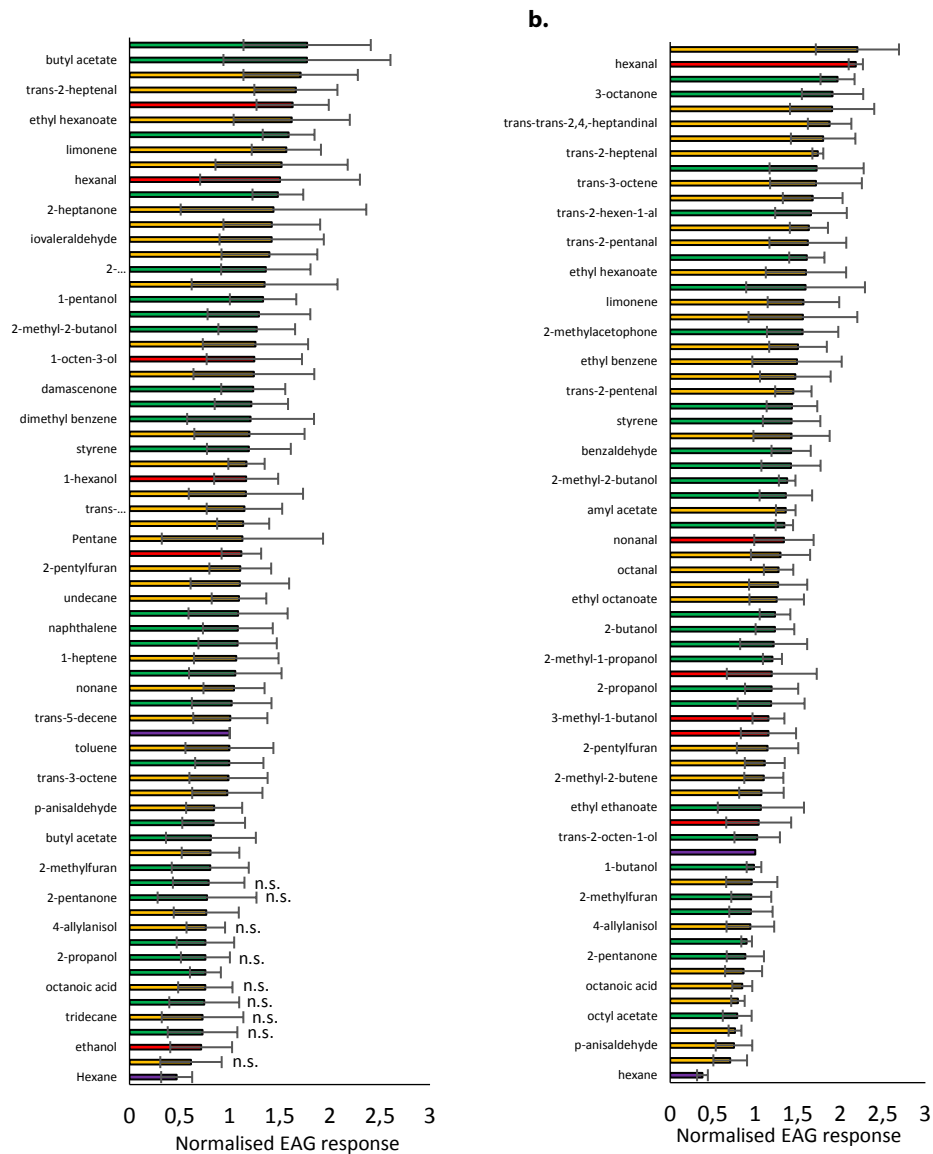
Figure 1 Average EAG responses of 8 CTC-12 strain (a) and 8 wild captured *Tribolium castaneum* (b), normalised against a DMD control, to 68 volatile organic compounds found in wheat germ oil or produced by grain associated fungi, plus the *Tribolium* aggregation pheromone, DMD as a positive control. Columns, arranged by descending EAG response, represent the average depolarization across eight individuals, and the error bars represent the standard deviations of the mean. Yellow bars represent volatile compounds found in wheat germ oil, green bars represent compounds identified as being products of grain associated fungi, red bars represent compounds we identified as being produced by both sources, and purple bars represent the two control compounds, DMD (positive control) and hexane (negative control). Compounds that did not elicit a significantly different EAG response compared to the hexane control are indicated with "n.s"; no compounds were found to be significantly different in the wild population beetles.
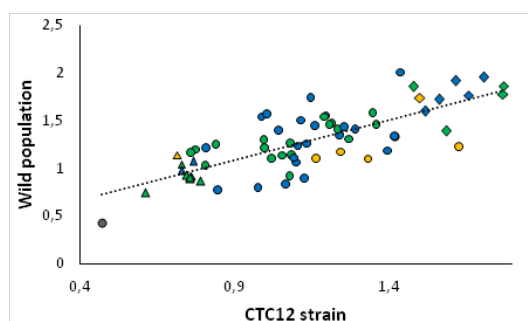
Figure 2 A bivariate plot showing the correlation between females of the CTC12 strain and females of the wild population strain in their average (n=8) normalised EAG responses to 68 different volatile organic compounds found in wheat germ oil or produced by grain associated fungi. Blue points represent volatile compounds found in wheat germ oil, green points represent compounds identified as being produced by grain associated fungi, yellow points represent compounds we identified as being produced by both sources, the grey point represents the control compound hexane. Pearson product-moment correlation indicated a strong positive correlation between the responses of the two strains to the different volatiles ($r = .809$, $n = 67$, $p < .001$). The ten compounds that elicited the largest average responses across both strains are indicated with diamond shaded points, and the ten compounds that ellicited the smallest average EAG responses are indicated with triangle shaped points. The responses to these indicated volatiles was also tested behaviourally using a y-tube olfactometer.
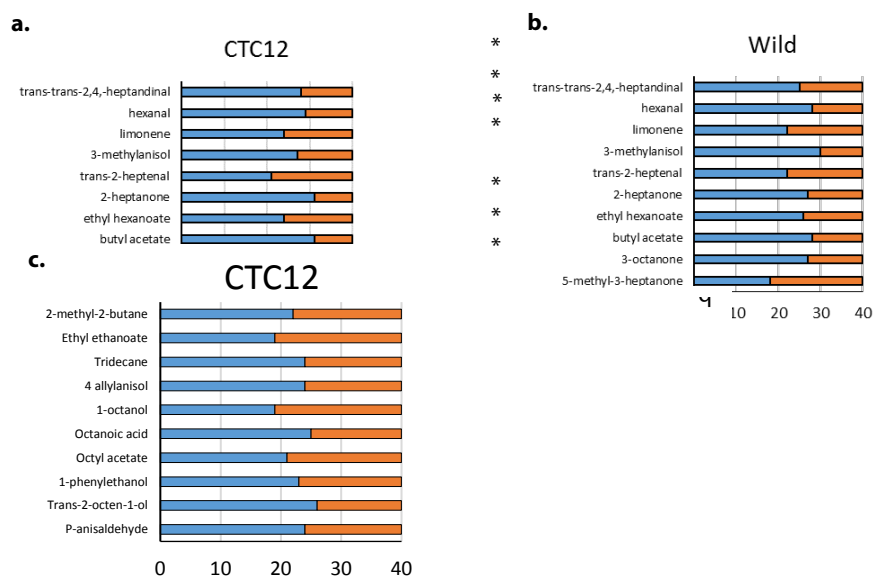
Figure 3 The attraction of female CTC-12 (a.) and wild population (b.) *T. castaneum* beetles to the ten volatile compounds that elicited the largest average EAG responses across both strains (see Fig. 2). The blue bars indicate the number of beetles (out of 40 individuals tested) that chose the Y-tube arm containing the test volatile, the orange bars indicate the number that chose the arm containing the hexane control solvent. Asterisks indicate a statistically significant bias for one arm of the Y-tube over the other (chi-square of goodness-of-fit <.05)

Y-tube olfactometer

Figure 3 shows the behavioural responses of female CTC12 (a) and wild (b) strain beetles to the ten compounds that elicited the highest EAG responses across both strains (indicated with diamond shaped points in Figure 2) and the responses of CTC12 strain females to the 10 compounds that elicited the smallest average EAG response across both strains (c) (indicated with diamond shaped points in Figure 2). The results show that, of the ten most attractive compounds, seven of the compounds elicited a statistically significant attractive response in the CTC12 strain (chi-square of goodness-of-fit p <.05), whereas five were shown to be significantly attractive to the wild population with all the compounds that were behaviourally attractive to the CTC12 strain also attractive to the wild population. None of the 10 compounds that elicited the smallest average EAG responses were found to be significantly attractive to the CTC12 strain.

Discussion

The results of our EAG and Y-tube olfactometer experiments give new insights into the physiological and behavioural responses of *T. castaneum* to common environmental volatile compounds that could have relevance to its future pest management. The results of the EAG experiments reveal that many of the compounds tested elicited large EAG responses relative to the DMD positive control, with around two-thirds of them eliciting larger responses than to DMD when used at the same concentration. This is encouraging given that insect aggregation pheromones (i.e. DMD) form the basis of many current lures. Strong antennal and behavioural responses were observed following exposure to a subset of volatiles found in wheat germ oil. This is perhaps unsurprising, given what was already known from the literature, and the current composition of insect lures. Interestingly, we also observed strong antennal and behavioural responses to volatiles from grain-associated fungi. *Tribolium castaneum* is known to have been associated with humans for at least 4,500 years, having been found sealed within Pharaonic urns in Egypt (Dawson, 1977). However, prior to the existence of anthropogenic food stores, *Tribolium* species must have fed on a different source of food. As many species in the same *Tenebrionidae* family as *T. castaneum* primarily feed on rotting tree bark, and other decaying plant matter, it is possible that this was the original food source of this species, before it switched to feeding on anthropogenic stored products. It has also been theorised that *T. castaneum* may have first adapted to feed on rotting grains stored in the burrows of rodents, and other sources of rotten grains, before switching to feed on mechanically processed grains stored by humans (Dawson, 1977). Therefore, *T. castaneum* may have co-opted an ancient ancestral attraction to fungal volatiles, derived from rotting plant matter, to find human stored grain products. Our findings lend some tentative support to this idea.

Although *T. castaneum* was attracted to volatiles from both wheat germ oil and grain associated fungi, there was no clear pattern between the responses to these two sources, with both groups of volatiles containing within them individual compounds that elicited very strong antennal responses, as well as volatiles that did not elicit strong responses. There was also no clear relationship between the type of compound and the size of the antennal depolarizations recorded. Some alcohols, ketones and compounds with methyl groups were found to elicit EAG depolarisations, while other compounds of the same chemical group did not. This agrees with a previous large-scale EAG screen which found no clear pattern between the size of depolarisations and the chemical class of the compounds tested in *T. castaneum* (Balakrishnan *et al.*, 2017). This suggests that this species is responding to very specific compounds associated with stored products, rather than to a broad range of chemically related compounds.

Before normalisation of the EAG responses, a striking difference was observed between the two *Tribolium* populations tested, with the depolarisations of the wild caught population typically being half the amplitude of the laboratory strain. However, after normalising the responses against a DMD positive control to correct for variation in antennal resistance over the course of taking recordings, the responses of both strains were found to be highly correlated. This demonstrates that the overall trend across the volatiles was the same in both strains, and could indicate that the composition of

odorant receptors is similar between both strains. However, to ensure we could record strong EAG responses, the concentration at which we tested these volatiles were much higher than would be encountered typically in a stored product environment, so it is possible that the responses to the two strains could still differ when they encounter these volatiles at more natural concentrations. It should also be noted that the results of the EAG on their own do not reveal how attractive these compounds are, only the degree to which they are detected at the insects' antenna. The amount of antennal depolarisation and the attractiveness of compounds are often not strongly correlated, and insects can have different responses when encountering blends of volatiles at different ratios (Bruce *et al.,* 2005). A previous study that examined behavioural differences between freshly caught and established laboratory populations of *T. castaneum* found little difference in their responses to traps baited with food and pheromone lures (Campbell, 2012). These results could suggest that fungal and wheat germ oil volatiles might elicit similar responses in *Tribolium* populations from diverse ecological backgrounds, which is important if these volatiles are to be used in a general-purpose lure and suggest that the results of previous behavioural studies conducted in lab strains should be applicable to wild populations, and vice versa. However behavioural experiments testing the responses to these compounds under more natural conditions would be needed to confirm this idea.

Owing to the very small response elicited to the hexane control volatile, all the volatiles tested in the wild population, and almost all the volatiles tested in the CTC12 strain, were found to elicit significantly different electrophysiological responses compared to this control. This is similar to the results of previous EAG experiments in *T. castaneum*, which found almost all of the compounds tested gave "a measurable EAG response" (Balakrishnan *et al.*, 2017). However, it was not clear from the study by Balakrishnan *et al*. (2017) what level of antennal depolarisation would predict a significant behavioural response from the beetles. When the ten volatiles that elicited the highest average EAG responses in the current study were tested for behavioural responses in the CTC12 strain, seven of them were found to be significantly attractive. In contrast, when the ten volatiles that elicited the smallest average EAG responses were tested, none of them were found to be significantly attractive. This would suggest that a certain threshold depolarisation must be reached before compounds become behaviourally attractive or that these compounds elicit a response that the Y-tube olfactometer does not measure, e.g. they are repellent or arrest the beetles by stimulation oviposition. However the results also show that even compounds that elicit relatively large EAG responses will not necessarily be attractive owing to complex relationship between odour perception and behavioural response in insects (Bruce *et al.*, 2005; Bruce and Pickett, 2011). It is possible that the same volatiles will be attractive when tested at different concentrations, or when tested together as a blend. Although attractiveness to most of the volatiles used in this study has not previously been demonstrated for *T. castaneum,* some of the volatiles have previously been used in research involving *Tribolium* species. For example, 3-octanone has been identified as a volatile that can be found in *Tribolium* infested flour, but is absent from clean flour (Abuelnnor *et al*., 2010), and this could explain the advantage of strong attraction of both strains to this volatile. In addition, hexanal has also been shown to be attractive to *T. confusum* when used in a blend with other plant volatiles (Wenda-piesik *et al*., 2016).

Taken together, the results of our EAG and behavioural experiments have revealed previously unidentified attractive compounds for *T. castaneum*, which have the potential to be used to improve the effectiveness of commercial *Tribolium* lures. There are also several wheat germ oil and fungal derived volatiles that elicited strong antennal depolarisations that have not yet been tested behaviourally, compounds that also have the potential to be highly attractive to *T. castaneum*. We are now exploring whether there are any synergistic effects of the attractive volatiles we have identified when they are encountered together. If fungal volatiles are indicators that grains are in a condition that *T. castaneum* can feed upon, it is likely that a stronger attractive response will be elicited when wheat germ oil and fungal volatiles are encountered together. This could be an important factor in adapting these volatiles for use as a *T. castaneum* lure. *Tribolium castaneum* has

been shown to be less attracted to lures when tested in an environment without a strong airflow (Campbell, 2012). We are therefore also doing behavioural experiments in environments closer to those encountered in a warehouse, which should provide better information about how attractive these compounds are to *T. castaneum* under real world conditions.

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The potential of host-specific volatiles from *Tribolium confusum* larval faeces for luring the ectoparasitoid *Holepyris sylvanidis*

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Abstract

The ectoparasitoid *Holepyris sylvanidis* (Bethylidae) attacks larvae of different stored product pest beetles. Previous studies on the olfactory host search of *H. sylvanidis* revealed that female parasitoids are strongly attracted to volatiles released from *Tribolium confusum* larval faeces, in particular to (*E*)-2-nonenal and 1-pentadecene. We suggested that these host-specific key compounds may serve the parasitoid as long-range attractants for host location. In this context, we propose that the attractive volatile blend could be used to establish a new approach within the biological control of stored product pests by guiding the parasitoid to its host and thus, increasing the host finding success. We investigated the potential of the identified host-indicating volatile cues to attract *H. sylvanidis* from a distance by offering the two key compounds to female parasitoids. Their walking behaviour and the covered distance were analysed on a Kramer sphere. Moreover, in semi-field trials both attractive volatiles were loaded onto rubber septa which were placed next to 4th instars of *T. confusum* at 1.5 m distance from the parasitoids. We studied the host finding success of *H. sylvanidis* by (i) measuring the mean time to locate and parasitise *T. confusum* larvae and (ii) counting the number of parasitised and unparasitised host larvae as well as the number of newly hatched parasitoids compared to the control without additional olfactory cues. First results showed that *H. sylvanids* females can locate the provided host larvae from a distance. Parasitistion of host larvae started four days after the release of parasitoids. No effect of the additionally offered host-specific key volatiles ((*E*)-2-nonenal and 1-pentadecene) on the parasitoid´s host finding success was observed at the given conditions and used amounts of compounds. Further studies are required to determine the right odour blend and concentrations for attraction of parasitoids over a distance and finally to show that the addition of host-derived kairomones may support the host finding success of *H. sylvanidis*.

Keywords: biological control, stored-product pests, semi-field trial, long-range attractants, Bethylidae.

Introduction

Over the last years, social concerns about the usage of synthetic pesticides for protection of food commodities or stored products against insect infestation have been increased considerably, mainly due to possible side-effects for humans (e.g. food contaminations, health risks for users) and environment (e.g. persistence of chemical residues) as well as well as the risk of developing resistance within pest populations (Field, 1992; Arias-Estevez et al., 2008). That in turn has led to an increased demand for alternative non-chemical pest management strategies (Phillips and Throne, 2010). Within the field of integrated stored product protection, the use of natural enemies (e.g. parasitoids) of stored-product pests as biological control method represents a promising and environmentally-friendly approach, but more research on the biology and behaviour of parasitoids is needed (Flinn and Schöller, 2012; Trematerra, 2012).

For instance, Adler et al. (2012) showed that the release of the larval ectoparasitoid *Holepyris sylvanidis* at a two-week interval was sufficient to control the local population of the confused-flour beetle *Tribolium confusum* in a grain mill. As a result, additional heat treatment, that had to be adopted in the past, was not necessary during the further experiment. Therefore, and

basend on previous studies and assumptions, *H. sylvanidis* is a potential candidate for the biological control of beetle larvae infesting stored products, in particular *T. confusum* and other *Tribolium* species (Evans, 1977; Fürstenau et al., 2016; Amante et al., 2017; Fürstenau and Hilker, 2017).

However, one important weak point in previous applications of parasitiods in general was the lack of specific traps and attractants which could help to monitor natural occurring beneficials as well as those additionally released. The development of suitable lures and traps for monitoring present populations of natural enemies requires profound and further research on the olfactory host finding process of the respective parasitoids with the aim to identify behavioural active, host-associated compounds for their application (Philipp and Thrones, 2010; Trematerra, 2012).

Previous studies on the odour-mediated host foraging behaviour of *H. sylvanidis* revealed that parasitoid females use volatiles released from larval faeces of *T. confusum* to locate its hosts. Two compounds of the faecal odour, (*E*)-2-nonenal and 1-pentadecene, were highly attractive to the parasitoid, particularly, in the presence of odour from host's feeding substrate, (i.a. wheat grist) (Fürstenau et al., 2016). Since the corresponding bioassays of this study were performed in a static 4-field olfactometer it still needs to be confirmed whether these two host-specific compounds ((*E*)-2-nonenal and 1-pentadecene) may act as long-range attractants for host location from a distance and whether these volatiles are possible candidates to lure and monitor *H. sylvanidis* individuals in the field. Therefore, the present study aimed to investigate (long-range) attraction effects of a two-component mix, consting of the specifically-host associated key compounds, on the host finding success and the efficiency of the parasitoid to locate *T. confusum* larvae in a semi-field trial from a distance.

Materials and Methods

Insects

Test insects (female *H. sylvanidis* and 4th instar host larvae of *T. confusum*) were taken from a permanent rearing at the JKI (Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany) as described previously (Fürstenau et al., 2016; Fürstenau and Hilker, 2017). According to Brindley (1930) and Sokoloff (1974), we defined 25-to-30-days-old *T. confusum* larvae (3-4 mm long) as 4th instars under our rearing conditions (25±1°C and 65±5 % relative humidity).

Preparation of the host-specific two-component mix (2CM)

The two-component mix, hereafter abbreviated to 2CM, was prepared by adding 2 mg (*E*)-2-nonenal (97%, purchased by Sigma Aldrich) and 1 mg 1-pentadecene (95%, purchased by TCI Europe) to 10 µl *n*-hexane (98%, purchased by Merck). The resulting solution was stored at -20°C until its use in subsequent bioassays.

Semi-field trial to evaluate the host finding success of H. sylvanidis from a distance

To investigate possible effects of two specifically host-associated compounds identified from *T. confusum* larval faeces on the foraging behaviour of *H. sylvanidis* females a semi-field trial was conducted. We evaluated the potential of 2CM to attract *H. sylvanidis* females over a 1.5 m-long distance and to improve the host finding success by guiding the parasitoid to its host. Experiments were performed in specifically manufactured boxes (0.75 x 2.0 x 1.0 m), consisting of a metal frame. Head and foot end as well as three side panels were made from gauze or cotton fabrics; four doors were embedded in the front side of the box. Each box was installed on a wooden panel. At the head end of the box we put a Petri-dish (Ø 5.5 cm) with fifty 4th instar host larvae of *T. confusum* on a plastic tray (23.5 x 30 cm) filled with wheat grist. Above the Petri-dish we put one odour dispenser which had been loaded with either 10 µl 2CM (treatment) or 10 µl of *n*-hexane (control) and evaporated for 24 h. As *H. sylvanidis* females generally transferres host larvae to hiding places (Ahmed et al., 1997) loose pipette tips were randomly put in each corner of one tray. Two boxes

for test and control trials each were placed in two separate rooms to avoid biased results due to interferences between test and control treatments. During the semi-field trial, room temperature and relative humidity depended on the outside conditions and were recorded by a datalogger; the average room temperature and relative humidity were 22 ± 1°C and 33 ± 7%, respectively.

At the beginning of the two-weeks-lasting experiment, twenty 1-to-8-days-old, mated parasitoid females were released at the opposite side of the box, 1.5 m away from the Petri-dish with host larvae. On day 1, 4 and 6 after the release of parasitoids we checked whether we could find paralysed and/or parasitised *T. confusum* larvae in the pipette tips and outside the tray. Pipette tips having paralysed and/or parasitised host larvae were replaced by new ones; all parasitised larvae were transferred to the climate chamber. After seven days the tray filled with wheat grist, the Petri-dish with *T. confusum* larvae and the pipette tips were renewed and the number of remaining *T. confusum* larvae found inside the Petri-dish and the wheat grist were counted. Twenty new parasitoid females were released in each box. As described above for 1st week, the number of *T. confusum* larvae outside the tray and the proportion of parasitised and unparasitised host larvae were measured. The semi-field trial was stopped after 13 days; each trial was repeated twice.

Since *H. sylvanidis* females drag the paraslysed host larvae to hiding places for parasitation we defined as a successful host finding event when *T. confusum* larvae were found in the pipette tips or outside the tray. In addition to the mean number of successful host finding events, we also calculated the parasitation rate and the ratio of hatched parasitoid (females and males) compared to the control. Data were statistically analysed by a Welch Two Sample *t*-test for the rat of successful host finding events and a Wilcoxon rank sum test with continuity correction for the parasitation rate. All analyses were done using the statistical programm "R" version 3.4.1 (R Core Team, 2017) with packages "car" (Fox and Weisberg, 2011) and "pastecs" (Grosjean and Ibanes, 2014).

Results and Discussion

In the present semi-field experiments a mix of two highly attractive, host-associated odours, (*E*)-2-nonenal and 1-pentadecene, identified from the volatile blend collected from *T. confusum* larval faeces was offered to *H. sylvanidis* test females in combination with host larvae to test the influence of these additional odours on the parasitoid's host finding behaviour. We measured the rate of successful host finding events by counting the number of *T. confusum* larvae found in hiding places (pipette tips) and outside the tray 1, 4 and 6 days after the release of parasitoids. In both treatments the number of *T. confusum* larvae which had been displaced by *H. sylvanidis* increased with the duration of experiment (number of experimental days; Tab. 1). On day 1 after start of the experiment, all host larvae were still in their respective Petri-dishes in test and control treatment. Six days after releasing the parasitoids, 4.88 (±1.63) and 1.25 (±0.75) displaced host larvae were counted in the control and the 2CM-treatment, respectively. Overall, *H. sylvanidis* could locate and displace ca. 25% of fifty host larvae offered to parasitoids in the control-treatment; the rate of successful host finding events was twofold higher than in the 2CM-treatment (ca. 13%) but did not differ significant (Tab. 1). In both treatments the number of parasitised host larvae was lower compared to the number of displaced host larvae. In the control treatment ca. 8% of the fifty offered host larvae were parasitised whereas the parasitation rate was fourfold lower in the 2CM-treatment (ca. 2%). When calculating the parasitation rate we excluded all larvae which were not found at the end of the experiment. Regarding the high number of not recovered host larvae (4.25±2.04 larvae in the control and 4.38±1.97 larvae in test treatment, Tab.1) one could assume that the actual parasitation rate might be higher in both treatments.

The host finding ability under storage-like conditions has been examined previously for a few parasitoid species (Steidle and Schöller, 2002; Adler et al. 2012; Niedermayer et al. 2016). For example, Niedermayer et al. (2016) showed that the two larval parasitoids *Lariophagus distinguendus* and *Anisopteromalus calandrae* could locate approximately 20% of wheat kernels infested by the granary weevil *Sitophilus granarius* when they were released at 1 m distance. In comparison, the rate of successful host finding events of *H. sylvanidis* was higher in the

control treatment (ca. 25%). Regarding environmental conditions, the field trial described before in comparison to our semi-field experiments differed considerably. The test parasitoids of *L. distenguendus* and *A. calandrae* were released into an open environment (two buildings of 150 m² and 45 m²) where the host location process might be influenced by several local predominat conditions such as air flow, light incidenc, variable temperature and humidity (Niedermayer et al., 2016). In contrast, we conducted our experiments in a constant dark and closed environment as we used 2 m long boxes placed in two separated rooms. The host location and ability of *H. sylvanidis* under storage-like conditions is not known yet and needs to be tested in further studies. However, Adler et al. (2012) already demonstrated that a mass release of laboratory reared *H. sylvanidis* is sufficient to temporally suppress the growth of a natural occurring *T. confusum* population in a grain mill. This result indicates that the rate of successful host finding events might be the same or even higher than what we measured in the here presented semi-field trial.

Tab. 1: Effect of additionally deployed host-specific volatiles on the host finding success of Holepyris sylvanidis in semi-field experiments (N=2).

	N° of displaced *T. confusum* larvae (mean ± SE [a])			N° of not found *T. confusum* larvae [b] (mean ± SE [a])	Rate (%) of host finding events (mean ± SE [a])	p-value [c]	Parasitation rate (%) (mean ± SE [a])	p-value [c]
	Days after parasitoid release							
Treatment	1	4	6					
Control (hexane)	-	3.38 ±1.53	4.88 ±1.63	4.25 ±2.04	25.00 ±5.96	0.126	8.50 ±2.35	0.054
2CM [d]	-	0.88 ±0.40	1.25 ±0.75	4.38 ±1.97	13.00 ±4.26		4.00 ±1.00	

[a] SE = Standard error of the mean.
[b] Number of *T. confusum* larvae which were not found in Petri-dishes, wheat grist, pipette tips or outside the tray during and at the end of the experiment
[c] To compare the different treatments a Welch Two Sample *t*-test for the host finding success events and a Wilcoxon rank sum test with continuity correction for parasitation rate were applied.
[d] 2-component mix of specifically host-associated compounds ((E)-2-nonenal: 2 mg, 1-pentadecene: 1 mg)

Initially our suggestion was that the addition of host specific volatiles (2CM) loaded onto dispensers may facilitate the host search of *H. sylvanidis* by attracting and guiding the parastioid to its host and thus, increasing the host finding success. Surprisingly, the rate of successful host finding events (13%) and the parasitation rate (2%) were both lower in the test treatment, offering 2CM, compared to the control (rate of successful host finding events = 25%; parasitation rate = 8%; Tab. 1). A possible explanation for the different performance when using 2CM might be that concentrations of both compounds ((E)-2-nonenal = 2 mg and 1-pentadecene = 1 mg) used here were (much) too high and therefore possibly reppelled the parasitoids instead of attracting them. Regarding this, in preliminary studies on a Kramer sphere, however, we observed noticeable differences in the walking behaviour of *H. sylvanidis* individuals when 2CM was tested compared to the control without offering volatiles. Usually, two characteristics of the walking behaviour of insects on the Kramer sphere is a higher walking speed and therefore, a longer track length when the test individuals are strongly attracted to an odour source as Thiery and Visser (1986) have shown for the Colorado potato beetle *Leptinotarsa decemlineata* and its preferred host plant, *Solanum tuberosum*. In contrast, *H. sylvanidis* females walked slower and covered a smaller distance in presence of 2CM compared to the control treatment. Additionally, *H. sylvanidis* frequently turned back while walking on the Kramer sphere or rested more time in the test treatment (personal observations). The reverse movements of *H. sylvanidis* in presence of 2CM probably indicate that parasitoid female re-examined the area for a potential host. A similar behaviour was observed when females of the larval parasitoid *Tiphia vernalis* could not find immediately a host at the end of a trail of their preferred host, the Japanese beetle *Popillia japonica*. Parasitoid females stayed nearby the trail's end and searched the area to locate potential hosts in the soil (Rogers and Potter, 2002). Therefore, we can not exclude that the application of two highly attractive, host-indicating compounds (2CM) may influence the host finding behaviour of *H. sylvanidis* by attracting or repelling the parasitoid. Further

studies on dispenser emission of 2CM are required to identify the correct blend (concentration, ratio etc.) and finally to show that these host-specific compounds can support the host finding success of *H. sylvanidis*.

Acknowledgement

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(Z, E)-9, 12-Tetradecadienyl Acetate (ZETA) disrupts mating of *Ephestia cautella*

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Abstract

The tropical warehouse moth *Ephestia cautella* is a major pest of stored products in Sri Lanka, and difficult to control using currently-available insecticides. The sex pheromone (*Z, E*)-9, 12- tetradecadienyl acetate (ZETA) emitted by the females attracts males of this species. Hence it can potentially be used in the management programs but the limited information on pheromone concentration and air movement impede the potential use of this pheromone in pest management programs. This experiment was conducted to determine the effects of ZETA concentration and air movement on the mating disruption of *E. cautella*. The male and female moths of *E. cautella* were introduced into a cubicle in which ZETA was placed at different concentrations. Later, the female moths were dissected to determine the presence/absence of spermatophore. All the pheromone concentrations tested recorded lower mating percentages than the hexane control. Mating disruption varied with the pheromone concentration and the availability of air flow. This study reveals that ZETA can be used to disrupt mating in *E. cautella*.

Keywords: *Ephestia cautella*, Mating disruption, Spermatophore, Concentration, ZETA

1. Introduction

The Tropical warehouse moth *Ephestia cautella* (Lepidoptera: Pyralidae) is a major pest of stored products (Hill, 1990) and reduce the quality of food commodities (Boshra, 2007). The current control measures by synthetic chemicals or extreme temperature exposure accompany disadvantages/limitations. Therefore, the grain-handling personnel seek for alternatives. The female moth releases the sex pheromone (Z, E)-9, 12-tetradecadienyl acetate (ZETA) to attract the males for mating (Kuwahara *et al.*, 1971). This is a promising pest management tool through mating disruption (MD) (Trematerra *et al.,* 2011) but certain information on the effective concentration and air movement on MD of *E. cautella* is not yet available. The objectives of this study were to evaluate the effect of pheromone (ZETA) concentration and the presence/absence of air movement on MD of *E. cautella*.

2. Materials and methods

Ephestia cautella adults were reared under ambient environmental conditions (30±2°C and 60±5% relative humidity), sexed at the pupal stage, and the adults emerged were used in the experiments. A cubicle (2.5 m×2.5 m× 2.5 m) having two opposite sides and the top covered by polythene, and the remaining two opposite sides covered by insect proof net was used to test the mating disruption. Each of the four pheromone concentrations prepared using commercially-available ZETA, diluted in hexane, was placed at the middle of the cubicle separately. Equal number of male and female adults of *E. cautella* was released into the cubicle at each concentration. The insects were recaptured after 24 hours and the female moths dissected to determine the mating status (Ryne *et al.*, 2001). The control experiments were conducted by using hexane solution. The highest mating disruption with respect to the pheromone concentration or the air flow was determined.

3. Results

Tab. 1 Mating disruption of *Ephestia cautella* at different ZETA concentrations (absence of air flow).

ZETA concentration (mg)	Mating disruption (%)*

0.05	25b
0.1	25b
1.0	37.5b
4.5	50a

*mating disruption (%) followed by the same letter are not significantly different at p=0.05 according to contrast option in binary logistic regression.

Tab. 2 Mating disruption of *Ephestia cautella* at different ZETA concentrations (presence of air flow).

Pheromone concentration (mg)	Mating disruption (%)*
0.05	37.5c
0.1	37.5c
1.0	62.5b
4.5	75a

*mating disruption (%) followed by the same letter are not significantly different at p=0.05 according to contrast option in binary logistic regression.

4. Discussion

This study reveals that MD of *E. cautella* increases with the increase in ZETA concentration and the presence of air flow. The higher MD with the presence of air flow compared to that without the air flow may be due to the increase in the dispersion of ZETA through the air.

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Suitability of Poaceae seeds for *Plodia interpunctella* development

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Abstract

One of the most important pests of stored grains is *Plodia interpunctella* (Hübner), whose larvae feed primarily on germinal part of the kernels, causing a reduction of seed germination and seed viability. This is detrimental for seeds of high category. However, seeds of different species within the same taxonomic family have different morphology (thickness of seed-coat, presence or absence of palea, palea loose or firmly attached to the seed etc.), which affects the susceptibility of seeds to *P. interpunctella* attack. The hypothesis was that seed hardness and the absence of palea could also significantly influence the life history of this pest. We assessed the suitability of different seeds from family Poacae (maize, wheat, barley, oats, ray, forage sorghum (variety), forage sorghum (hybrid), Sudan grass and millet) for *P. interpunctella* development and seeds susceptibility to pest attack (expressed in Susceptibility index –SI). The following parameters were monitored: larval mortality, adult emergence, mean developmental duration (from egg to adult) and female fecundity. Observations were carried out weekly, for 49 days. Data were statistically analyzed using Duncan's multiple range Test. The highest larval mortality, the lowest number of emerged moths and the lowest fecundity were recorded on millet, Sudan grass

and forage sorghum (variety and hybrid). However, the shortest larval development (27.8 days) and the highest fecundity (109.5-115.6 eggs) were on standard laboratory diet, maize and wheat. Morphometric measures of moths indicate that on unsuitable mediums like millet, Sudan grass, and different sorghum varieties the body lengths were statistically significantly shorter (0.5-0.6 cm) compared to other treatments (0.8-0.9 cm). According to the SI, the most susceptible were maize, wheat, barley, oats and ray, while moderately resistant were Sudan grass and millet. Testing kernel hardness and continuous improving of kernel resistance to storage insect pests could provide lower losses in stored grain quality and quantity.

Key words: *Plodia interpunctella*, Poaceae seeds, development, life history parameters

1. Introduction

Post-harvest losses and reduction of seed quality is one of the main restraints in achieving food security in developing and under developed countries (Rounet, 1992). During storage, the presence of insects is one of the major causes of deterioration of grain quality, reduction of grain weight, nutritional and market value. Indian meal moth, *Plodia interpunctella* (Hübner), is one of the most important polyphagous pests of grains, processed cereals and their products, oilseeds, nuts and manufactured products (Perez-Mendoza, 2003; Rees, 2004; Ozyardimci et al., 2006 Mohandasset al., 2007). It can be found on whole and/or damaged grains in storages, but since larvae feed mostly on germinal part of the seed and a bran layer (Almaši, 1984; Rees, 2004; Silhacek and Murphy, 2005), they lead to the reduction in seed germination and viability, which is detrimental for seed of high category.

Seeds of all cultivated Poaceae species (grains) are vulnerable to insect attack in warehouses because of usually prolonged period of storage. The growing importance of cereal (grain) production, primarily wheat, maize, barley and oats, lies in the fact that grains are the major carbon hydrate source in human and animal nutrition (FAO, 2011). Recently, there is also a growing interest in sorghum production because it has the potential to be used as bioenergy crop (Berti et al., 2013) and it is an attractive forage crop for many tropical and subtropical areas (Naeini et al., 2014). In 2013, sorghum was cultivated on over 300 thousand acres in Europe, while the world production of sorghum took place on the surface of over 42 mill acres (FaoStat, 2014) which also indicates at the growing importance of this crop.

The susceptibility of different grains (Poaceae seeds) to *P. interpunctella* attack and suitability for pest development depend on different characteristics of grains. In the first place it depends on the type of grain (hulless seeds like wheat, maize and rye, or seeds with palea like oats, barley, millet, Sudan grass), the type of palea (firmly attached to the seed – millet and Sudan grass, or loose palea -oats and barley) and the grain hardness (depends on grain density, structure of the grain, and the level of moisture). As a rule, grains without palea have higher density (Anonymous, 2017). Also, there can be a difference in seed characteristics between hybrids and varieties of the same species. For example, a variety of forage sorghum has palea firmly attached to the seed, while a hybrid of forage sorghum (crossing of male line of Sudan grass and female line of grain sorghum) does not have palea, since the female line is the grain sorghum.

This work aimed to assess susceptibility of different Poaceae seeds (wheat, maize, barley, oats, ray, millet, three genotypes of sorghum) for *P. interpunctella* attack and suitability for insect development.

2. Material and methods

2.1. Seed commodities

The experiment was carried out with seeds of nine different cultivated species from family Poaceae. Maize (*Zea mays* L.), wheat (*Triticum vulgare* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), ray (*Secale cereale* L.) and millet (*Pennisetum glaucum* L.) were used as nutrient medium for *P. interpunctella* development. Three varieties of sorghum (*Sorghum bicolor* Moench) were also used: a hybrid of forage sorghum, a variety of forage sorghum and Sudan grass. According to the agronomic classification based on different methods of sorghum cultivation and use, *S. bicolor*

species is divided into agronomic forms: grain sorghum, forage (sweet) sorghum, broomcorn and Sudan grass (Sikora and Berenji, 2011). Forage sorghum hybrids are obtained by crossing grain sorghum as the female parent and Sudan grass as the male parent (Pataki et al., 2010). This process of breeding provides seeds of sorghum without palea, which is the reason why it is more susceptible to insect attack. All seeds were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Republic of Serbia, vegetation season 2016.

The grains were not treated with insecticides and prior to the experiment and were exposed to deep freezing (-80 °C) in order to eliminate the presence of other pests and/or superficial harmful organisms.

2.2. Experimental design

P. intepunctella parental population originates from a laboratory population reared in plastic containers, at 28 ±1 °C, R.H. 60 ± 10% and 14:10 (L:D) photoperiod, on a standard laboratory diet (SLD) for *P. intepunctella* (Silhacek and Miller, 1972). 50 one-day-old eggs were placed on 100 g of grains into 0.25 L glass jars. Jars were sealed with a cotton swab for proper aeration. The experiment was set in 4 replicates and carried out at the same conditions as the rearing of parental population.

The following life history parameters of *P. intepunctella* were monitored: larval mortality, mean developmental duration (from egg to adult), adult emergence, adult lifespan and female fecundity. The observations were carried out weekly, until the last larva pupated. Once the emergence of adults began, assays were checked each 24 h and the number of the emerged moths was recorded. Newly emerged unmated moths from the same treatment were paired and each pair was isolated in a separate test tube until the oviposition. The fecundity was defined as the total number of eggs laid after the mating.

The susceptibility of different Poaceae seeds was assessed based on Susceptibility index (SI) described by Dobie (1974):

$$SI = \frac{(\ln(F_1))}{D} \, 100$$

ln – The natural logarithm of the mathematical constant *e*

F1 – average number of emerged moths per treatment

D – average developmental duration (egg to adult) in days

Seeds were rated as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) according to Mensah (1986) as follows:

$0 \leq SI \leq 2.5 \, (R)$

$2.6 \leq SI \leq 5 \, (MR)$

$5.1 \, SI \leq 7.5 \, (MS)$

$7.6 \leq SI \leq 10 \, (S)$

2.2. Statistical analysis

Data were statistically processed using Duncan's multiple range Test to analyse the differences between life history parameters on different grains, in software package SPSS 21 (P>0.05).

3. Results and discussion

3.1. Larval mortality

The highest mortality of *P. intepunctella* larvae was recorded on millet (34.9%) and Sudan grass (21.5%) and was significantly higher compared to other grains (0.9-15.3%). The lowest larval mortality was on SLD (0.7%), during the entire experiment (Tab. 1). The difference between treatments was statistically highly significant (F=300.66**, P<0.01). According to Subramanyam

(1995), the mortality of *P. intepunctella* larvae can reach 28% on yellow maize, while in this work it was significantly lower on maize (12.8%).

3.2. Mean development duration

The results on *P. intepunctella* developmental duration on nine different Poaceae seeds and SLD are presented in Tab. 1. The fastest development was on SLD (27.8 days), while the slowest was on millet (49.8 days) followed by forage sorghum hybrid, forage sorghum variety and Sudan grass (41.8, 42.0 and 42.0 days, respectively). The differences between developmental duration were statistically highly significant (F=96.14**, P<0.01). As reported by Williams (1964), duration of *P. intepunctella* development could range from 36 to 327 days. Developmental rate depends on maize variety (Abdel-Rahman et al., 1968) and also kernel damage. Mbata (1990) reported that the shortest development from egg to adult on the whole maize kernels was 31.2 days (tested on 13 varieties) and the slowest was 37.0 days, which was similar to the results of this work. Also, developmental dynamics of *P. intepunctella* depends on nutritive quality and mechanical state of food (Locatelli and Limonta, 1998; Vukajlović and Pešić, 2012; Kočović, 2014). Many researchers reported that life-cycle of *P. interpunctella* ranges from 27 to 52 days depending on factors such as temperature, food odor, presence of oil in diet, type of food, size and physiological state of females, availability of drinking water, food source and temperature (Allotey and Goswami 1990; Johnson et al., 1992; Nansen and Phillips, 2003; Mohandass, 2006). However, diet is the most important factor for determining the developmental period of the insects (Mbata 1985; Johnson *et al.,* 1992; Nansen and Phillips, 2003; Silhacek, et al., 2003; Silhacek and Murphy, 2005) and according to Vukajlović et al. (2017), when reared on whole wheat and rye kernels, this moth successfully completes life-cycle.

3.3. Adult emergence

The highest number of emerged adults was recorded on SLD (36.0), wheat (30.0) and maize (28.0), with statistically significant difference between treatments (F=172.12, p<0.01). The lowest number of emerged adults was on millet and Sudan grass (6.2, and 7.5, respectively). Other seeds were also less suitable for larval development, based on the number of emerged moths (10.5-18.5 moths). Essien (2006) reported that emergence of adult insect can be enhanced by the diet chemical composition, which was proven in this work, based on other life history parameters.

3.4. Female fecundity

The highest female fecundity (115.6 eggs) was recorded for females reared on SLD (Tab. 1). Between different grains, females reared on maize and wheat laid significantly higher number of eggs (110.2, and 109.5 eggs, respectively) which was at the same level of significance with the number on SLD. Females reared on millet laid the lowest number of eggs (16.2 eggs). The difference in fecundity among females reared on different grains and SLD was statistically highly significant (F= 432.43**, P<0.01). The food source is an important factor for determining fecundity of moths which can be influenced by different diets (Mohandass et al., 2007; Fathipour and Naseri, 2011; Madboni and Pour Abad, 2012), thus the low fecundity indicates at relatively poor nutrient medium (Arbogast, 2007). Values of *P. interpunctella* fecundity reported in the literature vary widely. Allotey and Goswami (1990) reported fecundity of 96.8 eggs per female on wheat and 174.2 eggs for moths reared on split maize kernels. According to Onaolapo (2017), the fecundity on formulated diet can reach 161 eggs, while Almaši (1984) reports only 26 laid eggs on whole wheat grains. In the research of Mbata (1990), among 13 tested Nigerian maize hybrids, some were more some less attractive for oviposition, regardless on the type of maize. This indicated at the presence of certain ques, i.e. ovipositional attractants that were not related to the type of maize. Babić et al. (2013) emphasized that dent type of maize kernels are the softest kernel type since it contains higher percentage of floury endosperm. Thus, we can speculate that the consumption of dent kernels is easier and the lower energy is needed for breaking the kernel pericarp, which might lead to higher mean fecundity.

3.5. Moth lifespan

The lifespan of moths differed depending on the grains, i.e. Poaceae species. The longest lifespan was recorded for moths reared on forage sorghum hybrid (9.5), millet (9.0), forage sorghum variety (8.5), barley (8.5) and Sudan grass (8.0), which was significantly longer compared to SLD and wheat - 6.0 days (F=6.11*, P>0.01). Subramanyam (1995) reported that the longevity of adult stage depends primarily on the environmental factors (temperature and humidity), occurrence of mating, opportunity for oviposition and the presence or absence of water for consumption.

3.6. Moth body lengths

Moths reared on SLD had the longest body sizes, 0.9 cm on average (Tab. 1). The smallest average body lengths were measured for moths reared on millet, Sudan grass, forage sorghum variety and hybrid (0.5-0.6 cm). The difference between body lengths of moths reared on different Poaceae seeds were statistically highly significant (F=66.32**, P<0.01). Akinneye (2009) reports that adult moths reared on the formulated diet produce the highest body lengths, which is in accordance with the results of this work since the longest body sizes were on SLD.

Tab. 1 *Plodia intepunctella* life history parameters on different Poaceae seeds and SLD

Commodity	Mortality of larvae (%)	MDD (days)	Adult emergence	Fecundity	Moth lifespan	Body lengths
Maize	12.8 ±1.25cd	28.3 ±0.58 c	28.0 ±2.08 b	110.2 ±2.64 a	6.5 ±0.56 c	0.8 ±0.08 ab
Wheat	2.9 ±0.85 e	32.0 ±0.50 c	30.0 ±1.82 b	109.5 ±0.96 a	6.0 ±0.81 c	0.8 ±0.02 ab
Barley	10.3 ±0.96 d	34.7 ±0.81 c	18.5 ±1.29 c	58.2 ±3.55 b	8.5 ±1.29 ab	0.7 ±0.09 ab
Oats	11.3 ±1.71 d	32.8 ±0.52 c	16.3 ±0.50 c	44.2 ±1.71 d	7.5 ±0.58 b	0.6 ±0.05 b
Ray	0.9 ±0.30 f	32.0 ±1.71 c	10.5 ±1.29 d	51.8 ±2.62 c	7.0 ±0.96 bc	0.6 ±0.05 b
Forage sorghum (hybrid)	13.0 ±1.29 c	41.8 ±1.25 b	14.0 ±1.71 cd	22.8 ±1.63 e	9.5 ±0.58 a	0.6 ±0.05 c
Forage sorghum (variety)	15.3 ±1.50 c	42.0 ±0.81 b	11.8 ±0.95 d	24.5 ±1.71 e	8.0 ±0.00 b	0.5 ±0.13 c
Sudan grass	21.5 ±1.29 b	42.0 ±1.00 b	7.5 ±0.96 e	27.5 ±1.71 e	8.0 ±0.81 b	0.5 ±0.09 c
Millet	34.9 ±1.03 a	49.8 ±1.29 a	6.2 ±1.26 e	16.2 ±1.55 f	9.0 ±0.00 a	0.5 ±0.21 c
SLD	0.7 ±0.37 f	27.8 ±1.29 c	36.0 ±1.00 a	115.6 ±3.40 a	6.0 ±0.58 c	0.9 ±0.08 a
F value	300.66**	96.14**	172.12**	432.43**	6.11**	66.32**

Mean values ±SD, Values with the same letter in the column are on the same level of significance, ** - P<0.01, * - P<0.05, ns - P>0.05

3.7. Susceptibility of Poaceae seeds to *P. intepunctella* development

The calculated SI (Tab. 2) indicates that maize and wheat kernels (SI=11.90, and 10.62, respectively) were the most susceptible to *P. intepunctella* attack (S), while the least suitable were millet and Sudan grass (SI=3.65, and 4.95, respectively), that were rated as moderately resistant (MR).

Grain resistance to environmental factors is influenced by the characteristics of the seed coat that covers its entire surface. The seed coat consists of extinct woody cells with thickening walls, made of cellulose, hemicellulose, lignin and other materials that provide high strength, and also additional resistance towards insect pests (Anonymous, 2017).

According to Weipert (1996) wheat kernels are much preferable to insect pests than rye, primarily because rye kernels are much harder and this is in accordance with the result of this work, where rye was less susceptible to *P. interpunctella* attack. Although, *P. intepunctella* larvae have strong mandibles, they do not easily break the pericarp of wheat and especially of rye kernels, so whole kernels are not the most suitable food for this moth (Locatelli and Limonta, 1998; Kočović, 2014), which is why *P. intepunctella* larvae are easily developed on crushed grain, rather than on whole ones (Lecato, 1976; Kočović, 2014). Barley seeds differ from the wheat and rye because palea is firmly attached to the seed coat and its share in the grain is 7-15% (Anonymous, 2017). This part of seed

may provide additional protection insect attack, so we can speculate that it is a reason for higher mortally of larvae. The outer layer of maize seed coat is more developed than in other cereals, but without palea. The total thickness of the maize seed coat (6 - 10% of the total grain weight) can be 0.5 mm, thus is easier to be damaged by insects, while for sorghum seeds, the seed coat thickness and the presence of palea depends on agronomic form.

Tab. 2 Susceptibility of different Poaceae seeds and SLD for development of IMM

Commodity	Susceptibility Index	Rating
Maize	11.90	S
Wheat	10.62	S
Barley	9.03	S
Oats	8.66	S
Ray	7.19	S
Forage sorghum (hybrid)	5.71	MS
Forage sorghum (variety)	6.28	MS
Sudan grass	4.95	MR
Millet	3.65	MR
SLD	12.80	S

R- resistant; MR – moderately resistant; MS – moderately susceptible, S - susceptible

Considering the above mentioned, it is obvious that aside from standard measures for control of storage insects, especially *P. interpunctella*, host plant resistance is one of the promising practices and more sustainable in integrated pest management, but also cheaper and ecologically safer (Abebe et al., 2009; Tefera et al., 2011).

Acknowledgement

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Population growth and development of *Liposcelis obscurus* Broadhead (Psocodea: Liposcelididae) at constant temperatures and relative humidities

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Abstract

The effects of nine temperatures (22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C) and four RHs (43, 55, 63, and 75%) on the population growth and development of the parthenogenetic *Liposcelis obscurus* Broadhead (Psocodea: Liposcelididae) were investigated in laboratory studies. Results showed that *L. obscurus* did not survive at 43% RH at all temperatures tested. At 55% RH, *L. obscurus* survived at 22.5, 25, and 27.5°C; none survived at 42.5°C and ≤63% RH. *Liposcelis obscurus* survived and the population increased 56–fold from an initial population of five adult females at 42.5°C and ≤75% RH. Population growth was highest at 40°C and 75% RH, where population increase was 215-fold. *Liposcelis obscurus* has three-to-five nymphal instars, and the percentages of third, fourth, and fifth instars were 52, 41, and 7%, respectively. Temperature-dependent developmental equations were developed for *L. obscurus* eggs, individual nymphal, combined nymphal, and combined immature stages. *Liposcelis obscurus* populations grew much faster at 30–42.5°C and 75% RH. These

data provide a better understanding of *L. obscurus* population dynamics, and can be used to develop effective management strategies for this psocid.

Keywords: psocid, stored-product, population growth, development rate, booklouse

1. Introduction

Psocids of the genus *Liposcelis* (Psocodea: Liposcelididae) have emerged as important pests of stored products worldwide over the last two-to-three decades (Nayak et al., 2014). Psocids are mostly found in grain food stores, food processing facilities, and they thrive on a variety of food products (Opit and Throne, 2008a; Athanassiou et al., 2010). Psocid infestations do not only cause grain weight loss but also result in significant germination failure by feeding on the germ and endosperm of seeds (Kučerová, 2002; Gautam et al., 2013). Psocids have a short generation time at elevated temperatures which allows them to rapidly colonize new habitats (Nayak et al., 2014). The economic importance of psocids in a commodity is not just limited to direct feeding and contamination but they can also lead to rejection of infested commodities from domestic and international markets (Nayak, 2006). Psocids are difficult to control using standard practices of protection and disinfestation (Wang et al., 1999; Beckett and Morton, 2003; Athanassiou et al., 2009; Huang et al., 2009).

In the US, *Liposcelis* and *Lepinotus* are two genera of psocids that are found in large numbers in grain storages and are of economic importance (Gautam, 2010; Opit et al., 2011). Four *Liposcelis* species of notable economic importance worldwide are *L. bostrychophila* Badonnel, *L. entomophila* (Enderlein), *L. decolor* (Pearman), and *L. paeta* Pearman, but examples of other species that are economically important include *L. corrodens* Heymons, *L. brunnea* Motschulsky, *L. obscurus* Broadhead, and *L. rufa* Broadhead (Lienhard and Smithers, 2002; Gautam et al., 2010).

The psocid species *L. obscurus* Broadhead has been found infesting storage structures in the US. *Liposcelis obscurus* is an obligate parthenogen (Mockford, 1993). The only ecological study conducted on *L. obscurus* published in scientific literature investigated the effects of temperature and food on the reproductive parameters of this species (Khalafalla, 1990). In the present study, objectives were to determine the effects of constant temperatures and relative humidities on the population growth of *L. obscurus* and to quantify the effects of temperature on the development of this species.

2. Materials and Methods

2.1. Insects

Cultures of *L. obscurus* used in this study were started using insects collected from peanut (*Arachis hypogaea*) warehouses in Oklahoma (USA). Voucher specimens of 100 *L. obscurus* preserved in 95% ethyl alcohol that were used in this study were deposited at the K.C. Emerson Entomology Museum at Oklahoma State University under lot numbers 119 (females). Psocids were reared on a mixture of 93% cracked wheat (*Triticum aestivum* L.) (Duster variety), 5% Rice Krispies (Kellogg North America Company, Battle Creek, MI), and 2% wheat germ (The Quaker Oats Company, Chicago, IL) (wt/wt) in 360-ml glass canning jars with mite-proof lids (Opit and Throne, 2008b). The top one-third of the inner surface of each jar was coated with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent psocids from accessing and gathering on the inside of the lid. Cultures were placed inside a growth chamber maintained at 30 ± 1°C in plastic boxes (42 x 29 x 24 cm high) painted black, which had saturated NaCl solution beneath perforated false floors to maintain a RH of 75 ± 5% RH. The boxes were painted black to mimic dark conditions in which psocids are typically found.

2.2. Effects of temperature and relative humidity on population growth

The effects of nine temperatures (22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C) and four RHs (43, 55, 63, and 75%) on the population growth of *L. obscurus* over a 30-d period were determined. The

inner sides of 108 Petri dishes (100 x 25-mm high) were coated with Fluon to prevent psocids from escaping. Into each Petri dish, 5 g of red colored diet, 1 g of cracked duster wheat, and 0.5 g wheat germ (hereafter referred to as diet) were placed. The mixture of red colored diet, cracked duster wheat, and wheat germ was used as diet because *L. obscurus* did not survive well on only cracked wheat diet. The plastic Petri dish lids were replaced. Red colored diet was made by mixing 100 g of Rice Krispies with a solution of 5 ml of red food dye (Global Chem Sources Inc., Cedar Grove, NJ) in 300 ml of water, drying the mixture in a mechanical convection oven (model HTM 85, Precision Scientific, Inc., Chicago, IL) for 6 h, and then grinding the dried mixture in a Wiley Mill. A U.S. Standard #20 sieve (0.85-mm openings) (Scientific Apparatus, Philadelphia, PA) was used to sieve the diet. Petri dishes with diet were randomly put in four plastic boxes (42 x 29 x 24 cm high) containing each of the saturated solutions of K_2CO_3 (43%), NaBr (55%), $NaNO_2$ (63%), and NaCl (75%) (Greenspan 1977) beneath perforated false floors to maintain the required RH. Petri dishes were kept at the four RHs to equilibrate the diet in them at room temperature for 4 wk. Each box had 27 Petri dishes.

To obtain 1- to 2-wk-old *L. obscurus* adult females required for the experiment, 300 female late-instar nymphs of *L. obscurus* were picked from culture jars and placed in six 9-cm Petri dishes with Fluon-coated sides. Each Petri dish had 5 g of colored psocid diet, 1 g of cracked duster wheat, and 0.5 g of wheat germ in it. The Petri dishes were placed on perforated false floors of one black Rubbermaid plastic box (32 x 18 x 13 cm). The late instar nymphs were maintained at 75 ± 5% RH for 2 wk.

After 4 wk of diet equilibration, five 1- to 2-wk-old adult *L. obscurus* were placed in each of the 108 Petri dishes containing equilibrated diet. Nine incubators (Thermo Fisher Scientific; Waltham, MA) were set at temperatures of 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C, where four plastic boxes (17 x 17 x 12 cm high) containing saturated solutions of K_2CO_3, NaBr, $NaNO_2$, and NaCl were placed. Three Petri dishes containing diet, equilibrated at room temperature and each RH, were randomly assigned to the corresponding RH box in all incubators. Methods by Gautam et al. (2010) and Aminatou et al. (2011) were then followed.

The experiment had three temporal replications, and the experimental design was a randomized complete block design (RCBD) with subsampling. Statistical procedures were done by using Statistical Analysis System software version 9.4 (SAS Institute, 2014). PROC MIXED was used for analysis of variance (ANOVA) to determine the effects of temperature and RH on the number of psocids in the Petri dishes. Data on psocid numbers were transformed using the square root transformation to stabilize variances before analysis. Untransformed means and standard errors are reported for straightforward interpretation. We used the least significant difference (LSD) test to determine differences among mean numbers of psocids produced at the various temperatures and RHs despite the quantitative independent variables, because we were not able to quantify the relationship using a biologically meaningful equation (TableCurve 3D) (Systat Software, Inc., 2002a).

2.3. Effects of temperature on development

Eggs were obtained by placing 1 g of red colored diet, 5 particles of wheat germ, and 30 adult female psocids of unknown age from our psocid cultures in each of eighty 35-mm-diameter Petri dishes (Greiner Bio-One, Kaysville, UT), which had a coat of Fluon on the sides. Procedures used to obtain the red colored diet were similar to those in Opit and Throne (2008). The Petri dishes were placed in two black Rubbermaid plastic boxes (30 x 23 x 9 cm high) that contained saturated NaCl solution (75% RH) beneath a perforated false floor. Boxes were placed in an incubator maintained at 40 ± 1°C. After 2 d, adult females were taken off, and the diet in each Petri dish was examined for eggs by using a dissecting microscope at 25x magnification. Procedures used for setting up the experiment to monitor development of eggs were analogous to those used by Opit and Throne (2018). Thirty centrifuge caps (associated with vial caps and Petri dishes) were randomly placed in each of nine Rubbermaid plastic boxes (37 x 22 x 13-cm high; 270 centrifuge caps total) that were painted black and contained saturated NaCl solution to maintain 75% RH. One box was placed in each of the nine incubators set to maintain treatment temperatures of 22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, 40.0, and 42.5°C. Temperatures above 42.5°C were not tested because preliminary

experiments had shown that *L. obscurus* eggs do not hatch at temperatures above 42.5°C. The experiment had two temporal replications. To estimate the incubation period of eggs and to mark insects after egg hatch to determine when one developmental stage ended and the next began, procedures analogous to those used by Opit and Throne (2018) were used.

2.4. Data analysis

In the determination of the effects of temperature on the duration of development of *L. obscurus,* PROC MIXED was used for analysis of variance (ANOVA). The experimental design for the analysis of the proportions of viable eggs and nymphs that developed to the adult stage was an RCBD. Regression (TableCurve 2D; Systat Software, 2002b) was used to describe the relationship between temperature and development time for the egg, individual nymphal, combined nymphal, and combined immature stages. Fitting curves with nonlinear regression showing the relationship between temperature and development time for the individual developmental stages were constructed using SigmaPlot version 10.0 (Systat Software, 2006). The selection of an equation used to describe the data was based on the magnitude and pattern of residuals, lack-of-fit tests, and whether the curve had a reasonable shape to describe the data. In the analysis of the proportions of viable eggs and nymphs that developed to the adult stage, the design for analysis was a RCBD. To analyze the proportions of viable eggs and nymphs, PROC MIXED was used for ANOVA after arcsine square-root transformation to stabilize variances.

The lower developmental threshold for *L. obscurus* was determined by fitting a linear equation to development rate (reciprocal of development time) and temperature data using TableCurve 2D (Systat Software Inc., 2002b). The upper developmental thresholds for *L. obscurus* developmental stages were found by determining the temperature at which the rate of development begins to decrease (Zilahi-Balogh and Pfeiffer, 1998). The upper developmental thresholds were obtained by fitting the appropriate equation to all the development rate and temperature data and by using the "EVALUATION" procedure in TableCurve 2D (Systat Software Inc., 2002b) to determine the upper developmental thresholds.

3. Results

3.1. Effects of temperature and relative humidity on population growth.

The nine temperatures and four RHs tested affected *L. obscurus* population growth (Fig. 1). No live *L. obscurus* were found at 43% RH for all temperatures; at 55% RH and 30–42.5C; and 63% RH at 42.5°C. Numbers of *L. obscurus* at 35 and 37.5°C and 75% RH were very similar—approximately a 143-fold increase in population, in 30 d, for each temperature. Population growth was highest at 40.°C and 75% RH, where population increase was 215-fold (Figure 1). At 42.5°C and 75% RH, *L. obscurus* populations increased 56–fold from an initial population of five adult females.
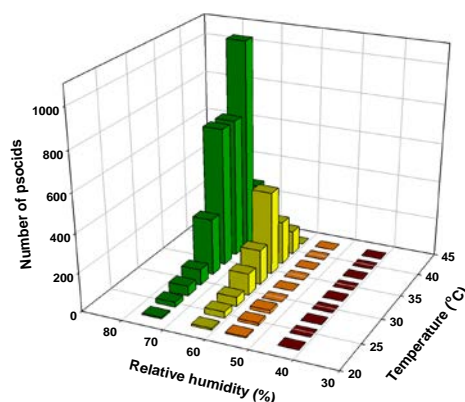
Fig. 1. Effects of temperature and relative humidity on *Liposcelis obscurus* population growth.

3.2. Effects of temperature on *L. obscurus* development.

3.2.1. Eggs

Incubation varied with temperature and the relationship between temperature and incubation time was well described by a quadratic equation (Fig. 2A). The optimal incubation temperature is 40.0°C, and development is completed in 4.1 d.

3.2.2. Nymphal and Combined Nymphal Stages

Duration of the nymphal and combined nymphal stages varied with temperature (Fig. 2B–E). Quadratic equations described the relationship between temperature and development time well for individual nymphal and combined nymphal stages. Temperature had a significant effect on development time for N1 (first instar), N2 (second instar), and N3 (third instar) (Fig. 2B–D); where development time decreased with increasing temperature. Based on analysis of data for all nymphs that developed to adults, combined nymphal development time averaged 28.6 d at 25°C and declined to 11.6 d at 40°C. However, developmental time increased slightly at 42.5°C and development is completed in 11.8 d, respectively. Based on the quadratic equation for the combined nymphal stages, the predicted optimal developmental temperature is 41.1°C and development is completed in 11.7 d.

3.2.3. Combined Immature Stages

The analysis of data for all individuals that developed to adults showed that temperature had a significant effect on total developmental time from egg to adult, and a quadratic equation fit the data well (Fig. 2F). Total developmental time from egg to adults averaged 42.7 d at 25°C and declined to 15.8 d at 40°C. However, developmental time increased slightly at 42.5°C and development is completed in 16 d. The upper developmental threshold was estimated as 43.9°C. The lower developmental threshold was estimated as 13.2°C using a linear equation that best described the development rate and temperature relationship. Based on this study, *L. obscurus* has three to five nymphal instars, and the percentages of third, fourth, and fifth instars were 52, 41, and 7%, respectively.

3.3. Effects of Temperature on Egg Viability and Nymphal Survivorship

Temperature affected egg viability ($F = 3.8$; df = 7, 7; $P = 0.049$), which ranged from 83% to 100% and averaged 91.5% for all temperatures. Temperature had no effect on nymphal survivorship ($F =$

1.0; df = 1, 1; $P = 0.50$). Proportions of nymphs surviving to adults at the nine different temperatures ranged from 65–73%.
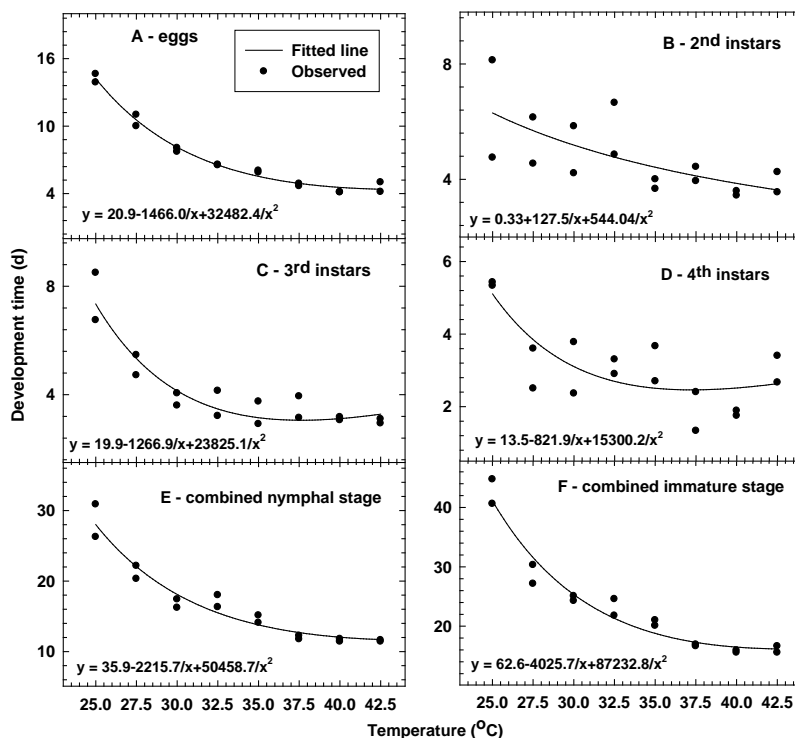
Fig. 2. Development of female *Liposcelis obscurus* at constant temperatures and 75% RH: (A) eggs, (B) second, (C) third, and (D) fourth instars, and (E) combined nymphal and (F) combined immature stages.

Discussion

Results from this study show that *L. obscurus* did not survive at 43% RH at any of the temperatures tested; at 55% RH and 30–42.5°C; and at 63% RH and 42.5°C. The optimal temperature and RH for population growth of is *L. obscurus* are 40°C and 75% RH. *Lepinotus reticulatus*, *L. brunnea*, *L. rufa*, *L. pearmani*, and *L. fusciceps* have also been reported not to survive at 43% RH (Opit and Throne, 2008b; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). Although *L. obscurus* survived and barely multiplied at 55% RH and 22.5–27.5°C over 30 d, data indicate it will not thrive at this low RH. At 63% RH, a low temperature of 22.5°C results in limited increase in population, and a higher temperature of 42.5°C kills all psocids. At 75% RH, a low temperature of 22.5°C results in limited increase in *L. obscurus* population. Rees and Walker (1990) observed that *L. bostrychophila, L. entomophila,* and *L. paeta* did not survive at low RHs (<60%). Knulle and Spadora (1969) stated that below the equilibrium RHs of psocids, death occurs. According to Devine (1982), high atmospheric water vapor of ≥60% RH is necessary for psocids to maintain body water levels by absorption; however, below this level, more moisture is lost than gained, which results in dehydration and death. At 30.0°C and 55% RH, *L. obscurus* did not survive, but *L. brunnea*, *L. rufa*, and *L. fusciceps* populations grew, although growth was slow (Opit and Throne, 2009; Gautam et al., 2010). *L. brunnea*, *L. rufa*, and *L. fusciceps* are probably well adapted in a manner that enables them to absorb atmospheric water vapor even when RH is as low as 55%.

The highest population growth for *L. obscurus* occurred at 40°C and 75% RH. RH of 75% has also been found to be optimal for the population growth of *L. reticulatus*, *L. rufa*, *L. pearmani*, and *L. fusciceps* but 63% RH was optimal for *L. brunnea*. Optimum temperatures for these species were 30C° for *L. fusciceps;* 32.5°C for *L. reticulatus*, *L. pearmani, and L. brunnea;* and 35°C for *L. rufa* (Opit and Throne, 2008b; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al. 2015). Optimal RH for *L. brunnea* (63%) explains why it mainly occurs in the relatively drier parts of US compared with other species (Gautam et al., 2010). However, its distribution may be limited by high temperatures of 35.0°C or higher (Opit and Throne 2009). Rees and Walker (1990) showed that the optimum conditions for *L. bostrychophila*, *L. entomophila,* and *L. paeta* are 30.0°C and 80% RH, 30°C and 70% RH, and 33°C and 70% RH, respectively. *L. rufa* barely survives at 40.0°C (Gautam et al., 2010). Therefore, higher temperatures may limit *L. rufa* distribution although it reproduces relatively well at lower RHs and temperatures (55% RH and 22.5–30.0°C) compared to *L. obscurus*. The optimum conditions for *L. obscurus* (40.0°C and 75% RH) imply that it is expected to have a broader distribution than *L. rufa,* and be more abundant in hot and humid areas. Based on this study, *L. obscurus* is capable of surviving and multiplying at moderately high rates at 42.5°C.

L. obscurus has three to five nymphal instars, and the percentages of third, fourth, and fifth instars were 52, 41, and 7%, respectively. *L. brunnea* females were also found to have three to five nymphal instars with a higher percentage having four nymphal instars (78%) compared with *L. obscurus* which has a higher percentage of insects with three nymphal instars. However, Khalafalla (1990), reports that the *L. obscurus* strains found in Egypt have exactly four instars. Opit and Throne (2008b), report that *L. reticulatus* (a parthenogenetic species) also has four nymphal instars. Males and females of bisexual *Liposcelis* species are found to have two to four and two to five nymphal instars, respectively (Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). Due to the additional number of instars female psocids have, the developmental period of females is longer than that of males. The evolution of a variable number of *Liposcelis* instars may be to prolong their survival in adverse conditions (Aminatou et al., 2011). According to Mockford (1993), psocids usually have four to six nymphal stages.

The optimal temperature for *L. obscurus* development from egg to adult was 40°C and development was completed in 15.8 d. The optimal temperature for development of female *L. badia*, *L. bostrychophila*, *L. reticulatus*, *L. pearmani*, and *L. tricolor* was 32.5°C and development were completed between 17 and 31 d (Wang et al., 2000; Dong et al., 2007; Jiang et al., 2008; Opit and Throne, 2008; Aminatou et al., 2011). For *L. brunnea*, *L. entomophila*, *L. decolor,* and *L. fusciceps*, the optimal temperature for development was 35.0°C and development was completed in 23.6, 21.7, 16.1, and 19.0 d, respectively; also, *L. paeta* and *L. rufa*'s development were completed in 11.5 and 21.6 d, respectively, at 37.5°C (Tang et al., 2008; Wang et al., 2008; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). At the optimal temperature of 40.0°C, development of *L. obscurus* from eggs to adult takes a slightly shorter time compared to other psocids that have been studied.

This study demonstrates how temperature and RH affect *L. obscurus* population growth and development. *L. obscurus* is not expected to be a serious pest in grain storages where temperatures are 27.5°C or less. Given that *L. obscurus* had a relatively higher population growth over a 30-d period compared to other *Liposcelis* species at higher temperatures of 35–42.5°C and 75% RH, we expect it to be (or become) a predominant pest in hot and humid areas. Finally, the temperature dependent equations developed for this species could be used to understand *L. obscurus* population dynamics and to develop effective management strategies.

4. Conclusions

Based on this study, *L. obscurus* is predicted to be more abundant and a pest in hot and humid areas of the world. That being said, to the best of our knowledge *L. obscurus* has only been reported twice — it was found infesting a peanut warehouse in Oklahoma, USA and in stored rice in Egypt. Possible

reasons for why *L. obscurus* has not been frequently reported may be due to lack of research or misidentification of this species.

Acknowledgements

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Circadian Rhythm of *Liposcelis entomophila* and *Liposcelis paeta* in Paddy Warehouse

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Abstract

Booklice is a small but serious stored grain pest, and understanding the circadian rhythm of booklice help to control. In this study, circadian activity of booklice were monitored with sticky traps in the grain bulk surfaces of two warehouses stored paddy rice in two different provinces in China. The results showed that the species of booklice were different and were *Liposcelis entomophila*, and *Liposcelisp paeta* for Nanning's and Zhanjiang's warehouses respectively. In term of *L.entomophila*, its activity intensity gradually decreased from 0 am to 12 pm and reached the lowest level of daily activity at 12pm. After this, there was a steady and straight upward trend, and the peak of its activity intensity is reached at 8 pm. Its circadian activity trend can be represented as: $y = -0.971x^3 + 21.88x^2 - 139.5x + 353.4$(x: time; y: quantity of booklice). Over the same period, the activity intensity of *L.paeta* varied greatly. It gradually increased, reached a peak at 8 am, dropped dramatically at 12 pm and then climbed the second peak at 6 pm.

Keyword: sticky trap, monitor, *L.entomophila*, *L.paeta*, circadian rhythm

1. Introduction

In control of stored grain pests, insect population dynamics monitoring and density inspection are important. The species, density, distribution, and damage status data of grain stored pests in the grain bulk can be timely detected, predicting the development trend of insects, avoiding unnecessary prevention cost and the economic losses, and providing scientific strategy for insect control (Bai Xuguang, 2002).

At present, the pest detection technology is traditional screening method. There are many disadvantages, such as, labor intensive, low efficiency, and imprecise. Base on these, trapping detections are developed. They are convenient, fast, environmentally friendly, and highly automated（LI Zhishen, 2014）. Sticky board trapping is one of these technologies and has been widely used in the monitoring and control of agricultural and forestry pests. However, there are few reports in stored grain insect pests. In this study, the sticky board trapping technology was applied to monitor the population dynamics of the booklice in two paddy warehouses in Guangdong and Guangxi, southern China. The aim in work reported in this publication was to assess sticky board trapping as an alternative to detection.

2. Materials and Methods

2.1 Test Warehouse

No. 32 large warehouses in Nanning Shajing Grain Warehouse examined was a length of 26.69 m and a width of 19.73 m, storage ability 1250t. The stored grain is indica rice and has been stored since September 2013. The moisture content and impurity ratio of this grain is 12.8%, and 1%, respectively.

No.4 large warehouses （50 m ×20 m） in Guangdong Zhanjiang North Station Grain Warehouse was selected for trails. The indica rice in this warehouse had 10.5% grain moisture content and been stored since August 2013. .

Both warehouses had been fumigated with phosphine.

2.2 Application of sticky board trap

Sticky board traps (20 cm × 25 cm, Beijing Ikoman Bio-Technology Co., Ltd.) were applied different positions of the surface of indica rice in each of two warehouses, including corner, under the fan, window, and check door. The numbers applied were four. The position of the trap location in the real warehouse is shown in Fig. 1.
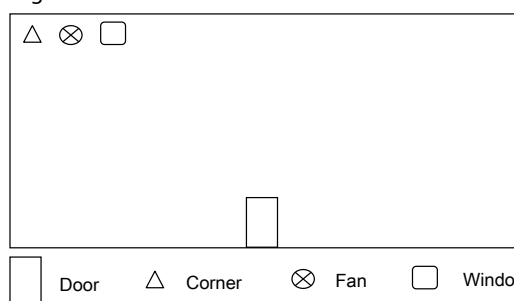
Fig. 1 sampling points in the warehouse

2.3 Observing the circadian rhythm of the booklice in warehouse

General procedures: the methods of assessment were based on procedures developed to measure booklice population on grain. The methods were:

- Counting of booklice in marked areas.
- Leaving sticky board traps for defined periods (2 hs), shaking out the booklices into trays and counting.
- Sampling grain with a bottom-opening probe, sieving (Φ 1.5 × 2.5 mm) and count insects.
- The whole test lasted for 24 hours.

The temperature and relative humidity of the pf the warehouses were monitored by Vaisala VAISALAHM34 High-precision Temperature and Humidity Table (VAISALA, Finland).

3. Results

3.1 Circadian rhythm of L.entomophila in paddy warehouse in Nanning Shajing Grain Warehouse

The species of booklice in Nanning Shajing Grain Warehouse was *L.entomophila*. Within 24 hours, the temperature ranged over time from 27.48 °C to 29.28°C and the humidity ranged from 68.95% to 73.05% (Fig. 2). The warehouse temperature showed an overall downward trend during the period from 12 am to 10 pm and a rise after 10 pm. During this period, the humidity showed an overall downward trend, except that it was an abnormally high point at 10 am. The trend continued until 2 pm. The quantity of booklice trapped generally decreased during the period from 12 am to 10 am, and reached a minimum at 12 pm. After 12 pm, the temperature in the warehouse experienced an increase, while this phenomenon appeared 4 hours later and 2 hours later in the humidity and the quantity of booklice trapped respectively. Therefore, the Circadian Rhythm can be inferred from the quantity of booklice attracted by the sticky trap at different time periods. The activity frequency of *L.entomophila* in the warehouse is correlated with the temperature and humidity in the warehouse. From early morning, the frequency of booklice gradually decreased, and booklice activities entered a relatively quiet period at 12 pm. After this, with the overall recovery of temperature and humidity, pest activity gradually became active again, and reached relative activity peak period from 6 pm to 2 am. The daily activity trend of *L.entomophila* can be expressed as $y = -0.971x^3 + 21.88x^2 - 139.5x + 353.4$ (x is the 24-hour time, and y is the quantity of booklice)
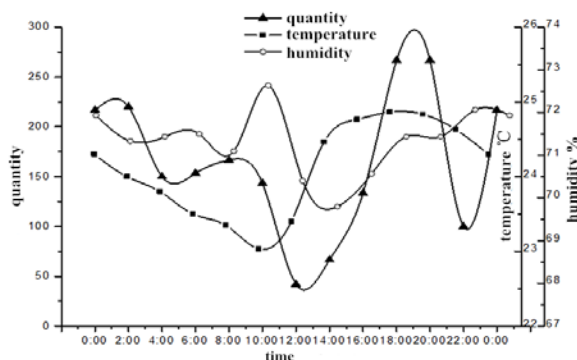
Fig. 2 Changes in Temperature, Humidity, and quantity of *L.entomophila* trapped with time in the warehouse of No. 32 Shajing Grain Depot

3.2 Circadian Rhythm of L.paetain Zhanjiang Warehouse

The species of booklice in Zhanjiang Warehouse is *L.paeta*. In Zhanjiang North Station State Grain Storage No. 4 warehouse, the temperature varied significantly with time, ranging from 27.30°C to 29.27°C. However, the humidity vary slightly with time, ranging from 74.40% to 79.27% and reached an unusually high point around 2 am. There was a certain correlation between the quantities of booklice and humidity after 10 am. The trend in Fig. 3 showed that the quantity of trapped *L.paeta* was relate to temperature. Interestingly, 12 pm was not the lowest temperature of the day, but the quantity of trapped *L.paeta* reached a minimum value as same as that of *L.entomophila* in Nanning Shajing. This might be caused by other factors expect temperature and humidity, such as insect daily activity rhythm. The peak activity of the *L.paeta* appeared at 2 pm.
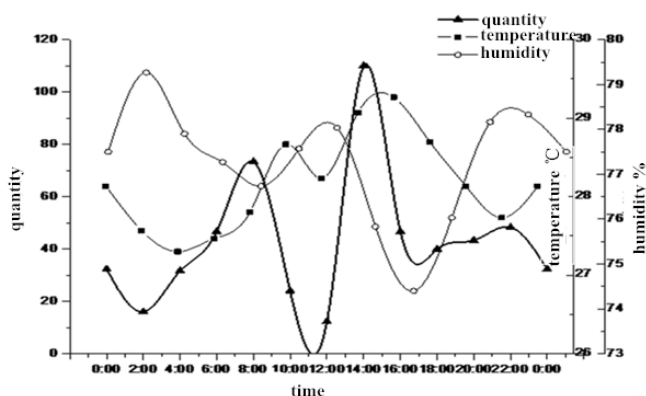
Fig. 3 Changes in temperature, humidity, and quantity of *L.paeta* trapped with time in Zhanjiang No. 4 warehouse

4. Discussion

In recent years, booklice has also became a new threat to global grain security (Zhang Shengfang, 1998), which is the problem that needs to be solved urgently (Muhammad Shoaibet al, 2010). The sticky trap in the study can be considered as a physical control method. It provides a new green and effective means for the prevention and treatment of booklice.

Since both the *L.entomophila* and *L. paeta* have obvious light-shielding properties (Yan Xiaoping et al, 2008), the principle of sticky trap remains to be further studied. It may be related to the fact that the *L.entomophila* prefers high humidity environment because of the stickiness. The glue on the glue sheet causes an increase in humidity.

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Development of a suitable rearing media for *Tribolium castaneum*

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Abstract

Tribolium castaneum is a serious pest of cereal flour and flour-based products, and thus a test insect in stored-product research. The composition of the rearing medium affects the progeny production, their performance and handling efficacy. The objective of this research was to develop a suitable rearing media for *T. castaneum*. The research tested wheat flour, crushed broiler feed, crushed dog feed and corn flour alone and in different combinations. Twenty adults of *T. castaneum* were introduced to each medium separately, and removed after 2 weeks. The progeny adults emerged in each rearing medium was determined. The progeny produced differed with the food medium. In general, the rearing media having a combination of ingredients produced more

progeny than a particular component alone. Different ratios of these food ingredients need to be tested to further increase the progeny production in *T. castaneum* and to determine the efficacy of these media on the progeny production in other species.

Keywords: *Tribolium castaneum*, rearing media, adult emergence, progeny

1. Introduction

Food security is a major issue throughout the world, and storage of food always has been a challenge due to infestation by insects (Wijayaratne et al., 2009; Wijayaratne et al., 2018). *Tribolium castaneum* larvae and adults cause quantitative and qualitative losses in stored products (Wijayaratne and Rajapakse, 2015), and hence appropriate control methods need to be developed. Thus *T. castaneum* is a test insect in many research and need to be handled in large numbers. A rearing media which facilitates healthy development of *T. castaneum* cultures and convenient handling of them is therefore important. Therefore this research was conducted to develop an effective rearing media for *T. castaneum* using food ingredients available at the local market.

2. Materials and methods

Tribolium castaneum reared in wheat flour at 30°C, 65% R.H. were used in the experiment. Wheat flour, crushed broiler feed, corn flour and crushed dog feed were used alone and in different combinations as given in Table 1. From each medium prepared, 100 g was weighed into a plastic vial and 20 adults (without sexing) were introduced into each vial. The adults were removed after two weeks. The progeny emerged in each container was counted at one and three months.

3. Results and Discussion

Progeny produced by *T. castaneum* varied with the medium (Figures 1-3). The medium that contained wheat flour, crushed dog feed and crushed broiler feed at 2:1:1 ratio produced the maximum adult progeny.

Tab. 1 Rearing media tested for progeny production by *Tribolium castaneum*.

Treatment	Wheat Flour(g)	Crushed Dog feed(g)	Crushed Broiler feed(g)	Corn flour(g)
A	100	-	-.	-
B	-	100	-	-
C	-	-	100	-
D	-	-	-	100
E	50	50	-	-
F	50	-	50	-
G	50	-	-	50
H	50	25	25	-
I	50	-	25	25
J	50	25	-	25
K	25	25	25	25
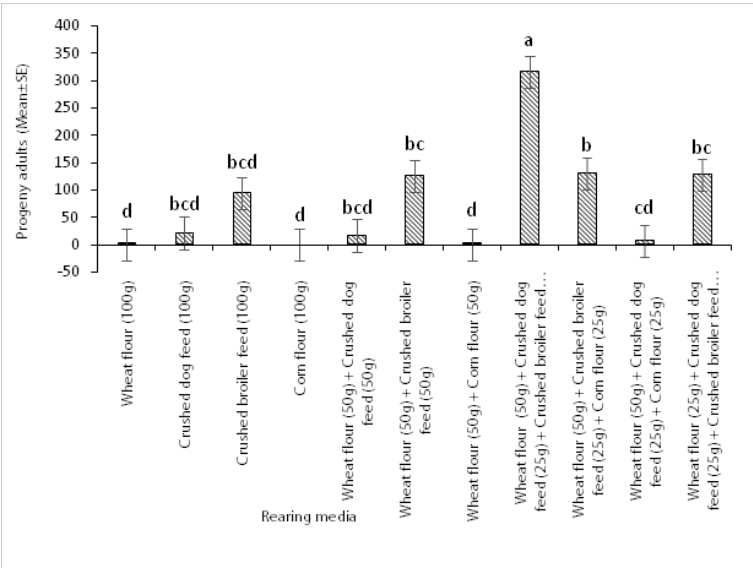
Fig. 1 Progeny *Tribolium castaneum* adults (mean±SE) emerged on different rearing media one month following infestation (n=4).
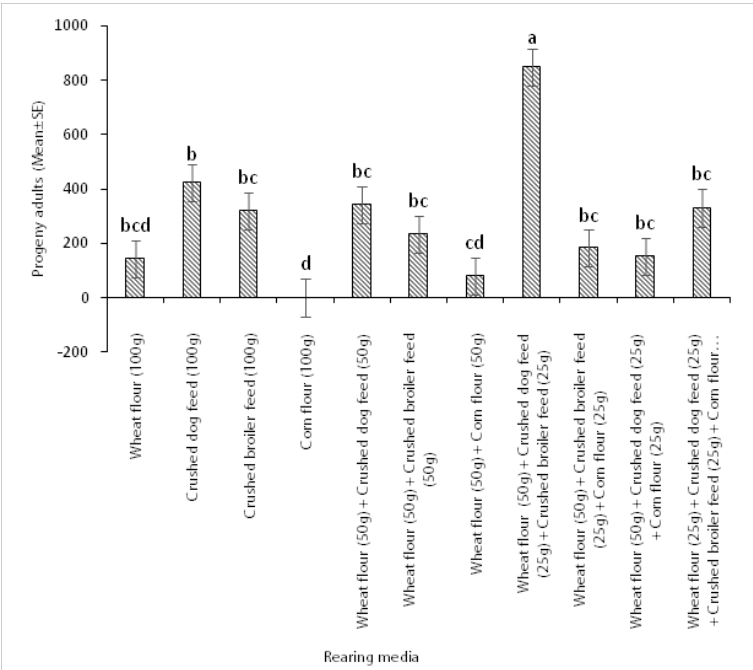
Fig. 2 Progeny *Tribolium castaneum* adults (mean±SE) emerged on different rearing media two months following infestation (n=4).
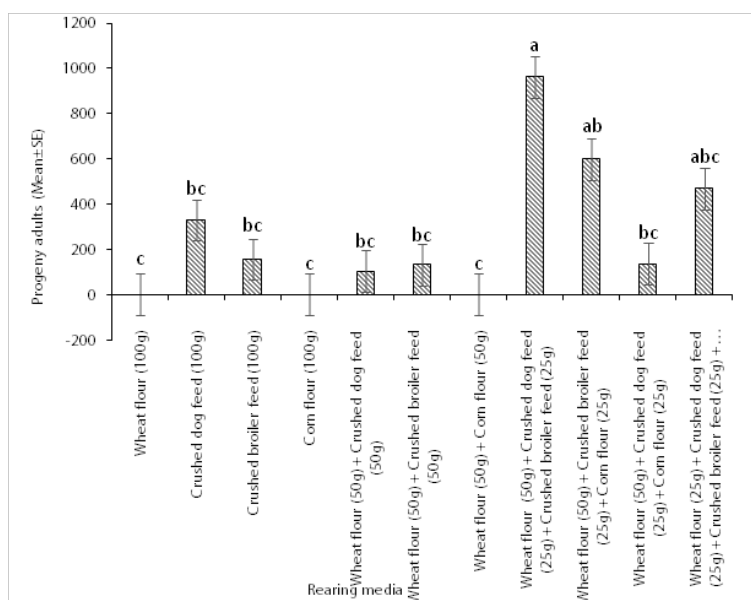
Fig. 3 Progeny *Tribolium castaneum* adults (mean±SE) emerged on different rearing media three months following infestation (n=4).

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Sitotroga cerealella (Olivier) resilience to extreme temperature and desiccation may explain its increasing pest status in changing climates

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Abstract

The mechanisms underlying *Sitotroga cerealella* survival under variable and increasing mean thermal and desiccation environments typical under global change is currently unknown. To understand how *S. cerealella* survives extreme abiotic stressors typical of stored-grain environments, we measured *S. cerealella* tolerance temperature and desiccation. The results showed that to survive desiccating grain storage environments, *S. cerealella* relied more on high body water content (BWC) (70.2 ± 3.72%) compared to lipid reserves (9.8± 0.81%). In desiccating environment, *S. cerealella* showed a reduced water loss rate (0.056mg/h) (equivalent of 1.81% of body water/hour) which would require 19.31 h to reduce the insect body water to its critical minimum (35.23% body water content at death), which is 50.20% of normal initial body water. Similarly *S. cerealella* exhibited high basal heat tolerance with critical thermal maximum of 46.09 ± 1.042°C and a heat knockdown time of 7.97 ± 1.64 minutes. Basal cold tolerance was relatively compromised (critical thermal minima of 4.52 ± 1.06°C and chill

coma recovery time of 5.80 ±1.17 minutes), following 1h at 0°C. We found no significant correlation ($P > 0.001$) between BWC and the measured thermal tolerance traits. Low water loss rates reported here may be an evolutionary resistance mechanism for desiccation tolerance. Observed abiotic stress tolerance may explain the ubiquitous distribution of *S. cerealella* in Africa which is likely to enhance its survival and increase its pest status under global change.

Keywords: storage insect pests, abiotic stress, thermal tolerance, desiccation tolerance, stored cereal grain, stress tolerance mechanisms.

1. Introduction

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) is a cosmopolitan primary coloniser of cereal grains in warm regions of the world (Hansen et al., 2004; Bushra and Aslam, 2014, Demissie et al., 2014). It is a dominant component of the cereal grain pest complex typical in small-scale farmers' stores along with *Sitophilus* species, *Prostephanus truncatus* (Horn.), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) (Hansen et al., 2004). It is one of the most problematic pests of sorghum (Mvumi et al., 2003; Mubayiwa et al., 2018) which is the second most important cereal in sub-Saharan Africa (SSA) (Mubayiwa et al., 2018). The high pest status of *S. cerealella* stems from its larval internal kernel feeding habit which minimises contact with insecticides (Bushra and Aslam, 2014). Moreover, this internal feeding has been reported to contribute to insecticide resistance (Bushra and Aslam, 2014). Tunnels made by the larvae result in grain quantitative and qualitative losses, exposure to secondary pests and microbial attack (Akter et al., 2013). In addition, the feeding frass and scales from dead moths reduce the aesthetic value and hence economic value of the grain. In consequence, it is imperative to understand its ecological characteristics specifically abiotic stress responses to enable the development of alternative non-chemical control methods. The current cereal grain storage methods by small-scale farmers in developing countries (Nukenine, 2010; Nyagwaya et al., 2010) promote the unabated proliferation of *S. cerealella*, making it one of the most abundant problem pests especially in small grains (Mvumi et al., 2003; Hansen et al., 2004). However, to-date no study has focussed on the abiotic stress tolerance of *S. cerealella*, especially temperature and desiccation, two environmental stressors mainly used as proxy to determining insects' survival (Kelley 2014) and potential pest status.

Most terrestrial arthropods including insects are vulnerable to extreme temperature stress (Chown and Nicholson, 2004) and water loss due to their high surface area to volume ratios (Gibbs, 1997; Weldon et al., 2013, 2016). Different species have developed different mechanisms to enhance dehydration tolerance to survive low relative humidity environments (Weldon et al., 2013) such as stored grain habitats. We hypothesize that *S. cerealella*'s ability to withstand desiccation and extreme temperatures typical in tropical climates where it is dominant, likely contributes to its enhanced survival and hence, continued increase in pest populations and grain damage. The objectives of the current study were therefore to determine (1) *S. cerealella* heat and cold tolerance in comparison to like species and in relation to prevailing tropical temperatures; (2) determine whether *S. cerealella* desiccation survival is due to high lipid or body water storage or both and how water loss control may contribute to the moth's desiccation survival.

2. Materials and Methods

2.1 Insect rearing and handling

Insects were reared in the Eco-physiological Entomology Laboratory at Botswana International University of Science and Technology. Field-collected moths were placed in sterilized maize grain (-15°C for 14 days followed 7 days of preconditioning at room temperature of 25 ± 1°C and 60± 5% RH) in 35 × 35cm^2 bugdorm insect cages (BugDorm®, MegaView Science Co., Ltd. Taiwan). The bugdorms were placed in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) maintained at 25± 1°C , 10:14 day and night photoperiod, and 65 ± 10% RH. The moths were fed on 10% sugar solution through the dental-wick method (Shelton et al., 2012) and supplied with randomly folded black-dyed filter paper for egg laying and resting. After 7 days, the moths were

removed from the grain leaving grain and filter papers with eggs. F_1 hatched larvae were allowed to feed on the grain until adult stage. Unsexed 2-3 day old F_1 adult moths were used for the experiments. To reduce mortality, moths damage and excessive loss of scales, resting moths were carefully handled by individual trapping into 0.6 ml polypropylene eppendorf tubes with minimum direct contact.

2.2 Body water and lipid content (BWC and BLC)

To determine Body water content (BWC), 50 moths were collected from the bugdorms and weighed (RADWAG®, Wagi Elektroniczne, Model As220.R2, Radom, Poland) to 0.0001 g in 0.6 ml numbered and uniformly perforated polypropylene eppendorf tubes. These were placed on eppendorf-tube-holders and transferred to a laboratory oven (UF160, Memmert GmbH + Co.KG, Germany) set at 60°C for 48 h. Moths were re-weighed after 48 h and the BWC was calculated as the difference between the initial and the final body mass. (Lease and Wolf, 2010)

For Body lipid content (BLC), 50 moths were individually placed in uniformly perforated, pre-weighed and numbered 0.6 ml polypropylene eppendorf tubes. These were dried in an oven (UF160, Memmert GmbH + Co.KG, Germany) at 60°C for 48 h. The moths were immediately weighed after drying to determine dry weight and transferred to unperforated 2ml eppendorf tubes containing 1.5ml diethyl ether. The tubes were gently agitated at 37°C for 24 h before the insects were removed and re-dried in the oven (60°C) for 24 h (Lease and Wolf, 2010). After drying, the moths were weighed and the BLC was calculated as the difference between the initial insect dry mass and the final (lipid-free) dry mass.

2.3. Water loss rates (WLR)

Desiccation tolerance experiments were conducted following standard protocols (Gibbs, 1997; Weldon et al., 2013). After initial mass of the moths were recorded, 50 moths were individually placed in numbered 0.6 ml uniformly perforated polypropylene eppendorf tubes. The tubes were placed in small loose granules of desiccant (Drierite, W.A. Hammond Drierite Co. Ltd, Xenia, USA) with < 10% RH at 25°C in a large airtight laboratory glass bowel. Vials were inspected every 3 h and mortality was recorded.

2.4. Critical thermal limits (CTLs)

Critical thermal limits (CTmin and CTmax) were measured using a protocol developed by Nyamukondiwa and Terblanche (2010). Ten individual moths were placed in a series of test tubes floating in insulated double-jacketed chambers or "organ pipes" connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol and subjected to a constant cooling or heating rate. Moths were first given 10 minutes to equilibrate at 25°C before the temperature was ramped up or down for CTmax or CTmin respectively at a three different rates; 0.12, 0.25 and 0.5°C/min. This was repeated three times to yield sample sizes of n = 30 individuals per treatment. A thermocouple (type T 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the middle (control) test tube to monitor temperature. In the current study, CTmax and CTmin were defined as the maximum or minimum temperature, respectively; at which each individual moth lost coordinated muscle function, following mild prodding with a thermally inert object (see Nyamukondiwa and Terblanche, 2010).

2.5. Chill coma recovery (CCRT) and heat knockdown time (HKDT)

CCRT and HKDTs were conducted following a method developed by Weldon et al. (2011). For CCRT, moths were individually placed in 0.6 ml eppendorf tubes and then loaded into a large zip-lock bag which was subsequently submerged into a water bath (Systronix, Scientific, South Africa) filled with 1:1 water: propylene glycol mixture, was set at 0°C for 1 hour. After 1 hour of exposure, the tubes

were removed from the water bath and transferred to a Memmert climate chamber set at 25°C, 65% RH for moth recovery. The chamber was connected to a camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) that was linked to a computer where observations were recorded. In this study, CCRT was defined as the time (in minutes) taken by an individual moth to recover and stand upright on its legs. For HKDT, ten moths were individually placed in 0.6 ml eppendorf tubes and placed in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) set at 48 ±1°C and 65±5% RH. Observations were recorded as explained for CCRT. In this study, HKDT was defined as the time (in minutes) at which moths lost activity following exposure to high (48°C) temperature. Both CCRT and HKDT were repeated three times (n = 30 individuals moths).

2.6. Data analysis

Data analyses were carried out in STATISTICA, version 13.2 (Statsoft Inc., Tulsa, Oklahoma). For CTLs, ramping rate was used as a factor in one–way Analysis of Variance (ANOVA) and statistically significant means were separated using Tukey-Kramer's post-hoc test. However, for BWC, WLR, LC, HKDT and CCRT mean values were presented and compared to like species, e.g *Plutella xylostella* (L.) and *Tuta absoluta* (Meyrick).

3. Results

3.1. Body water content, water loss rates, water loss at death and body lipid content

Sitotroga cerealella had a mean body water content (BWC) of 1.80 ±0.517 mg with an average body mass (BM) of 2.61 ±0.691 mg resulting in 70.2% BWC. The water loss rates of 0.056 mg/h (1.81% BW/h) were low, showed that *S. cerealella* would need 19.31 h of exposure to a desiccating environment (≤ 10% RH) to reach its critical body water at death, 35.23% of initial body water. There were no significant correlation ($P > 0.001$) between BWC and thermal traits (CTLs, HKDT and CCRT). The BLC of (9.8± 0.81%), was relatively low compared to BMC indicating that lipids were unlikely used as a source of metabolic water.

3.2 Thermal tolerance (CTmax, CTmin, HKDT and CCRT)

At a benign ramping rate of 0.25°C/min, *S. cerealella* had a CTmax of 46.1±1.04°C which was significantly higher ($F_{(2, 87)} = 83.921$, $P < 0.0001$) than the CTmax at lower ramping rate (0.12°C/min) , 42.8±1.19°C (Fig 1A). However, higher ramping rate (0.5°C/min) did not have a significant effect on CTmax (Fig. 1A).
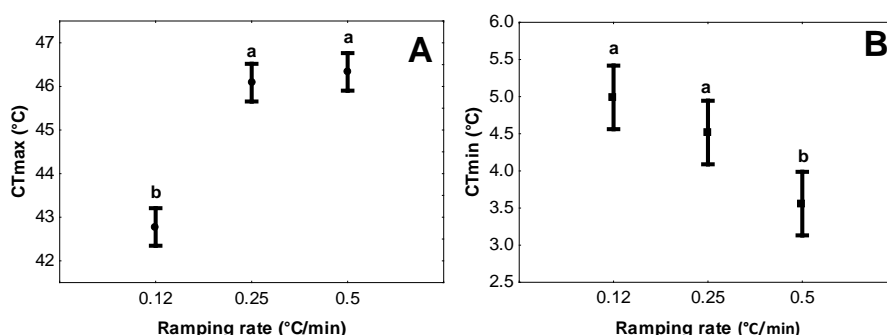
Fig. 1 The critical thermal limits (CTLs) for *S. cerealella* at different ramping rates: (A) critical thermal maxima (CTmax); and (B) critical thermal minima (CTmin).

Similar to CTmax, the CTmin for *S. cerealella* was significantly affected by the ramping rate. At the benign ramping rate of 0.25°C/min, *S. cerealella* showed a CTmin of 4.5±1.06°C, which was significantly higher ($F_{(2, 87)} = 11.443$, $P = 0.0004$) than the CTmin at a higher (0.5°C/min) ramping rate (3.6±0.98°C) (Fig. 1B). However, lower ramping rate (0.12°C/min) did not have a significant effect on CTmin (Fig. 1B).

Tab. 1 Thermal traits of *S. cerealella* compared to like species.

Insect species	CTmax (°C)	CTmin (°C)	HKDT (min)	CCRT (min)	Reference
S. cerealella	46.1±1.04	4.5±1.06	7.9±1.64	5.8±1.17	Current study
Plutella xylostella	46.6±0.52	-3.2±0.41	3.8±0.65	2.48±0.40	Machekano et al., 2018a
Tuta absoluta	44.1±0.43	-5.2±0.23	*	*	Machekano et al., 2018b

*Denotes that data on that thermal trait were not available.

Table 1 shows the comparison of thermal traits between *S. cerealella, P. xylostella and T. absoluta*. The CTmax is comparable to *P. xylostella* and *T. absoluta* (Machekano et al., 2018a & b, upcoming). Compared to other economic Lepidopterans *S. cerealella* shows almost double the time (7.9±1.64 minutes) needed to be knocked down by heat stress (HKDT) compared to *Plutella xylostella* (3.8±0.65 minutes). Its CTmin (4.5±1.06°C) however, was higher (4.5±1.06) than *P. xylostella* (-3.2±0.41°C) and *T. absoluta* (-5.2±0.23°C) (Table 1). In addition, the chill-coma recovery time (CCRT), was almost two-fold that of *P. xylostella* (Table 1). To understand whether *S. cerealella* was likely to cope with prevailing field temperatures, we compared its critical thermal limits to field meteorological data for the 2015-2016 seasons (Fig. 2).
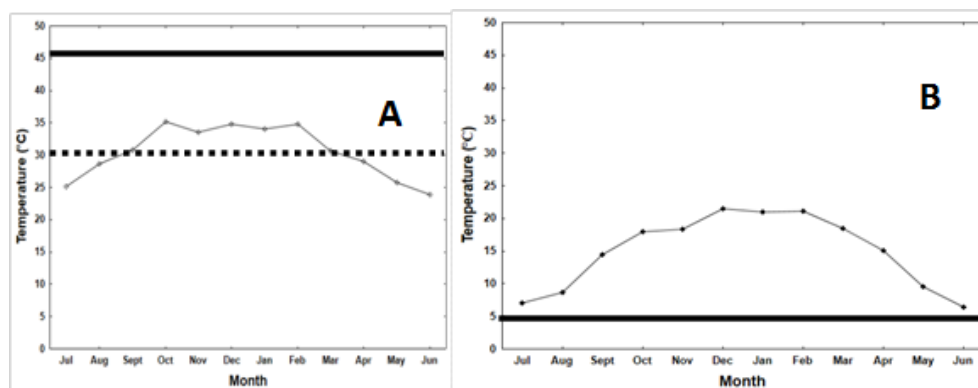
Fig. 2 (A) The mean maximum field temperature data in Botswana (2015-16) (black curved line) compared to *S. crealella* CTmax (black solid line) and optimum temperatures (black dotted line), (B) the mean minimum field temperatures (black curved line) compared to *S. cerealella* CTmin (black solid line).

The difference between the highest recorded temperature (35.2°C) (October, 2015) and *S. cerealella* CTmax was~10°C (Fig. 2A), evidence of a very high thermal safety margin (Deutsch et al., 2008) for *S. cerealella* considering a 30.0°C optimum temperature (Hansen et al., 2004; Demissie et al., 2014). Similarly, the mean field minimum temperatures were well above *S. cerealella*'s CTmin (Fig. 2B), indicating that it is not strained by low temperatures in nature under typical tropical conditions. Like other economic Lepidopterans (Table 1), *S. cerealella* is likely not under thermal physiological stress under current and projected global change. Such adaptations to increasing temperatures may contribute to high pest activity, short generation time and high population growth with potential deleterious effects on stored cereal grains.

4. Discussion

Sitotroga cerealella's high BMC (70.2%) of total body mass, was comparable to desiccation-resistant *Drosophila* species observed by Gibbs and Matzkin (2001). This entailed that *S. cerealella* likely used high body water storage to tolerate low relative humidity, typical of grain environments to survive desiccation. This is so because, high levels of internal body water content extends the time required for dehydration to critical levels that would induce mortality. The observed low water loss rates showed that *S. cerealella* would need 19.31 h of exposure to a desiccating environment to reach its critical body water at death. This suggests that, *S. cerealella* likely uses two mechanisms; high body water and low water loss rates to tolerate desiccation in dry stored-grain habitats. The exact physiological mechanisms used by *S. cerealella* to reduce water loss need further investigation. Lack of significant correlation ($P > 0.001$) between BWC and thermal traits (CTLs, HKDT and CCRT) further suggests that high body water content solely played a significant role in desiccation tolerance. This is supported by the relatively low body lipids (9.8± 0.81%), which explains that lipids were unlikely to be a source of metabolic water but probably energy for this species (Arrese and Soulages, 2010). Demissie et al. (2014) reported that low relative humidity did not have a significant effect on the growth and development of *S. cerealella* except egg hatching, suggesting that apart from the egg stage, all stages of *S. cerealella* are capable of tolerating desiccation.

Our data and previous reports suggest that *S. cerealella* is heat-tolerant. Its CTmax is comparable to the thermally resilient *P. xylostella* (Machekano et al., 2018a) and the invasive *Tuta absoluta* (Machekano et al., 2018b, upcoming). However, on the low temperature scale, *S cerealella* showed compromised CTmin (4.5±1.06°C) compared to like species. The time taken by the moths to recover from chill-coma was almost two-fold that of *P. xylostella*. Both CTmin and CCRT responses suggest limited low temperature tolerance for *S. cerealella*. High temperature tolerance likely explains why it is a major pest in the warm tropical climates especially SSA (Hansen et al., 2014; Bushra and Aslam, 2014) as similarly reported for fruitflies (Nyamukondiwa et al., 2010) and stemborer species (Mutamiswa et al., 2017).

Only low ramping rate had an effect on CTmax, but higher ramping rate (0.5°C/min) did not significantly shift the CTmax. This result suggests the inability of *S. cerealella* to adjust its CTmax in the short-term or rapid heat hardening but only in the long term. Similar to CTmax, the CTmin for *S. cerealella* was significantly affected by the ramping rate. On the low side of the temperature scale, only the higher ramping rate (0.5°C/min) significantly dipressed the CTmin. This result suggests faster ramping rates enhanced low temperature tolerance, measured as CTmin. This plastic effect has been observed in similar Lepidopterans (Machekano et al., 2018a and b) and likely facilitates adaptation to novel but stressful environments (Nyamukondiwa et al., 2010). Compared to other economic Lepidopterans, *S. cerealella* showed two-fold time needed to be knocked down by heat stress compared to *Plutella xylostella* further attesting its thermal resilience on the higher side of the temperature scale.

The difference of ~10°C between the highest recorded field temperature and *S. cerealella* CTmax, is evidence of a very high thermal safety margin (Deutsch et al., 2008) from its 30.0°C optimum temperature (Hansen et al., 2004; Demissie et al., 2014). Similarly, the mean field minimum temperatures were well above *S. cerealella's* CTmin (Fig. 2B), indicating that it is not strained by low temperatures in nature under typical tropical conditions. Like other economic Lepidopterans (Table 1), *S. cerealella* is likely not under thermal physiological stress under current and projected global change. Such adaptations to increasing temperatures may contribute to high pest activity, short generation time and high population growth with potential deleterious effects on stored cereal grains.

We conclude that *S. cerealella* uses high body water storage and low water loss rates to tolerate desiccation in low humidity stored grain habitats. *Sitotroga cerealella* shows high basal heat but not cold tolerance, and coupled with potential plasticity and behavioural regulation, this may aid its survival under abiotic stressful environments. Grain cold treatment may be used as an effective pest control method against *S. cerealella*.

Acknowledgements

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Suitability of hemp seed for reproduction of stored-product insects

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Extended Abstract

1. Introduction

Hemp, or industrial hemp, is a high value alternative crop that has seen major increases in acreage in Canada since commercial production was legalized in 1998. The term industrial hemp applies to non-psychoactive varieties of *Cannabis sativa* L. There have been reports of insect infestations on stored hemp seed in Manitoba. The moths *Plodia interpunctella* (Hübner) Indianmeal moth, and *Ephestia küehniella* (Zeller) Mediterranean flour moth feed on hemp seed (Hagstrum and Subramanyam, 2009). Our objectives were to determine which stored-product beetles can reproduce on hemp and the effect of dockage and seed moisture content.

2. Materials and Methods

Twenty adult insects were placed on 15 g of hemp seed at two different moisture contents (~8% or ~15%) and two different dockage levels (~0% or~15%) and held at 30°C and 60-70% relative humidity. The number of live and dead insects were counted at 3, 5, 7 and 9 weeks. Only live adults were returned to vials.

3. Results and Discussion

These insect populations increased over the 9 weeks; red flour beetle [*Tribolium castaneum* (Herbst)], drugstore beetle [*Lasioderma serricorne* (F.)] saw-toothed grain beetle [*Oryzaephilus surinamensis* (L.)], warehouse beetle (*Trogoderma variabile* Ballion). These insect populations did not increase: rusty grain beetle [*Cryptolestes ferrugineus* (Stephens)], lesser grain borer [*Rhyzopertha dominica* (F.)], rice weevil [*Sitophilus oryzae* (L.)], flour mill beetle (*Cryptolestes turcicus* (Grouvelle), confused flour beetle (*Tribolium confusum* Jacquelin du Val), cigarette beetle [*Stegobium paniceum* (L.)]. In general, higher dockage led to higher populations. The effect of moisture content was variable.

Keywords: *Cannabis sativa,* reproduction, dockage, moisture

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The use of long-lasting insecticide netting to prevent dispersal of stored product insects

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Abstract

The lesser grain borer, *Rhyzopertha dominica*, and red flour beetle, *Tribolium castaneum*, are two notorious primary and secondary pests of stored products. Extensive research has been done to prevent the establishment and subsequent infestation of the insects in stored product facilities. Long-lasting insecticide netting (LLIN) on mosquitoes has proved effective in controlling the spread of malaria, but little research has been conducted on the LLIN's behavioral effects of stored product insects. In this study, a movement and dispersal assay were performed. In the movement assay, the video-tracking software, Ethovision, recorded the movement of *R. dominica* and *T. castaneum* after 1-10 min exposures to LLIN or control netting and a waiting period of 1 min, 24 hr, 72 hr, or 7 days after netting exposure. In the dispersal assay, *R. dominica* and *T. castaneum* were observed after 5 minutes of exposure to LLIN or control netting to measure the insects' ability to reach new food patches at three different distances. The results from the movement assay showed a significant reduction in horizontal movement and significant increase in angular velocity for beetles exposed to LLINs, indicating that movements were more erratic and less directed. The dispersal assay revealed that exposure to LLIN had a significant effect on the dispersal ability of both *R. dominica* and *T. castaneum* with averages of 0-3 from a group of 20 beetles reaching the new food patch. These results indicate that LLINs can be an effective tool for the prevention of stored product insect establishment and colonization.

Keywords: polyethylene netting, integrated pest management, behavior, sublethal effects, bed nets

1.Introduction

Together, the major three stored grains in the US (corn, soybean, and wheat) alone represent a value of $85.9 billion (NASS, 2018), much of it exported to help feed the world's growing population. The world's population is estimated to reach 9 billion people by 2050 (Godfray et al. 2010), and agricultural output will have to more than double by that point (Ray et al. 2013). Insects are our main competitors for food on the planet, resulting in 10-50% yield loss of products after they have been harvested from the field. The key to many integrated pest management programs (IPM) for stored products is sanitation to prevent infestation by insects (Phillips and Throne, 2010). However, this is often difficult because of the success with which stored product insects can immigrate to new facilities (McKay et al. 2017; Campbell and Arbogast 2004).

In particular, the lesser grain borer, *Rhyzopertha dominica*, and red flour beetle, *Tribolium castaneum*, are two notorious primary and secondary pests of stored products. These represent radically different life histories among stored product insects. *Tribolium castaneum* is a secondary feeder (Hagstrum and Subramanyam 2006), feeding on already broken grain, is a relatively weaker flier, and is mostly confined to facilities and local areas around which grain is processed (Drury et al. 2009; Ridley et al. 2011). By contrast, *R. dominica* is a primary feeder, boring into whole kernels, depositing eggs, and developing inside the grain (Hagstrum and Subramanyam 2006), while also being a strong flier (Edde et al. 2006) and long-distance disperser (Mahroof et al. 2010).

Extensive research has been done to prevent the establishment and subsequent infestation of the insects in stored product facilities. One potential alternative management tactic that has not been evaluated for control of stored product insects is long-lasting insecticide netting (LLIN). Since the 1990s, LLINs have proved effective in reducing mosquito populations to control the spread of malaria (Lengeler 2004; Kitchen et al. 2009; Alonso et al. 1991) and to kill vectors of other arthropod-borne diseases (Dutta et al. 2011). LLINs are constructed such that insecticide moves to the surface of the netting material over time, producing multi-year residual efficacy (Martin et al. 2007). In the past few years, LLINs have been evaluated for their utility in protecting crops before harvest in agriculture. This has included as a kill mechanism in traps for the brown marmorated stink bug (Kuhar et al. 2017; Morrison et al. 2017; Rice et al. 2018). Most recently, LLINs are now being considered for their ability to control post-harvest insects (Scheff et al. 2018; Rumbos et al. 2018). However, one challenge with currently available LLINs is that stored product insects are small enough to pass through the netting material, and it takes extended durations of exposure to elicit outright mortality. As a result, a natural question is whether the netting will have sufficiently pronounced effects on the behavior of stored product insects to prevent their dispersal after contact.

For LLINs to be an effective control measure, they must be compatible with the biology and behavior of stored product insects. Pyrethroids, which are the active ingredient in many LLINs, may have deleterious behavioral side effects in some arthropods, such as repellency (Katz et al. 2008). This would prevent the use of LLINs from effectively intercepting pests as they immigrate to stored product facilities. However, Scheff et al. (2018) importantly found no evidence of long-distance or contact repellency from LLINs against *T. castaneum* and *R. dominica*. Nonetheless, there are several other considerations that must be met for LLINs to be behaviorally compatible with the behavior of stored product pests and be potentially effective as a control tactic. Specifically, LLINs must 1) swiftly decrease the locomotion and result in the loss of coordinated movement by stored product insects, and 2) prevent dispersal to new food patches after brief contact with the material. In this study, a movement and dispersal assay were performed. We employed either a deltamethrin-incorporated polyethylene netting at 0.6% a.i. (ZeroFly, Vestergaard-Frandsen, Inc., Switzerland; LLIN hereafter) or netting with identical physical characteristics but not insecticide (control netting).

2. Materials and Methods

In the movement assay, the video-tracking software, Ethovision, recorded the movement of *R. dominica* and *T. castaneum* for 2 h after 1, 5, or 10 min exposures to LLIN or control netting and a waiting period of 1 min, 24 hr, 72 hr, or 7 days post-exposure (Fig. 1). The movement variables characterized were the total distance moved (cm) by adults and their mean angular velocity (deg/s). In the dispersal assay, *R. dominica* and *T. castaneum* were observed after 5 minutes of exposure to LLIN or control netting to measure the insects' ability to reach new food patches at three different distances (25, 75, or 175 cm) at the conclusion of a 48-h period. These were performed in laboratory spaces and environmental chambers under standardized abiotic conditions (30°C, 65% RH, 14:10 L:D). The data did not conform to the assumptions of normality, and thus were log-transformed prior to running the final model. Data were analyzed with an ANOVA, and upon a significant result from the model, Tukey's HSD were performed for post-hoc pairwise comparisons. All data was analyzed using the R Software (R Core Team, 2017).

3. Results

In the movement assay (Fig. 1), brief bouts of 1-min exposure to LLINs resulted in the same numbers of affected and dead as longer 10-min exposure to the same nets for both species (ANOVA: *T. castaneum*, $F = 0.073$; df = 2, 404; $P < 0.93$); *R. dominica*, $F = 1.38$; df = 2, 407; $P = 0.25$). Importantly, movement was descreased by 3-fold for both species after exposure to LLINs (*T. castaneum*: $F = 102$; df = 1, 404; $P < 0.0001$; *R. dominica*: $F = 28.2$; df = 1, 407; $P < 0.0001$). There was some recovery of *T. castaneum* at 72 h and 7 d, but not for *R. dominica*. Movement was immediately reduced by half after exposure, and by 24-72 h later, movement was reduced by 4 to 9-fold compared with adults exposed to control netting. The behavioral effects of exposure extended out to 7 d later for both species where movement was still reduced by half or more compared to control netting-exposed adults. Angular velocity was elevated for LLIN-exposed adults compared to those exposed to control netting (*T. castaneum*: $F = 289$; df = 1, 404; $P < 0.0001$; *R. dominica*: $F = 38.1$; df = 1, 407; $P < 0.0001$), though this effect attenuated by 7 d after exposure for *R. dominica*, but not *T. castaneum*.

The dispersal assay (Fig. 2) revealed that exposure to LLIN had a significant effect on the dispersal ability of *T. castaneum* ($F = 2151$; df = 1, 89; $P < 0.0001$) with averages of ~1 from a group of 20 beetles reaching the new food patch after exposure to LLINs, whereas almost the full set of 20 control netting-exposed adults reached the novel food patch. Out of over 1,400 *R. dominica* tested, not a single LLIN-exposed adult reached the novel food resource ($F = 701$; df = 1, 54; $P < 0.0001$). The distance that adults had to disperse did not impact their ability to disperse; the primary determinant was whether they were exposed to netting with insecticide. The dispersal distance did not affect the dispersal capacity of either species for the range of distances tested (*T. castaneum*: $F = 1.59$; df = 2, 89; $P = 0.21$; *R. dominica*: $F = 2.31$; df = 2, 54; $P = 0.11$).
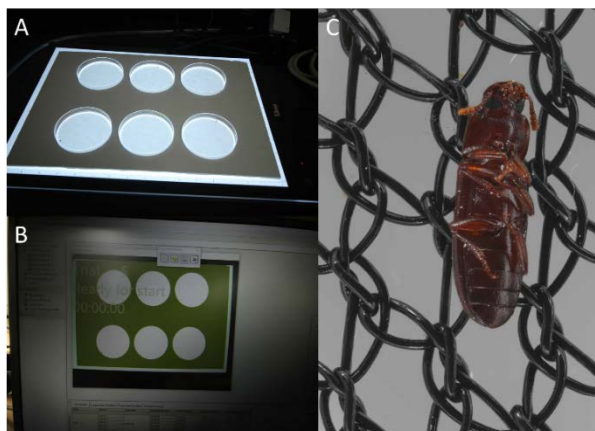
Fig. 1 The movement assay used with A) individual adult *Tribolium castaneum* or *Rhyzopertha dominica* placed in 100 × 15 mm petri dishes, 2) their movement tracked with a video camera and sent to software on a computer, and C) the effect of netting on the mobility of *T. castaneum*. Please note that figures (photographs or graphs) shall be provided in the best possible resolution without frames.
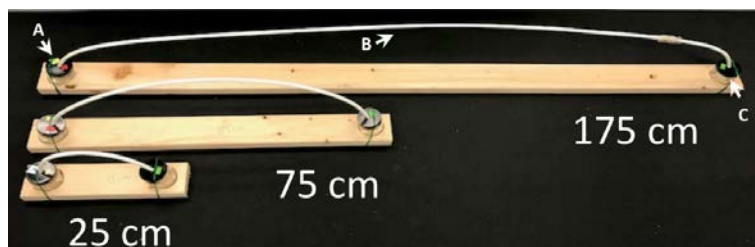
Fig. 2 The dispersal assay that tested the ability of *Tribolium castaneum* and *Rhyzopertha dominica* to move to a novel food resource after a 48 h period, with A) 20 adults introduced in the introduction chamber, B) a single line of twine threaded through the system from the bottom of the introduction chamber to halfway down the jar with the novel food resource so movement was only unidirectional, and C) a dispersal chamber with 15 g of organic, unbleached flour.

4.Discussion

This is the first study to examine, in-depth, the sublethal effects of exposure to LLINs on any stored product insects. We have shown here that even brief exposure times of 1-min are sufficient to induce the same dramatic decreases in movement and increase in disorientation as longer 10-min exposures compared to controls. Exposure to LLINs reduced adult movement of both species by 3 to 4-fold. In addition, a moderate exposure time of 5-min was sufficient to substantially reduce or effectively prevent the dispersal of adult stored product insects, with *R. dominica* the more susceptible of the two species studied. Radically diminished dispersal capacity held steady even after a 2-3 d period during which adult *T. castaneum* or *R. dominica* could have recovered, but did not. As a result, this suggests that while mortality may be initially incomplete after exposure, brief bouts of contact with LLIN are adequate in preventing adults from reaching novel food patches. Overall, these results indicate that LLINs are a promising tool for the prevention of stored product insect establishment and colonization.

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Evaluation of the attractiveness of an organic litter compared to breeding substrate

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Abstract

In a pet shop warehouse, stored food pest insects can develop in various preserved animal feeds (dog's pasta, puffed rice, kibble). However, there is another commodity that is rarely considered, such as the organic litter which is composed of bran, flours and other residues of the screening of corn that may result attractive to the same pest insects. The purpose of this laboratory test was to evaluate the attractiveness of organic litters on *Plodia interpunctella*, *Tribolium confusum*, *Oryzaephilus surinamensis* in comparison with breeding substrate. The results confirmed that the test insects were attracted by the breeding substrate rather than by the organic litter.

Keywords: stored food pest insects, pet food, organic litter, *Plodia interpunctella*, *Tribolium confusum*, *Oryzaephilus surinamensis*.

1. Introduction

The stored food insects can attack several food products, the importance in stored food losses are estimated about 16% (World Bank et al., 2011). Food industries pest are also involved in losses in pet food industries. Roesli et al. (2003) reported that it is possible to record up to thirty insect species belonging to 20 families in four pet stores chain during February to August 2001. The most common and abundant species were *Plodia interpunctella* (Hübner), *Oryzaephilus mercator* (Fauvel), *Tribolium castaneum* (Herbst) and *Sitophilus* spp.

In the pest food stores this attack by stored pest food can cause damage to various preserved animal feeds (dog's pasta, puffed rice, kibble). However, there is another commodity that is rarely considered, such as the organic litter which is composed of bran, flours and other residues of the screening of corn that may result attractive to the same pest insects. In July 2017, a package of dog's pasta found infested by *O. surinamensis* in a pet food store was delivered to LEAA (Laboratory of applied entomology Agroblu). The origin of infestation was investigated, and it was discovered it had developed from a pallet of ecological litter for pets. Thanks to these events, the aims of the present work is to investigate the attractiveness of organic litter applied to *O. surinamensis*, *P. interpunctella* and *T. confusum.* in comparison with a balanced diet, typical of breeding. This test was combined to a development evaluation test of the same species on such substate.

2. Materials and Methods

2.1 Insects

The insects used in the test was provided by the Agroblu Laboratory of Applied Entomology (LEAA: via Isonzo 20, Rozzano- Milano – Italy) where are reared at 26 ± 2 °C 70% RH and photoperiod light darkness 16L:8D. The test organisms used were typical insects infesting food industries and also collected in pet stores. For the test were employed larvae II instar of *Plodia interpunctella* (Hübner), adult stage of *Tribolium confusum* (Jaqcquelin du Val) and *Oryzaephilus surinamensis* (Linnaeus). Table 1 reported the species and stage used for the test.

Tab. 1 Species, stages and substrates used for the test.

Insect	Stage	Quantity	Substrate TNT	Substrate T
P. interpunctella	II instar larvae	20	Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran	Organic litter
T. confusum	Adult	20	Semolino, brewer's yeast, bran	Organic litter
O. surinamensis	Adult	20	Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran	Organic litter

2.2 Substrate

The test was conducted to compare the attractiveness of a commercial organic litter and a balanced diet normally used for breeding. *P. interpunctella* and *O. surinamensis* were reared on a diet composed as follow honey 15%, glycerin 5%, white flour 20%, semolina 20%, yellow flour 15%, oatmeal 5%, sesame 15%, bran 5%.

T. confusum diet was composed 70% semolina, 29% bran and 1% brewer's yeast.

The composition of the organic litter, vegetable granules, obtained by extracting and drying the fibrous part of the corn's ear, was reported in table 2.

Tab. 2 Composition of organic litter.

Component	Range
Raw ashes	1 – 2%
Raw protein	0,5 – 1,5%
Raw lipidis	0,1 – 1%
Raw fiber	33 – 40%
Extraction inazotati	50 – 60%
Humidity	4 – 10%.

2.3 Y-olfactometer

To test the preference between the two substrates, a plexyglas Y-olfactometer was used. The arms of the structure were 10 cm long and 3 cm wide. At the end of the arms a plastic panel (3x3 cm) was fixed with a 0,5 cm wide hole to allow the air flow created thanks to an extractor fan. To allow the assessment, the Y-olfactometer was fixeded on a rectangular plexiglass panel. On the top of the structure, another rectangular plexiglass panel was placed, featuring a rubber gasket to avoid insects escape.
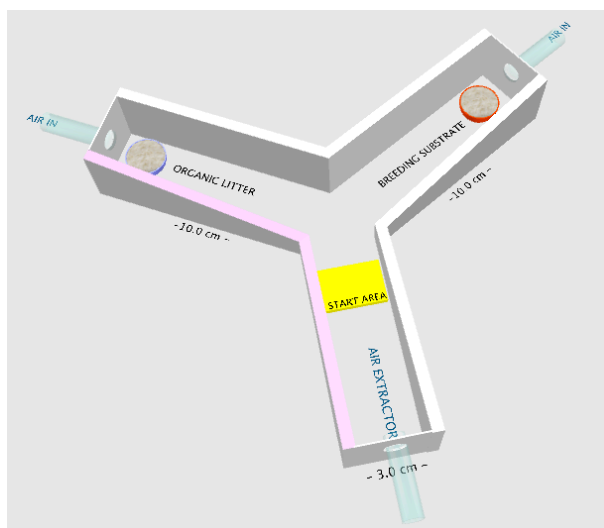
Fig. 1 Scheme of the plexyglass Y-olfactometer setup for the test

2.4 Test System

The Y-olfactometer was set as follow:

- Principal arm: starter point of insect;
- Arm 1: TNT (breeding substrate);
- Arm 2: T (organic litter);

The position of the two alternative substrate was randomized in the replicates. (figure 1).

2.5 Replicates

The test was replicated 20 times.

2.6 Test site

The test was conducted in the Peet Grady Room of LEAA at $26 \pm 2\ °C$ 70% RH and photoperiod light darkness 16L:8D.

2.7 Application method

2.7.1 Coleoptera

For *O. surinamensis* and *T. confusum* 50 adult insects were sampled from the breeding and placed in a Petri dish for 72 hours. After this period a single insect was placed in the principal arm of the olfactometer to choose between two alternative substrates.

2.7.2 Lepidoptera

For *P. interpunctella*, 50 larvae at the II instar, high trophic activity development stage, were collected and placed in a petri dish for one hour. The larvae were placed in the principal arms individually to choose between two alternative substrates.

2.8 Evaluation method

2.8.1 Coleoptera

To ensure accurate assessments, the insects were observed up to their choice for 5 minutes, as choices adopted after 3 minutes do not statistically differ from choices taken within the first 3 minute (Wakefield et al. 2004).

2.8.2 Lepidoptera

For *Lepidoptera* the assessment lasted longer because the choice took place in max 25 minutes. The choice of substrate and the time of choice was recorder and compared with t-test.

3. Results

The results showed that all the species taken into consideration, preferred to head towards the substrate typically used for breeding (table 3).

Tab. 3 Percentage of choise between T and TNT.

Test insect	T-Organic litter	TNT-Breeding substrate
Plodia interpunctella	15%	85%
Tribolium confusum	30%	70%
Oryzaephilus surinamensis	20%	80%

The observed mean values were significantly different for $p<0.05$ (t-test) for all the species and stages considered (table 4).

Tab. 4 Mean of insects that have chosen the test substrate (organic litter). Means followed by "*" are significantly different ($p<0,05$) than the response to the control (t-test).

Test insect	T-Organic litter	TNT-Breeding substrate
Plodia interpunctella	0,15*	0,85
Tribolium confusum	0,30*	0,70
Oryzaephilus surinamensis	0,20*	0,80

The mean time of choice of the two alternatives was recorded and compared with t-test. *P. interpunctella* larvae took more time than Coleoptera to choose, the mean time of choise of the preferred substrate for Coleoptera was respectly 3.21 minutes for *T. confusum* and 1.63 minutes for *O. surinamensis*, only the mean choice time of *T. confusum* between organic litter and breeding substrates was statistically significant for $p<0.05$ (t-test) (table 5).

Tab. 5 Mean time used by insects in chosen the test substrate (organic litter). Means followed by "*" are significantly different ($p<0,05$) than the response to the control (t-test).

Test insect	Choice time (min)	
	T-Organic litter	TNT-Breeding substrate
Plodia interpunctella	11,67	12,76
Tribolium confusum	5,00*	3,21
Oryzaephilus surinamensis	1,75	1,63

4. Discussion

The results obtained show that the test insects in front of a choice between a balanced diet substrate and a commercial litter, prefer the first substrate. This result complete and integrate the information available in literature (Phillips *et al*., 1994, TsuJi, 2000, Mowery *et al*., 2002). These data are preliminary and require further investigations on the possible attractiveness of organic litter compared to other commodities stored in pet food shops by other stored food pest or its attractiveness in interaction with other volatile components.

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Evaluation of the difference in the development of stored insect pests on organic litter

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Abstract

On July 2017 in a warehouse of pet food shop in Italy an infestation of *Oryzaephilus surinamensis* was found on a pallet of organic litter, near an infested pallet of dog's pasta. In order to investigate the origin of the infestation, and to support the risk assessment by the pest control operator, one test was conducted at Agroblu Laboratory of Applied Entomology (LEAA) to observe the feasibility of development of *O. surinamensis*, *Plodia interpunctella* and *Tribolium confusum*, in a substrate of 2,5 g of organic litter and to compare it to a balanced diet substrate.

The results showed that only *T. confusum* was able to develop with no statistical difference both on the breeding diet and the organic litter.

Keywords: stored food pest insects, organic litter, *Plodia interpunctella*, *Tribolium confusum*, *Oryzaephilus surinamensis*

1. Introduction

The stored food insects have a high economical importance because of they contribute to the post harvest losses around 16% (World Bank et al., 2011). Food industries pest are also involved in losses in pet food industries. A limited number of surveys were conducted to determine insect species associated with retail grocery, and pet stores, however there are some experience that record the presence in warehouses and in the pet food stores of the common stored pest insects (Loschiavo and Okumura, 1979; Platt et al., 1998, Roeslli et al., 2003). This autor reported that the common species recorder in a pet store were *Plodia interpunctella* (Hübner), *Oryzaephilus mercator* (Fauvel), *Tribolium castaneum* (Herbst) and *Sitophilus* spp.

In a pet store, stored pest food can cause damage to various preserved animal feeds and to another commodity that is rarely considered, such as the increasingly popular organic litter for cats, hamsters, reptiles and amphibia. Organic litter is composed of bran, flours and other residues of the screening of corn that may result attractive to the same pest insects. On July 2017, an infestation by *O. surinamensis* of a package of dog's pasta has been reported in a pet food store. The origin of infestation was investigated and was discovered it had developed from a pallet of ecological litter for pets. Thanks to these events, the aims of the present work is to investigate the development faeseability for *O. surinamensis*, *P. interpunctella* and *T. confusum* on organic litter in comparison with a balanced diet.

2. Materials and Methods

2.1 Insects

The insects used in the test was provided by the Agroblu Laboratory of Applied Entomology (LEAA) placed in Via Isonzo 20, Rozzano Milan, where are rearing at 26 ± 2 °C 70% RH and photoperiod light darkness 16L:8D.

The test organisms used were typical insects infesting food industries and also collected in pet stores.

For the test were employed eggs 72 h laid of *Plodia interpunctella* (Hübner), adult stage of *Tribolium confusum* (Jaqcquelin du Val) and *Oryzaephilus surinamensis* (Linnaeus). Table 1 reported the species and stage used for the test.

Tab. 1 Species, stages and substrates used for the test.

Insect	Stage	Quantity	Substrate TNT	Substrate T
P. interpunctella	Eggs 72 h laid	50	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran	Organic litter
T. confusum	Adult	10 adults	Semolino, brewer's yeast, bran	Organic litter
O. surinamensis	Adult	10 adults	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran	Organic litter

2.1 Substrates

The test was conducted to compare the level of development on organic litter with a substrate normally used for breeding. *P. interpunctella* and *O. surinamensis* were reared on a diet composed as follow honey 15%, glycerin 5%, white flour 20%, semolina 20%, yellow flour 15%, oatmeal 5%, sesame 15%, bran 5%.

T. confusum diet was composed 70% semolina, 29% bran and 1% brewer's yeast.

The composition of the organic litter, vegetable granules, obtained by extracting and drying the fibrous part of the corn's ear, was reported in table 2

Tab. 2 Composition of organic litter.

Component	Range
Raw ashes	1 – 2%
Raw protein	0,5 – 1,5%
Raw lipidis	0,1 – 1%
Raw fiber	33 – 40%
Extraction inazotati	50 – 60%
Moisture	4 – 10%.

2.3 Test unit

The substrates whit the test species were put into small plastic container 6 cm diameter and 6,5 cm high. The container was covered with a plastic twist cap provided with a small hole 1cm diameter, covered with a special filter that avoid the escape of insects and allow the air exchange.

2.4 Test site

The test was conduced in the Peet Grady room of LEAA at 26° ± 2 °C 70% RH and photoperiod light darkness 16L:8D in 4 replicates.

2.5 Application method

2.5.1 Lepidoptera

For the test with *P. interpunctella* the eggs were collected from breeding and examineted at the stereo-microscope to verify their integrity and to exclude the presence of mites. After the check, 8 groups of 50 eggs were sorted each in one plastic container four of which were filled with the balanced diet (TNT) and the other four have been filled with organic litter (T) to give the possibility to the newborn larve to feed.

2.5.2 Coleoptera

For *O. surinamensis,* 8 groups with 10 adults were sorted in 8 plastic containers 4 with balanced diet (TNT) and 4 with test substrate. For *T. confusum* 10 new born larvae per group were arranged as above.

2.6 Evaluation method

After the start of the test, the experimental units were checked every seven days and the development stage of the test species at the time of the assessment was noted. In according to the scale shown in table 3 the qualitative data was converted in number for statistical analysis.

Tab. 3 Table of conversion for data analysis.

Index	Stage achieved (at least one individual)
0	No development
1	Newborn larvae
2	Mature larvae
3	Pupae
4	Adults

The obtained data was statistically elaborated with t-test. For all test species the test was stopped at the appearance of adults.

2. Results

The results showed a difference in development in all the species. Only *T. confusum* showed the ability to complete the life cycle to organic litter successfully. The table 4 below showed the results observed.

Tab. 4 Table of results (mean of four replicates). Means followed by "*" are significantly different ($p<0,05$) than the response to the control (t-test).

P. interpunctella	*T. confusum*	*O. surinamensis*

	T	TNT	T	TNT	T	TNT
T 1	0	0,25	2	2,5	0	0
T 2	0,25*	1	2,75	2,5	0*	3,25
T 3	0,25*	1,5	3,5	3,75	0*	2,75
T 4	0,5*	2	4	4	0*	2,5
T5	0,5*	3,25	4	4	0,25*	4

P. interpunctella was not able to develop on organic litter, indeed young larvae failed to develop in any replicates. *O. surinamensis* too showed difficulties in develop on organic litter and only one adult emerged by one replicates in all the test.

T. confusum showed a significative difference in development only in the first assessment. The following assessments showed no significant difference to the control.

3. Discussion

These data confirmed and integrated the avaible information in literature about the influence of diet on development of stored pest, with reference to *P. interpunctella* and *O. surinamensis* (Fields *et al.*, 1992; Johnson *et al.,* 1995, Hagstrum and Milliken, 1988; Waldbauer and Bhattacharya, 1973).

With reference to *T. confusum*, this study showed that it was able to complete successfully his development on organic litter. These data are preliminary and require further investigations on the possible development on organic litter by other stored food pest in addition to adjustments to the experimental protocol.

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Unusual cases of product contamination by 'wandering' larvae of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae)

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ABSTRACT

Upon hatching, the larvae of the Indian meal moth (IMM), *Plodia interpunctella*, disperse vigorously. Within a few hours, they establish themselves on the crevices of food or enter packaged product through small openings and cracks. When on food the larvae intensively feed in or near a tunnel-like case made of frass and silk they web together. The number of larval instars varies from five to seven, depending on temperature, humidity and available food quality. Most mature larvae leave the food medium and search for a suitable place to spin a cocoon in which they pupate or hibernating (diapause). At the end of larval development, the larvae usually chews a hole in a packaging foil, and leave the medium to pupate outside in corners and cracks and also behind

items on walls. Fully grown larvae of the IMM may travel a considerable distance before pupating in a location that is frequently away from the larval food source. It will be proven and illustrated that during this time larvae the IMM may penetrate the packaging material of some household items that were not their food source. Unusual cases of product contamination by 'wandering' larvae will be described. Client claims are thus frequent as only a few larvae in a package with their webbing and frass are very repulsive to homeowners. Impact of product contamination by 'wandering' larvae of the IMM to the firm marketing the products will be discussed.

Key words: Indian meal moth, *Plodia interpunctella*, larva, food products, contamination, client claims

1. Introduction

Females of the Indian meal moth (IMM), *Plodia interpunctella* (Hübner), lay 200-400 eggs singly or in a small batches on food products or near them (Mullen and Arbogast, 1977), sometimes spatially aggregated in some fashion (Arbogast and Mullen, 1978). These eggs are rounded or elongated (0.3 x 0.6 mm) and white, turning orange over time. The larvae (L1) that emerge from these eggs are very small and barely visible to the human eye. They usually disperse vigorously in a search for food, and after detection of the food odor they move into its direction and find food source finally (Sedlacek et al., 1996). Within a few hours, they establish themselves on the crevices of food or enter packaging through cracks and crevices. The larvae eat the stored products in or near a tunnel-like case of frass and silk they web together (Mueller, 2010). Larvae feed greedily on various food products, grow quickly and molt. Fully grown larvae called also the 'wandering larvae' are 9-19 mm in length, with an average of about 1,25 cm. Their color is usually dirty white, but may range from pink to brown to a greenish tinge. The number of larval instars varies from five (Allotey and Goswami, 1990) to seven, depending on temperature and available food (Tzanakakis, 1959).

Thus, the IMM larvae contaminate food products with their presence and webbing containing larval excreta (frass) and exuvia (cast skins). Customers usually find the food product to be infested when larvae grow up and produced a vast amount of webbing.

What is more, the fully grown larvae of the IMM (the wandering larvae) leave the food medium, and they may travel a considerable distance before pupating in a location that is frequently away from the larval food source. Before and during this migration the larvae may penetrate the packaging material of many household foods (Robinson, 1996). Simply, some products (food and non-food) kept in storage are indirectly contaminated by wandering larvae that usually search not for food, but for pupation sites. The presence of larvae within these products causes consumer complaints and rejection of these products.

This paper will prove and illustrate the cases that during search for pupation sites larvae of the IMM may penetrate the packaging material of some household items that were not their food source. Unusual cases of product contamination by 'wandering' larvae will be described. Client claims are thus frequent as only a few larvae with their webbing and frass in a package are very repulsive to homeowners. Therefore, the impact of product contamination by 'wandering' larvae of the IMM to the firm marketing the products will be also discussed.

2. Materials and Methods

Within last 3 years, a company "Trojszyk" Entomological Consulting, Warsaw, Poland, received various products contaminated by pests or sometimes pictures illustrating the pest contamination as customer complaints. These were provided with the orders of manufacturers to determine an insect pest to the species, and to explain each case of product contamination. Among requests there were some cases of unusual contamination of different food and non-food products, and they were accompanied by the repulsive customer claim. These non-typical cases were selected, analyzed and presented in this paper. Cases were illustrated with the pictures, a part of them done by the customers, therefore some of them are of low quality.

3. Results

The typical and unusual cases of collateral contamination product by IMM larvae from another food sources are illustrated and described below.

Case No. 1:

A consumer provided a chocolate bar that was contaminated only with large fecal pellets (frass) and delicate webbing that were produced by the fully grown IMM larvae. No larvae and no other signs of pest activity were noticed.

This chocolate bar was traditionally packed, and the aluminium foil and paper were used as packaging materials. Wrapping materials did not constituted a barrier that prevented the larval invasion. Thus, fully grown larvae visited the product for a short time, and they laid down only fecal pellets and a few threads of silk webbing on the surface of chocolate bar. Probably, a nearby product was infested and it formed a source of wandering larvae.

Fig. 1 Excreta (frass) of the IMM larvae on the surface of the chocolate bar with no other signs of the larval activity.

Case No. 2

Chocolate and nut candies were provided for the evaluation. The thorough investigation of these candies under a microscope revealed that these candies were contaminated only on the external surface of candy wrappers (Fig. 2 & 3). No signs of pest activity were found on the candy surfaces.

A live pupa of the IMM (Fig. 2) and remains of pupal skin (Fig. 3) left by an emerging moth were found in spaces of the wrapper that were suitable for pupation. Silk cocoon was produced during warm months as it was made of delicate silk webbing. The product was contaminated by the fully grown larvae that were interested only to find no food but a proper space for pupation. Some other products (may be other candies of the same lot) were contaminated by the pest, and these products were a source of larvae.

Case No. 3

IMM larva was found under a cap of the bottle with mineral water (Fig. 4). It was fully grown larva (wandering stage) within the cocoon made of delicate silk webbing. Thus, the cocoon was produced during the summer months. Mineral water as a content of the plastic bottle was not contaminated by larva or larval excretes. Possible entries to the space confided by a cap are indicated in Fig. 5 with arrows. This is essentially collateral damage from another food product.

Fig. 2 A live pupa of the IMM within the candy wrappers.

Fig. 3 Remains of pupal skin left by an emerged IMM moth

Case No. 4:

A customer consumed one third of content of the ketchup jar, and on the following day found larva under the jar lid (Fig. 6). The consumer documented this case of product contamination by the picture, and reported a complaint to the manufacturer.

The larva found under the jar lid build already a delicate cocoon, indicating the summer contamination. This larva seems to be freshly pupating, thus it contaminated ketchup jar 1-2 days ago, and it happened in the customer house, not at a premise of the manufacturer nor in the retail food store.

Fig. 4 Larva of the Indian meal moth under the cap of bottle with mineral water.
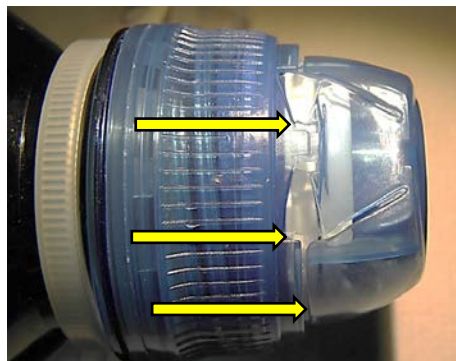
Fig. 5 Possible entries for the larva of the Indian meal moth

Fig. 6 IMM larva in a pupal cocoon found under jar lid.

Fig. 7 Pupa of the IMM in a hibernation cocoon found under lid of jam jar

Case No. 5

The Case No. 5 is similar to the Case No. 4. A pupa of the IMM was found under a lid of the jam jar (Fig. 7) that was freshly bought at the local retail food shop. The pupa was alive, and its body color was deep brown. Pupa was confined within a dense silk cocoon. Pupal stage of the IMM lasts 15-20 days under prevailing room temperatures about 20oC (Sedlacek et al., 1996). Thus, the cocoon was spun during a cold month (made of a dense webbing), and development of pupal stage was advanced (deep brown coloration of pupa). The customer provided the receipt indicating that the jam jar was purchased a few days ago. All these facts prove that the pest contaminated the jam jar at the retail food shop.

Again, the final product was contaminated by wandering larva of the IMM. That larva left the product in which it was feeding and developing, and after searching around for the hiding place larva finally found a proper place under the lid of glass jar, constructed a silk cocoon there and pupated.

Case No. 6

An unusual food contamination by live larvae of the IMM was reported on November 2016 by a customer which opened the originally closed glass jar, and found two moving larvae on the surface of jam (Fig. 8). Label data indicate that jam was manufactured on March 2016. Thus, the product was not infested at the manufacturer site, as it was not possible for IMM larvae to survive more than a half of year on the surface of jam kept in a tightly closed glass jar.

Simply, larvae of the IMM fall down on the surface of jam when a customer removed a lid off the jar, devastating a silk construction of the cocoon that was formed outside of jar, just under its lid.

Fig. 7 Pupa of the IMM in a hibernation cocoon found under lid of jam jar

Fig. 8 Two live larvae of the Indian meal moth on the surface of the final product

Case No. 7

The most unusual case of product contamination is illustrated by Fig. 9. It presents several fully grown larvae of the IMM within the diapers (baby nappies). Packaging of diapers was not insect-proof, and wandering larvae readily penetrated into the bag with diapers. They were not searching for food, but only for a good hiding place for pupation. A nearby product was heavily infested by larvae of the IMM, and it should be removed from a premise as soon as possible to prevent the further spreading of wandering larvae.

Discussion

Indian meal moth (IMM) is a world-wide insect pest of stored products and processed food commodities. Cox and Bell (1991) noted that this moth 'has the widest distribution of all moths generally infesting stored foods and is truly a global pest'. Also, it is one of the most troublesome pests in retail shops or private households. Infestation of the stored food products by IMM can cause a direct product loss and indirect economic costs through pest control costs, quality losses, and considerable amount of consumer complaints.

The cause of consumer complaints are the IMM larvae or pupae in cocoons found within the product. Only one larva or a few larvae in a package with their webbing and frass are very repulsive to homeowners, and very costly to the companies that market the products. Consumer complaints about the presence of insects on or in packaged products can affect the reputation of the brand or manufacturer.

Fig. 8 Two live larvae of the Indian meal moth on the surface of the final product

Fig. 9 The wandering larvae of the IMM found a good hiding place for pupation within the diapers (nappies).

When full grown, IMM larvae usually leave by the hole chewed in packaging material of the product and pupate in a suitable place outside the package (Robinson, 1996). They then emerge from their food source and can travel some distance before spinning their cocoons in various crevices or at wall/ceiling junctions. Pupation usually occurs not only in the vicinity of their food (Mueller, 2010), but also away from their food source. Some larvae spin their cocoons in the food medium just below the surface, but cracks, crevices or other protected places, typically in dark locations, are preferred by the others. One infested package of product in a store can be a source of larvae that search out other products usually to pupate on the surface or interior spaces of the other packages. Thus, a collateral contamination with pest from another food products should be considered (Fig. 1-9). Therefore, control treatment with insecticides should be followed by the advanced inspection including a search for pupal cocoons in corners and cracks and even … behind items on walls. All food and non-food product must to be checked, even those perfectly sealed packages that contain non-food for the IMM larvae (Fig. 6 & 7) as well as the diapers (baby nappies) (Fig. 9).

Cocoons spun by the pupating larvae can be differentiated from those made by the hibernating or diapausing larvae. The hibernaculum (i.e., hibernation cocoon) is dense and completely closed (Fig. 7), whereas the pupal cocoon is flimsy, loose fitting, tapering, and opens anteriorly to permit exit of the adult (Fig. 6). Following hibernation, the larva opens a hole in the hibernaculum and either spins its pupal cocoon inside, or comes out and constructs the pupal cocoon nearby. It appears that larvae spin pupal cocoons outside the hibernaculum only when the hibernaculum is not large enough to include the pupal cocoon (Sedlacek et al., 1996). The differentiation between the pupal cocoons and dense hibernation cocoons should be always conducted when we want to explain the case of product contamination by IMM. For example, when a product was produced in February and some

pupal cocoons were found in August, then one may conclude that the product was not contaminated at the manufacturer site.

Diapause provides a means for the species to overwinter or survive periods of adverse environmental conditions at higher latitudes in unheated situations. The extent to which different strains diapause varies greatly, and those from the tropics or long reared in laboratories showing a reduced capacity. Diapause induced in response to short photoperiods (Bell, 1976), low temperature, or high population pressure (Tsuji, 1963) may greatly extend the developmental periods. At the limits of its range, IMM may have only one to two generations per year, but as many as eight generations per year may occur in warmer climates (Tzanakakis, 1959; Stratil & Reichmuth, 1984). Therefore, the use of the larval developmental time under the prevailing room temperatures is cumbersome to determine the moment of the product contamination by the IMM larvae. Only during warm months it is possible to indicate the time (and place) of product contamination when live larvae of the IMM or live pupae in the pupal cocoons are found within the infested product.

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Susceptibility of dried berries to infestation by *Plodia interpunctella* (Lepidoptera: Pyralidae) in correlation with total sugar content

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Abstract

By assessing the degree of resistance of stored products to infestation by insect pests and correlating it with physical, chemical and nutritional characteristics of products, we could gain a real insight in these pests feeding preferences, and consequently in their biology and ecology. The aim of this study was to assess the degree of resistance of five dried berry species (strawberry, raspberry, blackberry, black chokeberry and cranberry) to infestation caused by the major pest of dried berries, *Plodia interpunctella*. Susceptibility was rated based on the Index of susceptibility (IS) for insect development and the Susceptibility rating. Dried cranberries were

absolutely resistant to infestation by *P. interpunctella* (IS = 0) - no larvae reached the adult stage. Four other dried berry species were also resistant (IS ranged 2.01 – 2.44). In other words, dried cranberries are very unsuitable food for *P. interpunctella*, while other four tested species were slightly more suitable. The content of total sugars in dried berries varied from 24.2% (black chokeberry) to 72.8% (strawberry), but important correlation between IS and total sugar content was not found. By analysing feeding preferences of *P. interpunctella*, we can undertake different pest-management strategies for protection of stored dried fruits.

Keywords: Indian meal moth, dried fruits, infestation, index of susceptibility, susceptibility rating.

Introduction

During all stages of storage process and in all types of storages, dried fruits could be infested by different stored product insect pests like *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Tribolium castaneum* (Herbst) and *Oryzaephilus* spp. Periodically, some polyphagous moths, beetles and mites could also be found (Simmons and Nelson, 1975; Hagstrum and Subramanyam, 2009; Johnson et al., 2009, Almaši and Poslončec, 2010). The most important pest of dried fruits is Indian meal moth, *P. interpunctella* (Johnson et al., 2009), which larvae eat the inside and out of the fruit and cover it with excrements and silk, making it unusable for human diet (Burks and Johnson, 2012). In other words, the biggest losses are in the quality of goods. This moth is of the greatest concern for dried fruits processors (Burks and Johnson, 2012), although studies report that it develops poorly on dried fruits in the laboratory (Arbogast et al., 2005).

In Serbia, the most commonly used dried fruits are prunes, raisins, dried figs and apricots. Recently, different types of dried berries are becoming more popular in human diet and production of dried berries is increasing (Statistical office of the Republic of Serbia, 2018). There are no published data about losses and damages in dried berries caused by *P. interpunctella*. But still, in personal communication with a lot of small producers in Central Serbia, it is emphasized that *P. interpunctella* makes a lot of damages in storages of dried fruits and berries. Besides fumigant control with sulfuryl fluoride and phosphine, the most important methods in control of this pest are sanitation, pest exclusion, sanitary facility design and environmental conditions in storages, especially temperature control (Heaps, 2012).

Dried fruits and berries also have their own susceptibility to infestation by *P. interpunctella*. It depends on the type of fruit, particularly mesocarp density and structure, and also on its nutritional quality and level of moisture. Dried fruits contain > 10% of moisture, which makes them very suitable substrate for the development of this pest. If dried fruits contained < 10% of moisture, it would be more resistant to infestation, but would also be unattractive to human consumers (Sood, 2011). Besides water, dried fruits contain a lot of sugars, commonly > 30% (Cvetković et al., 2009). Proteins and fats, which are very important for insect development, are found in very small amount in dried fruits. Therefore, we hypothesize that the total sugar content could be an important factor that influences the suitability of dried fruits and berries to development of *P. interpunctella*. Based on this hypothesis, the aim of this study was to assess the degree of resistance of five dried berry species (strawberry, raspberry, blackberry, black chokeberry and cranberry) to infestation caused by *P. interpunctella* in correlation with total sugar content.

Materials and Methods

Parental *P. interpunctella* population used in this study was reared for ~50 generations in the Laboratory of General and Applied Entomology, Faculty of Science, University of Kragujevac, Serbia. The population was reared in climate chamber (27 ± 1°C, R.H. 60 ± 10% and photoperiod 14:10 (L:D)), in transparent plastic containers (1.2L in volume) and fed on standard laboratory diet (Silhacek and Miller, 1972). About 100 one-day-old moths *in copuli* were transferred from rearing containers to oviposition jars and one-day-old eggs were used in assays.

Suitability of five dried berry species commonly used in Serbia were tested as a nutrient medium for *P. interpunctella* larvae: strawberry (*Fragaria* × *ananassa* Duchesne), raspberry (*Rubus idaeus* L.), blackberry (*Rubus fruticosus* L.), black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) and cranberry

(*Vaccinium macrocarpon* Aiton), all bought in a local market. 100 mL of each dried berry species were measured and placed into separate glass jars (250 mL in volume). Assays were repeated 12 times for each dried berry species, with a total of 60 replications (jars). In each jar, 50 *P. interpunctella* eggs were added. Jars were then sealed with cotton swab, coated with cotton cloth, for proper aeration. The experiment was carried out in the same environment conditions as described for the rearing of the parental moth population.

Once the emergence of adults began, jars were checked every 24 h and the number of emerged adults and mean developmental duration (MDD) for each adult were recorded. The mean developmental duration was calculated as the average time (in days) from the start of the experiment to the emergence of each adult.

The degree of resistance of five dried berry species to infestation caused by *P. interpunctella* was calculated based on the Index of susceptibility (IS) for insect development (Dobie, 1974)

$$IS = \frac{\left(\ln\left(F_1\right)\right)}{D} \quad 100$$

where F1 represents the mean number of *P. interpunctella* adults that emerged in twelve replications during the experimental period, while D represents MDD. Susceptibility rating (SR) was based on the calculated Indices of susceptibility as suggested by Mensah (1986):

- IS value 0.0 – 2.5 = resistant;
- IS value 2.6 – 5.0 = moderately resistant;
- IS value 5.1 – 7.5 = moderately susceptible;
- IS value 7.6 – 10.0 = susceptible;
- IS > 10 = highly susceptible.

The analyses of total sugar content in dried berries were conducted in Accredited Laboratory (ISO/IEC 17025:2005) of the Center of Hygiene and Human Ecology, Institute of Public Health Kragujevac, Serbia. Total sugar content was determined according to the Luff-Schoorl method for determination of total sugars after inversion, as described in Anonymous (1983). The results are expressed as mass percentage of the sample. Each value was measured three times and averaged with standard error.

Data were statistically analysed using IBM SPSS Statistics 21 software package. Means of Indices of susceptibility and the total sugar content were compared using the Bonferroni test (p < 0.05). Correlation between the Indices of susceptibility and the total sugars content was calculated using Pearson coefficient of correlation.

Results

Indices of susceptibility and susceptibility ratings of five analysed dried berry species are presented in Tab. 1. Cranberries were absolutely resistant to infestation by *P. interpunctella* (IS = 0.00). In this assay, one week after the beginning of the experiment, all larvae died. Four other dried berry species were also resistant to infestation by *P. interpunctella*, with higher IS values, ranging from 2.01 (black chokeberry) to 2.44 (blackberry). Index of susceptibility of cranberry was significantly lower than those of four other tested dried berry species (p < 0.0005). There were no significant differences established among any of four other dried berry species (p = 1.0).

The results of the total sugar content in five dried berry species are presented in Tab. 1. Dried strawberry had the largest content of total sugar (72.8%), while dried black chokeberry had the lowest (24.2%). Correlation between IS and the total sugar content was negative and weak, statistically insignificant (r = -0.230; p = 0.709).

Tab. 1 Mean values of Index of susceptibility (± SE), susceptibility rating and the total sugar content (%) of five dried berry species to infestation by *Plodia interpunctella*

Dried berry	Index of susceptibility (IS)	Susceptibility rating (SR)	Total sugar content (%)
Strawberry	2.26 ± 0.22 [a]	Resistant	72.80 ± 1.56 [a]
Raspberry	2.37 ± 0.41 [a]	Resistant	51.43 ± 0.80 [d]
Blackberry	2.44 ± 0.36 [a]	Resistant	60.40 ± 0.36 [c]
Black chokeberry	2.01 ± 0.18 [a]	Resistant	24.20 ± 0.52 [e]
Cranberry	0.00 ± 0.00 [b]	Resistant	66.74 ± 0.26 [b]

Vertical mean values of Index of susceptibility and total sugar content having different letters in superscript are statistically different by one-way ANOVA test and Bonferroni test at $p < 0.05$.

Discussion

Due to our knowledge, there are no data about life history of *P. interpunctella* on dried berries, although this moth is one of the most important pests of dried fruits in the world (Hagstrum and Subramanyam, 2009; Sarwar, 2015).

Values of indices of susceptibility and resistance of five tested dried berry species to infestation by *P. interpunctella* could be attributed to their nutritional and moisture content. A few studies showed that nutritional content is of primary importance for successful development of *P. interpunctella*, while moisture content is of secondary importance (LeCato, 1976; Sambaraju and Phillips, 2008; Burks and Johnson, 2012; Predojević et al., 2017). In this experiment, we hypothesized that total sugar content, as major nutrient in dried fruits, could be an important factor that influences the susceptibility of dried berries to infestation by *P. interpunctella*. Our result showed that total sugar content, if used alone as parameter and tested only at five dried berry species, did not show its influence. Sugars are very important for insect development, not alone, but in combination with other nutrients. For example, Arbogast et al. (2005) reported that *P. interpunctella* fails to develop on raisins in the laboratory, while in storage it completes development, thanks to the fungal presence, because the conidia of fungi supports neonate larval development.

In this experiment, five tested dried berry species were resistant to infestation by *P. interpunctella*. Dried cranberry was the most resistant. Value of IS for cranberry was 0.00, because seven days after the beginning of the experiment all larvae were dead. Values of IS of four other tested dried berry species were higher, especially for blackberry (2.44). These results indicate that damages of *P. interpunctella* to tested dried berries were small, but important for the quality of goods, because it makes them much less desirable for human consumption.

Numerous studies showed that some dried fruits are not as suitable for development of *P. interpunctella* as some other types of food (like nuts, maize etc.), especially in laboratory conditions, but still, it is the most important pest of dried fruits. Johnson et al. (1995) reported that prunes are unsuitable food for *P. interpunctella*, because only 0.7% of individuals emerged as adults and MDD lasted between 80 and 160 days. Studies of Johnson (2004), Sambaraju and Phillips (2008), Almaši and Poslončec (2010) and Vukajlović et al. (2017) also indicated that development of *P. interpunctella* on prunes lasts very long, while the number of survived individuals is low. Similar results were published for dried apricots (Almaši and Poslončec, 2010; Vukajlović et al., 2017) and dried cherries (Vukajlović et al., 2017). Recent study showed that dried apricots, prunes and cherries were resistant to infestation by *P. interpunctella*, with IS valued 0.00, 0.78, 1.01, respectively (Vukajlović et al., 2017).

By assessing the degree of resistance of different dried fruits to infestation by *P. interpunctella* and correlating it with physical, chemical and nutritional characteristics of dried fruits, we could gain a real insight in this pest feeding preferences, and consequently in its biology and ecology. On the other hand, by knowing these facts, we can undertake different pest management strategies for protection of stored dried fruits.

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Behaviour of the Angoumois grain moth (*Sitotroga cerealella* Oliv.) in different grain substrates and assessment of losses

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Abstract

The Angoumois grain moth, *Sitotroga cerealella*, is a primary stored grain pest, which development occurs within a single grain. The respond of the pest to various offered grain substrates was studied in no choice laboratory experiment (temperature 27±1ºC; relative humidity 60-80%), by rearing moth populations on entire grains (corn, wheat, barley, sorghum, millet, tall fescue and Kentucky bluegrass) and mechanically damaged grains (corn in fractions with/without embryo, polished rice). The pest behaviour was determined by observation of the entrance and exit hole position on different grains. The food consumption was estimated after adult emergence, by measuring of mass losses of infested grains. Mass losses were correlated with quantitative and qualitative grain parameters. The development was successfully accomplished in all grain substrates, except in Kentucky bluegrass. Strategies of larval penetration and exit hole position depended on morphological properties of grains. As a rule, the development of an individual was completed in a single grain, but in polished rice the transfer from one to another grain was observed. The highest loss of infested grain was recorded in corn grains (55.48 mg), the lowest in tall fescue grains (2.40 mg). Positive correlations were detected between the mass losses and protein, lipid and sugar content, negative in relation to cellulose and ash content.

Keywords: grain, Poaceae, *Sitotroga cerealella*, behaviour, losses.

Introduction

The Angoumois grain moth (AGM), *Sitotroga cerealella* (Olivier), is worldwide distributed primary stored grain pest. In tropical, subtropical and temperate regions with warmer climate it can also affect cereals in the field (Vukasović, 1940). The complete development of an individual generally occurs inside the kernel and therefore the survival directly depends on quantitative and qualitative properties of the available food resources, provided by a single infested kernel. The females of *S. cerealella* lay the eggs by attaching them to the grain surface, being stimulated by mechanical contact of the abdomen with the tight interspaces between the kernels (thigmotactism). After hatching, the larva penetrates the kernel and once inside it continues to feed and develop. Before the pupation, the mature larva extends its feeding chamber to the outside of the kernel, leaving intact only the epidermis of the bran, a symptom of infestation that is visible from outside as a circular transparent "window". After emerging, the adult pushes the "window" and leaves a small characteristic round exit hole. The development and survival of an individual is strongly directly depending on the available food resources, which are determined and limited (quantitatively and qualitatively) by a single inhabited kernel, by itself.

The objective of the present study was to evaluate the convenience of different cereal species/types of grains (whole grains or mechanically modified grain kernels) as feed for the AGM by comparing penetration modes of neonate larvae in different offered grain types, positions of adult exit holes, as well as by evaluation of the final results of infestation: number of emerged adults, mass losses of overall infested grain substrates and single infested kernels, as well as to determine on which extent the survival of AGM and consecutive losses depend on quantitative and qualitatative properties of grains.

Materials and Methods

The respond of the pest to various offered grain substrates was studied in no choice laboratory experiment in controlled conditions of temperature (27±1ºC), relative humidity (60-80%) and photoperiod (16h/8h light/dark), by rearing AGM populations on grains of different Poaceae plant species, including whole and mechanically damaged kernels of the following plant species:

- Corn, *Zea mays* L. (NS SC 444), whole grains
- Corn, *Zea mays* L. (NS SC 444), fraction with embryo
- Corn, *Zea mays* L. (NS SC 444), fraction without embryo
- Wheat, *Triticum vulgare* Host (Balkan), whole grains
- Barley, *Hordeum sativum* J. (NS 27), whole grains
- Rice, *Oryza sativa* L. (population), polished (white) grains

- Sorghum, *Sorghum vulgare* Pers. (NS-šećerac), whole grains
- Millet, *Panicum mileaceum* L. (population), whole grains
- Tall fescue, *Festuca arundinacea* Schreb. (NS- visoki vijuk), whole grains
- Kentucky bluegrass, *Poa pratensis* L. (population), whole grains.

The two fractions of corn grains were obtained by cutting each grain transversally at the line approximately corresponding to the middle grain length.

Eggs of the same age, with ongoing embryogenesis confirmed by the detection of the formed larva under the transparent chorion, were transferred to plastic jars (500 ml), filled with a grain substrate (10 different substrates, 4 replications, 100 eggs per unit). The successful hatching was confirmed by observations of empty transparent chorions.

Each of the 40 experimental units contained 37.5 g of a grain substrate, equivalent to the average weight of 100 undamaged corn grains, which as being the largest grain species could hypothetically assure the development of at least 1 larva/grain.

Quantitative and qualitative parameters of each grain species/type regarded: the measurement of the mass of uninfested kernels (with precision of 0.01 g), determination of sugar content (mg/g of dry matter), protein, lipid, cellulose and ash content (% of dry matter) and energy value (J/g) of each grain substrate before exposition to AGM. Standard methodology was applied for determination of protein content (Kjeldhal method), lipid content (Soxlet method) and cellulose content (Sharner-Kurschuer method), while the ash content was determined by heating at 550ᵒC. Sugar content was determined by the method of liquid chromatography (VAN RIEL AND OLIEMAN, 1989) and the energy value by the method of calorimetry described in KRAJCOVIC AND REGAL (1976).

Emerged adults were extracted from the jars on daily basis and the total number of emerged adults was recorded in each experimental unit. The termination of the eclosion was assured by absence of newly hatched adults in the period of 14 days after the last recorded adult emergence.

The pest behaviour in relation to different offered grain species/type was determined by visual observation of the mode of penetration of neonate larva into akernel (i.e. position of entrance holes), as well as by the position of the exit holes of the emerged adults. Determination of infested kernels was conducted under magnification of binocular microscope Wild M400.

After the termination of adult eclosion, the measurements of the mass of infested substrates (expressed in grams) and single infested kernels (in milligrams; up to 50 kernels with a single exit hole/unit) were conducted in each experimental unit and compared with the related values obtained before the infestation in order to evaluate the mass losses. Average mass loss of an infested kernel (expressed in mg and %) represented directly the total feed consumption (i.e damage) of a single individual in larval stage that successfully developed and survived until adult emergence. Furthermore, the obtained data on mass losses of infested grain substrates and number of emerged adults served for calculation of average mass losses per survived individual (adult). Comparison of the results for each obtained quantitative paramether (number of adults, mass losses of grain substrate, mass losses of grain substrate/adult and mass losses of infested kernel) was conducted by Duncan Multiple Range Test for significance level of p = 0.05.

Finally, the impact of qualitative grain paramethers to the expression of the number of emerged AGM adults and to the mass loss paramethers (mass losses of grain substrate, substrate/adult and infested kernels) were identified by determination of correlation coefficients and the level of their significance. The statistical analyses were conducted in Statistica 13.2 software (Dell Inc. Dell Statistica (data analysis software system) version 13. 2016. (http://software.dell.com).

Results

The development of AGM was successfully accomplished in all tested grain substrates (with whole and mechanicaly damaged kernels), except in Kentucky bluegrass. Neonate larvae were not able to bore the entrance hole in Kentucky bluegrass, probably because of the structure of the husk. Indeed, during the carefully observation of the behaviour of neonates on Kentucky bluegrass grains under

the microscope magnification, it was remarked that the trichomes on the husk represent the mechanical barreier limiting the larval movement and causing letal injuries to larval body. Thus, Kentucky bluegrass may not be considered by us as a host plant species.

Observations of exit holes on infested kernels of other grain species/types demonstrated that usually only one AGM individual can complete the development in a single kernel. An exception was recorded in the substrate with whole corn kernels, where appart the most frequent combination of one individual per single kernel, also two AGM individuals could inhabit the same kernel and successfuly accomplish the development.

Entrance and exit holes

The position of the entrance hole of newly hatched larvae (neonate) on the kernel surface of the offered grain species/type indicated that larvae perform different strategies of penetration depending on grain the properties. In whole, undamaged grains (corn, barley, wheat, sorgum, millet, tall fescue), as a rule, the entrance holes were detected in the zone of the germ (embryo). In such cases, the exit holes were always located at the opposite end (latero-terminally), indicating that during the feeding a larva is following the direction of the longitudinal axis of the grain. Moreover, in some of the infested wheat kernels, the entrance hole was also detected on the dorsal side, in the zone of endosperm and in such cases the exit hole was located at the oposite, ventral side of the kernel. In millet grains, covered by tightly adhering husk (*palea* and *lemma*), which represents a hard, insuperable mechanical barrier, the precise location of the entrance hole coincided with the micropyle, as a naturaly present opening. In tall fescue, the larvae were able to bore the entrance hole through the husk covering at the ventral side of the kernel, whether in the zone of the germ (down below or through the *rachila*) or in the zone of endosperm, and the exit holes were detected on the terminal side of the kernel, exclusively. The hull present on barley kernels did not represent any kind of barrier for larval penetration in the germ zone.

The position of the entrance hole in the zone of the germ was also detected in mechanicaly alterated corn kernels containing the germ, and in this case the the exit hole was also located on the oposite side laterally, but never directly on in the transfersal cut.

In mechanicaly damaged corn kernels without the germ (fraction without embryo), the penetration of a larva occured in the zone of the transfersal cut, through the space left after the removal of the germ. The exit holes in this grain type were always located on the latero-terminal, intact part of the grain, as in the case of the entire corn grains.

Finally, polished white rice (free of husk and bran), the entrance hole of AGM was found on different parts of the kernel, with no particular rule, but in this substrate type the transfer of the larva from one to another kernel was remarked, suggesting that the food resource obtained by a single grain is not satisfying the food requirements of a larva to complete the development. After consuming the most of the single kernel, a larva was able to pass to the next one in order to continue the feeding. The transfer from one kernel to another occurred within the silky tunnel produced by the larva, which served as protective „bridge" between the kernels. In some cases few (up to 6) kernels were connected with silky threads and some of them were damaged only superficially. The pupation occured whether inside or outside the kernel in the silky cocoon.

Losses

The suitability of different offered grain species/types for AGM was estimated by the number of emerged adults, mass losses of the grain substrates, including the mass losses calculated per emerged AGM individual (Tab.1) and mass losses of infested kernels (Tab. 2).

As demonstrated in Tab. 1 highly significant differences ($p<0.01$) in mass losses of grain substrates, number of emerged adults and estimated consumed mass of substrate per emerged (i.e. survived) individual were recorded among different grain species/type.

Tab. 1 Mass losses of different grain substrates caused by *Sitotroga cerealella* infestation and estimation of the individual consumption based on the number on emerged adults

Grain substrate	Mass losses of grain substrate*			Number of emerged adults**		Consumed mass of substrate per individual	
	Mean (g)	Sd	Mean (%)	Mean	Sd	Mean (mg)	Sd
Corn	4.86 a	0.59	12.97 a	79.75 b	2.75	60.99 a	7.24
Corn fraction without embryo	4.16 b	0.09	11.09 b	68.75 c	6.75	60.82 a	4.80
Corn fraction with embryo	4.24 b	0.13	11.30 b	88.25 a	4.57	48.11 b	2.76
Barley	2.71 c	0.19	7.22 c	86.75 ab	9.78	31.33 c	1.49
Wheat	2.05 d	0.08	5.46 d	84.00 ab	5.72	24.42 d	1.10
Rice-polished	0.94 e	0.08	2.49 e	19,50 d	1.91	48.08 b	2.96
Sorghum	0.97 e	0.04	2.59 e	70.00 c	4.24	13.93 e	0.97
Millet	0.16 f	0.03	0.42 f	19.25 d	2.87	8.17 f	0.27
Tall fescue	0.09 f	0.01	0.25 g	21.00 d	2.45	4.41 f	0.27
Kentucky bluegrass	0.00 f	0.00	0.00 h	0.00 e	0.00	.	.
Analysis of variance F	337.40		863.61	199.31		181.44	
p	<0,01		<0,01	<0,01		<0,01	

*Each experimental unit initially contained 37.5g of grain substrate
**Each experimental unit was initially infested with 100 individuals in egg stage
Mean values labeled with the same letters are not significantly different according Duncan Multiple Range Test for significance level of p = 0.05. In the process of statistical analyses the % values were transformed in arcsin √%

The calculation of mass losses of the whole substrates took in account the losses caused by entire population of hatched larvae which consumed the feed, including also those that eventually died during the development. The highest mass loss was recorded in substrate with entire corn kernels (4.86 g; 12,97%), followed by corn in fractions without and with embryo, barley, wheat, sorghum and polished rice, millet and finally tall fescue with the lowest registered mass loss (0.09 g; 0.25%). In the substrate with Kentucky bluegrass, neither emerged adults nor mass losses were recorded. The highest average number of emerged adults was recorded in substrates with corn fraction with embryo, barley and wheat (88.25, 86.75 and 84.00, respectively), followed by the substrate with entire corn grains (79.75), which was not significantly different from the number of adults recorded in barley and wheat. Significantly lower number of adults were recorded in sorghum and corn fraction without embryo (70 and 68.75), the lowest in tall fescue and millet (21 and 19.25, respectively).

Another general picture of the infestation consequences in each substrate is demonstrated by estimation of the consumed mass of the substrate per emerged AGM individual (Tab.1). Here, the ordination of the substrates following the decreasing values is similar as in the case of comparison of mass losses of the substrate, with the highest consumption/AGM individual recorded in the case of corn entire grain and fraction without embryo (60.99 mg and 60.82 mg, respectively), the lowest in the cases of millet and tall fescue (8.17 mg and 4.41 mg, respectively). Surprisingly high consumption of grain supstrate per AGM individual was recorded in polished rice (48.08 mg) where low number of adults emerged. The estimated individual consumption in rice was not significantly different from the value recorded in the population reared in corn fraction with embryo (48.11 mg), where high number of adults emerged. In order to survive larvae of AGM reared in polished rice, had to consume as high quantity of feed as in corn fraction with embryo and therefore the consumption of more rice grains was required and obtained by the transfer from one rice kernel to another.

The most precise quantification of the larval damage was provided by calculation of the mass losses of single infested kernels (Tab.2), that were determined before the measurement by the presence of both entrance and exit holes. The average mass of a kernel before and after the infestation, as well as the resulting average mass loss of single infested kernel were significantly different depending on the grain species/type. All offered grain species/types had significantly different mass before infestation, with the highest value recorded in corn with entire kernel (375.03 mg), the lowest in Kentucky bluegrass (0.40 mg). As previosly mentioned, in Kentucky bluegrass the infestation of

the kernel was not observed and no losses in substrate mass were recorded. Therefore, within the serial of tested species/types recognized to host AGM, the lowest kernel mass that provides sufficient food resources for the successful development of AGM was recorded in tall fescue (2.80mg).

Tab. 2 Mass losses of single infested kernels of different grain species/type caused by *Sitotroga cerealella*

Grain species/type	Mass of a single kernel				Loss of a single kernel mass		
	Before infestation		After adult emergence		Mean (mg)	Sd	Mean (%)
	Mean (mg)	Sd	Mean (mg)	Sd			
Corn	375.03 a	0,29	319.55 a	7,67	55.48 a	7.61	14.75 a
Corn fraction without embryo	227.30 b	2,12	175.00 b	1,40	52.30 a	1.67	23.01 b
Corn fraction with embryo	143.28 c	0,79	95.60 c	2,51	47.68 b	2.67	33.26 c
Barley	51.43 d	0,17	21.90 e	0,68	29.53 c	0.66	57.41 f
Wheat	50.10 e	0,08	26.60 d	0.33	23.50 d	0.37	46.90 d
Rice-polished	19.63 g	0,30	5.73 g	0.17	13.90 e	0.20	70.83 g
Sorghum	20.75 f	0,06	9.75 f	0,10	11.00 e	0.14	53.01 e
Millet	7.43 h	0,05	1.95 gh	0.06	5.48 f	0.10	73.74 h
Tall fescue	2.80 i	0,00	0.40 h	0.00	2.40 f	0.00	85.71 i
Kentucky bluegrass	0.40 j	0.00	.	.	.	.	.
Analysis of variance — F	114831.83		6328.5		223.15		1472.8
Analysis of variance — p	<0,01		<0,01		<0,01		<0,01

NOTES: Mean values labeled with the same letters are not significantly different according Duncan Multiple Range Test for significance level of p = 0.05. In the process of statistical analyses the % values were transformed in arcsin √%

In accordance with the availability of the mass of food resources determined by a single kernel (Tab.2), the highest average mass loss of an infested kernel (i.e. larval consumption) was recorded in grain species/types having the highest kernel mass (whole corn grain and in corn-endosperm fraction, 55.48 mg and 52.30 mg, respectively), the lowest in millet and tall fescue (5.48 mg and 2.40 mg, respectively). Despite the low number of emerged adults in kernels of low mass (e.g. millet and tall fescue), it was demonstrated that AGM is able to survive with remarkably limited amount of feed, consuming about 10-23 times lower mass of kernel than in optimal conditions provided by wheat, barley or corn. The lowest average mass loss of an infested kernel expressed in percentages was recorded in whole corn kernels (14,75%), the highest in grains of tall fescue where 85.71% of grain mass was consumed. Obviously, the utilization of food resources in grains of low kernel mass is significantly higher.

Statistically highly significant correlations were detected between the paramethers of chemical composition of the grain substrates and number of emerged adults (Tab.3). Sugar, protein and lipid content had positive influence to the development of AGM, while cellulose and ash content had negative influence to the number of emerged adults. Highly significant positive correlation was also detected between the energy value of a substrate and number of emerged adults. Similarly, higly significant influences were also recorded when chemical composition parameters, as well as the energy value of grain substrates were correlated with mass losses of grain substrate, and with mass losses of single infested kernels. The impact of each of the tested chemical composition parameter to the mass loss of the supstrate per emerged adult (i.e. consumption of a survived individual), was also higly significant: positive when it regarded the influence of sugar, protein and lipid content and negative regarding the influence of cellullose and ash content. The only not significant correlation coefficient was established between the energy value of the substrate and mass losses of the grain substrate per emerged adult.

Tab. 3 Qualitative parameters of different grain species/types and correlations with the number of emerged adults of *Sitotroga cerealella* and mass losses parameters

Grain species/type	Chemical content					Energy value (kJ/g)
	Sugar (mg/g)	Protein (% d.m.)	Lipid (%d.m.)	Cellulose (% d.m.)	Ash (% d.m.)	
Corn	15.45	10.10	3.88	2.02	2.54	17.437
Corn - fraction without embryo	16.60	14.01	2.56	1.75	3.36	18.566

Corn - fraction with embryo		16.03	16.52	3.22	1.50	3.08	18.911
Barley		15.00	11.66	2.39	3.52	4.09	17.334
Wheat		14.56	12.53	2.10	2.33	2.81	16.196
Rice - polished		11.53	9.11	1.95	2.36	3.36	14.866
Sorghum		13.56	10.51	2.11	2.21	2.16	17.258
Millet		10.02	11.49	2.76	3.28	5.21	16.487
Tall fescue		9.23	5.06	1.47	3.71	6.83	16.911
Kentucky bluegrass		9.85	4.21	1.64	3.55	5.91	16.722
Correlation coefficients	Number of emerged adults	0.91**	0.76**	0.59**	-0.60**	-0.73**	0.56**
	Mass losses of grain substrate (g)	0.91**	0.68**	0.79**	-0.73**	-0.61**	0.65**
	Mass losses of substrate/adult (mg)	0.72**	0.47**	0.60**	-0.71**	-0.53**	0.32
	Mass losses of infested kernel (mg)	0.88**	0.61**	0.75**	-0.70**	-0.51**	0.66**

d.m.- dry matter   ** highly significant (p<0.01)

Discussion

AGM is worldwide distributed oligophagous pest species that usually attack cereal grains in extensive storage conditions. In temperate regions, as demonstrated by Trematerra (2015) in Southern Italy, the infestations with AGM occurre both during preharvest plantation and postharvest storage, and therefore the author highlighted that warehouses, field-plots and wild hosts distributed on the teritory can each serve as sources of both reproduction and aggregation, depending on the time of the year. Stored grains of plant species that are frequently reported as hosts of AGM are corn, wheat, barley, sorghum, rice, ray, oatand millet, but apart these most usually cultivated cereals it can also develop in grains of some spontaneous Poaceae species of few genera, such as *Lolium* L., *Eleusine* Gaertn., *Phalaris* L. and *Echinochloa* Palisot de Beauvois (Balachowsky, 1966; Dakshinamurthy and Regupathy, 1988). So far, the only available report on successful development of AGM in grains of tall fescue (*Festuca arundinacea*) is given by Ignjatović Ćupina (2001).

Apart the cereal species commonly known as hosts of AGM (corn, wheat, barley, sorghum), this study confirmed the adaptability of the pest to survive in small grain species, such as millet and tall fescue, as well as in mechanically damaged kernels of common host species (corn grain fractions with and without embryo, polished rice), but not in Kentucky bluegrass. Despite the less favorable quantitative and qualitative conditions in host species of small grains, the survival of AGM was still evident at different extent, reflected by lower number of emerged adults and lower mass losses of the infested substrate.

Usually a single grain kernel is infested by only one, single AGM larva. The best conditions for the development of AGM are provided by corn grains, which offer enough food resources for the development of even more than one AGM individuals (up to 3) per single kernel, and such behavior was rarely recorded also in wheat grains (Grandi, 1951; Balachowsky, 1966; Vukasović et al., 1972; Manojlović, 1987). According Prakash et al. (1982), the grain resistence to pest infestation depend on physical and biochemical grain properties, as well as on the pest feeding and/or oviposition preference.

Different modes of penetration of AGM neonate larvae into the kernel are described depending on the plant host species. In corn grains the penetration takes palce in the germ zone (Vukasović et al., 1972), where the bran is thinnest and additionally such strategy provide the most nutritious matters contained in the germ during the initial feeding of the young larva. The same strategy of penetration in the germ zone in sorghum kernels was reported by Wongo (1990) and Wongo and Pedersen (1990).

In the present research the boring of larvae in the germ zone was observed in grains free of hull (corn, corn-fraction with embryo, sorghum, wheat), but also in husked grains (barley, millet and tall fescue). According to Vukasović et al. (1972) penetration of AGM larvae in the germ zone of wheat

kernels seems quite unusual. Penetration in the zone of endosperm in wheat kernels was detected in the present study, but not exclusively (some larvae preferred the germ zone for penetration).

Few authors observed that the hull of rice kernels represents an important protective structure that prevent the penetration of pest insects into the kernel and hard, thick and intact hull represents a resistance factor that affect the penetration of AGM larve (RUSSELL AND COGBURN, 1977; COGBURN AND BOLLICH, 1986; RAGUMOORTHY AND GUNATHILAGARAJ, 1988; SAUPHANOR, 1988; COGBURN *et al.*, 1989; TAKESHITA AND IMURA, 1990).

In grains completely covered by intact hull, which reperesents a mechanical barrier, the penetration may occure through the abscission scar of the central vascular bundle, as described in rough rice kernels by COGBURN *et al.*, (1983). In this research, such beheviour was observed in tall fescue kernels. Similar strategy of penetration was observed in millet kernels thightly enclosed by *palea* and *lemma*, where the larva also chose the natural opening (micropyle) to bore into the kernel. However, the hairy hull structure of Kentucky bluegrass represented the insuperable mechanical barrier for larval penetration into the kernel. The larval mortality of 100% in this grain substrate was obviously attributed to the husk structure, not to the grain size (i.e. food resources).

The imperfect hulls of barley kernels did not represent a barrier for penetration of larvae in the germ zone. Similar observation was reported by COGBURN *et al.* (1983) who stated that rice varieties with imperfect hull favored the infestation by AGM.

Furthermore, the hardness and thickness of the bran also contribute the resistance to penetration of insects into the kernel, as demonstrated in sorghum kernels where the lowest infestation occurred in varieties with the hardest bran layer (SHAZALI, 1985). In the present research, the penetration hole in corn fraction without embryo was always detected on the side of the transfersal cut (free of the bran), where the larvae penetrated through the crevice left after removal of the germ. The possibility of larvae to penetrate mechanically damaged corn kernels suggests that such substrates are equally susceptible to infestation by AGM as whole grains. ALLOTEY AND MOLOKO (2015) recorded higher emergence rate of AGM reared in substrates with whole grains of maize varieties than in mechanically altered grains (cut and ground grains). However, in the present study where the fractions with and without embryo were separately considered, the obtained results demonstrated the highest adult emergence rate in corn fraction with embryo, followed by whole corn grains and fraction without embryo. In contrast, in sorghum and millet varieties ALLOTEY AND MOLOKO (2015) recorded the highest number of adults in substrates with broken grains and such results were explained by the earlier exposure of endosperm for larval feeding and easier exit path between the broken grains. In the present study, small grain species, were offered to AGM only as whole grains. Nevertheless, the emergence rate of 70% of AGM reared in sorghum whole grains was similar to the values recorded by ALLOTEY AND MOLOKO (2015) in both whole or broken sorghum grains (66,3% and 68,8%, respectively), depending on the tested variety.

The most interesting behavior of AGM was observed in polished rice, where no particular rule of larval penetration was observed. Lacking the external mechanical barrier in this type of mechanically processed substrate the larvae were able to pass from one kernel to another by producing the silky tunel between the kernels, and additionally during the transfer some of the surrounding kernels were damaged superficially. Superficial damages on adjacent kernels were reported in rare cases when the food resource provided by a single kernel is scarce (BALACHOWSKY, 1966). Lacking the epidermis layer in polished rice kernels, the exit holes of AGM aduls did not have a typical "window" appearance. Whether the transfer to another kernel was conducted with ultimate pupation inside the kernel, or the pupation occurred outside in the interspace between the kernels in the cocoon, the silky threads connecting the kernels and frass were evident. At a first appearance, such infestation symptom might be incorrectly linked to some other stored product pests that are feeding externaly, such as *Nemapogon granella* (Linnaeus) or *Plodia interpunctella* (Hübner).

Appart the positive association of the integrity of the rice husk with the resistence to pest infestation, COGBURN AND BOLLICH (1986) emphasized that hardness and texture of the kernel surface are of a crucial importance for oviposition and further development, and suggested that nutritive compounds also play a role in grain resistance. However, several aspects are involved in grain resistence to infestation with store products pests, such as the absence of preferences (for oviposition and/or feeding), physical and chemical grain properties and changes during the grain processing (PRAKASH et al., 1982). Furthermore, the grain resistance to infestation depends on the grain size, as observed among different cultivars of pearl millet (SEIFELNASR AND MILLS, 1985). Numerous studies were conducted in order to estimate the survival rate and mortality of AGM in grains of different plant species, as well as in different varieties of the same plant species. According MANOJLOVIĆ (1987), the mortality rate during the post-embryonic developlent of AGM reared under the same conditions in corn hybrids of larger grain size ranged between 36.2% and 40.05%, while in smaller size hybrid it was 41.46%. In the same study, the mortality rate in wheat variety of smaller grain size was also higher (46.02%) than in the varieties of larger kernels (36.2-40.05%). The research conducted by COGBURN (1989) demonstrated that the survival rate of AGM reared in different species of rice with deliberately broken hulls ranged between 0.0% and 40.2% and significantly depended on the size and mass of the kernel. The resistence of different stored rice varieties, expressed through the number of emerged AGM adults was positively and highly significantly correlated with the weight loss (RIZWANA et al., 2011). In different tested varieties of sorghum, SRIVASTAVA (1996) determined significantly different mortality rates during the postembryonic development ranging between 7.47% and 41.64%.

In the present study a series of different grain species/types were tested to AGM infestation. Appart the external physical characteristics of the kernel that influenced the larval penetration, quantitative and qualitative grain properties had significant impact to the survival of AGM (i.e. number of emerged adults) and consecutive mass losses, as expected. Substrates with higher mass of kernels favored the development of AGM resulting in higher number of adults, higher mass losses of the infested kernels and higher mass losses of grain substrates as a whole. In kernels of lower mass, the number of emerged adults was significantly lower, but in such kernels the available food resources were efficiently utilized by the survived individuals (e.g. consumed kernel mass of 73.74% and 85.71% in mellet and tall fescue, respectively). The mass of consumed feed by an individual in such small kernels (5.48 mg and 2.40 mg in mellet and tall fescue, respectively) was significantly lower than in optimal conditions, such as provided by whole corn kernels where the consumption was 55.48 mg. Despite the low mass of consumed feed in sorghum kernels (11 mg/larva; 53,01% of kernel mass), the survival rate (i.e. number of emerged adults) in this small grain substrate was surprisingly high (70%), not significantly different from the survival rate recorded in corn fraction without embryo, but still significantly lower than in corn fraction with embryo, whole corn grains, barley and wheat. Similarly to our results, BORZOUI et. al (2017) also recorded significantly lower food consumption per larval individual in sorghum grains (about 27 mg), than in wheat and maize grains (about 52 mg and 65 mg, respectively), as well as significantly lower survival rate of immature stages reared in sorghum (about 46%) than in maize (65%), barley (68%) and wheat (90%). Our results demonstrated the high adaptability of AGM populations to survive in limited conditions of available feed, determined by the mass of kernels. Nevertheless, the food resource available in polished rice kernel was not sufficient to an individual, and therefore, appart the consumation of 13.90 mg of internaly infested kernel (70.83 % of the kernel mass), the larva was able to move to the next kernel and continue the feeding (supeficialy or internaly). Therefore, the mass losses of polished rice substrate calculated per number of emerged individual was as high as recorded in corn fraction without embryo. However, the ability of AGM to transfer and infest more polished rice kernels did not result in high number of survived individuals.

Obvuiously, the chemical composition and the energy value of the feed were also significantly involved in the survival of AGM and consequently had the impact on mass losses parameters (mass losses of whole grain substrate, mass losses of grain substrate per adult and mass losses of infested

kernels). Reserches conducted by some authors on different varieties of the same plant species were not able to identify significant correlations between the chemical composition parameters (sugar, lipid, protein, starch, ash content) on one side and mass losses or pest survival rate (or index of population growth) on the other side. Such results were obtained in the studies conducted by Pandey and Pandey (1983) in varieties of corn, as well as in the research of Rizwana et al. (2011) in rice varieaties. However, Demissie et. al (2015) also tested different varieties of corn for susceptibility to AGM and found significant positive correlation between the ash content and the number of emerged adults, while the impact of the moisture and phenolic content to the number of total progeny emerged was significantly negative. In addition, the same authors also have not found statistically significant correlations between other studied parameters of biochemical composition of the feed (i.e. content of crude oil, crude carbohydrate, crude proteins, crude fibre, amylose and amylase) and the total emerged progeny, as well as no significant correlations between biochemical composition paramethers and the percentage of weight loss, with the exception of crude proteins and amylose content that had significantly positive impact to the grain weight loss. Recent research of Safian Murad and Batool (2017) demonstrated that varieties of wheat with higher protein and carbohydrate content, higher grain weight and lower grain hardness were more susceptible to AGM, since in such varieties significantly higher number of AGM adults emerged, and higher values of percent damage and percent weight loss were recorded. In our present research, where different plant species/types of grains were compared in relation to AGM development and consecutive losses, the impact of biochemical composition of the feed became clearly evident. Sugar, protein and lipid content were positively and highly significantly correlated with the number of emerged adults and mass losses parameters, while negative, also highly significant correlation was detected with the cellulose and ash content. The energy value of the feed had also statisticaly significant positive impact to the number of emerged adults, to the mass losses of the whole substrate and mass losses of infested kernels.

Based on the presented results, it can be concluded that kernels of small grain species with intact hulls, higher cellulose and ash content, lower sugar, lipid and protein content (e.g. millet and tall fescue), as well as mechanically/morphologically dammaged kernels (e.g. polished rice) had negative impact to the development of AGM populations, which resulted in lower number of survived individuals and lower mass losses of grain substrates. However, in such substrates AGM individuals could survive by consuming surprisingly low mass, but high percentage of available feed limited by a single kernel, as demonstrated in millet and tall fescue, or by successful transfer and infestation of more than one kernel in order to compensate the insufficient food resources, as observed in the substrate with polished rice.

Acknowledgement

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Progeny production by *Stegobium paniceum* in different spices

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Abstract

Spices have long been an important component in the preparation of food, and some have medicinal properties as well. *Stegobium paniceum*, the drugstore beetle, has been detected in spices but no detailed information is available on its infestation in certain locally-available spices. Objective of this study was to find out the degree of infestation by *S. paniceum* in ten different spices. Twenty adults of *S. paniceum* were introduced into a vial containing a particular spice, maintained for two weeks and shifted out. These were maintained under ambient environmental conditions and the progeny adults emerged in each medium was counted at two week intervals for three months. The progeny produced varied with the food medium; the highest progeny was recorded in coriander whereas the lowest progeny was recorded in cinnamon, clove, dill seeds, cardamom, chilli, pepper corn and turmeric powder. This study reveals that *S. paniceum* infests a wide array of spices at different levels. This information is important for taking necessary steps to protect the spices from the infestation of *S. paniceum*.

Keywords: *Stegobium paniceum*, Progeny, Spices, Infestation

1. Introduction

Stored-product losses are more in tropical countries than in temperate regions (Wijayaratne et al., 2018). Sri Lanka has the reputation for producing good quality spices. Drugstore beetle, *Stegobium paniceum* is a pest of stored spices (Cabrera, 2014). Infestation of spices kept in storage by *S. paniceum* is reported but a proper investigation has not yet been performed. Therefore, the objective of this study was to find out the infestation level of *S. paniceum* in ten spices locally available and frequently used as indigenous medicine.

2. Materials and Methods

Ten spices were used in this study: coriander, cardamom, pepper corn, cumin seeds, dill seeds, clove, fennel seeds, chilli pieces, cinnamon and turmeric powder. Drugstore beetles were reared in coriander medium inside the incubator at 30^0C and 60% RH. The progeny adults aged one month were used in the experiments. Twenty adults of *S. paniceum* were introduced into a vial containing 12 g of a particular spice, maintained for two weeks and sifted out. Four replicates from each treatment were maintained. Progeny adults emerged in each medium was counted at one month intervals for three months.

3. Results and Discussion

The progeny production differed with the spice and the duration. Highest infestation recorded in coriander. No progeny was produced in cardamom, pepper corn and turmeric powder.
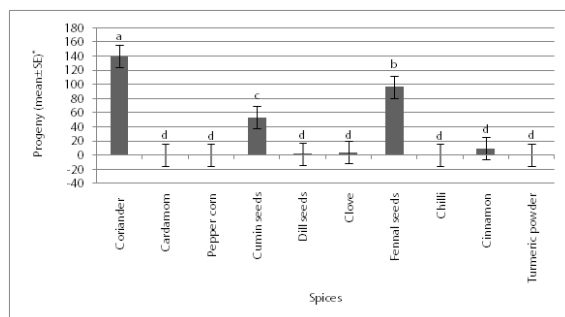
*Progeny produced in spices followed by the same letter are not significantly different according to Tukey's test.

Fig.1 Progeny adults emerged (mean±SE) in different spices one month following initial infestation.
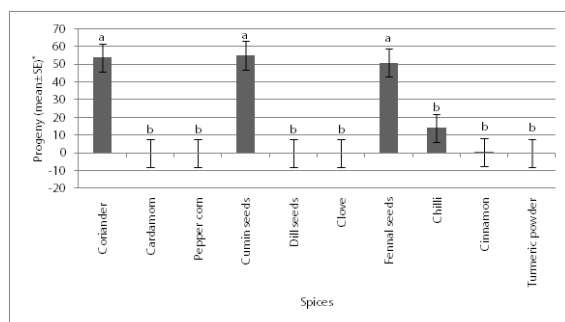
*Progeny produced in spices followed by the same letter are not significantly different according to Tukey's test.

Fig. 2 Progeny adults emerged (mean±SE) in different spices two months following initial infestation.
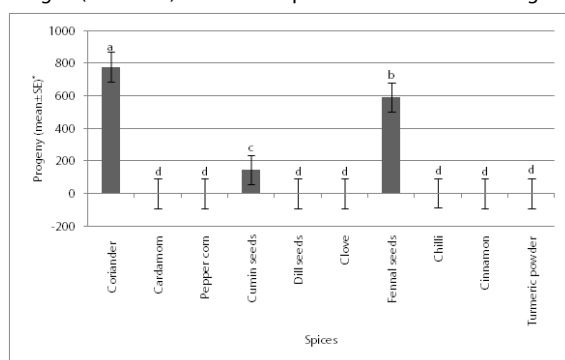
*Progeny produced in spices followed by the same letter are not significantly different according to Tukey's test.

Fig. 3 Progeny adults emerged (mean±SE) in different spices three months following initial infestation.

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The developmental parameters of the minute brown scavenger beetle *Dienerella argus* (Coleoptera: Latridiidae)

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Abstract

Adults and larvae of *Dienerella argus* (Reitter) (Coleoptera: Latridiidae) feed on fungi and are frequently found in indoor, moldy areas. The basic biology of this species, other than its feeding habits, has not been determined. In this study, the developmental parameters of the beetle were investigated using dried hyphae and conidia from three fungi that are common in living areas. The developmental periods of the beetle on *Cladosporium cladosporioides*, *Penicillium citrinum*, and *P. decumbens* were examined at 16, 20, 24, 28, 32 °C / 70–75 % RH under dark conditions. The low developmental threshold temperatures and thermal constants calculated from egg to adult emergence were 10.5 °C and 526 DD (degree day), 9.0 °C and 500 DD, and 10.9 °C and 370 DD on *C.*

cladosporioides, *P. citrinum*, and *P. decumbens*, respectively. These developmental parameters indicate that these beetles can breed year-round in indoor areas that are in air-conditioned facilities.

Keywords: *Dienerella argus*, developmental period, low developmental threshold temperature, thermal constant, *Cladosporium*, *Penicillium*

1. Introduction

Latridiidae is a family of small (1–3 mm) mycophagous beetles that includes 761 species according to Rücker (2015). These beetles are named minute brown scavenger beetles, or plaster beetles for some indoor species, and are found in moldy areas, on debris and occasionally on flowers. At least 30 species have been listed as stored product pests, although they do not directly affect stored foods but feed entirely on the fungi that grow on foods (Hinton 1941). Most species associated with stored foods are dispersed worldwide, perhaps due to the international transportation of food commodities. *Dienerella argus* (Reitter) is one of these wide-ranging species that was introduced into Japan (Mito and Uesugi 2004). Stored-product pest species usually adapt to indoor environments and are frequently found in moldy areas such as plaster walls in a damp-dried state; under floors, garrets, and internal wall structures; and in air-conditioning and refrigeration systems, where dew condensation occurs, as well as hospitals or sterile drug processing areas (Carlton 1988; Robinson 2005; Tanaka 1986; Tani and Ito 2006). In manufacturing industries, the populations of these beetles sometimes increase explosively inside factories or warehouses, causing insect contamination in products. These beetles may possibly cause sanitary problems by spreading fungus spores (Robinson 2005). For example, Tani and Ito (2006) isolated the fungi *Cladosporium* spp. and *Penicillium* spp. from the body surface of *Dienerella costulata* (Reitter) and three fungus genera including *Aspergillus* from another latridiid beetle. Currently, little is known about the basic biology of these species, except for feeding habits. Since a successful artificial rearing method has been established for the beetles using dried hyphae and conidia of fungi, the low developmental threshold temperature and thermal constant of the beetle on three fungi, *Cladosporium cladosporioides*, *Penicillium citrinum*, and *P. decumbens*, were examined.

2. Materials and Methods

2-1. Collection and rearing method of *Dienerella argus*

Adult beetles were collected from the floor using a vacuum cleaner at a laboratory in the Manufacturing Technology Center, Japan Tobacco Inc. (Tokyo), where an outbreak of this insect had occurred. The insects were preserved on the dried hyphae and conidia of *Cladosporium cladosporioides* (NBRC6348) on potato dextrose agar (PDA). Approximately 30 adults that were 1–4 weeks old were placed on dried fungi in a petri dish and kept under 27 ℃, 75 % RH and dark conditions. The adults were removed after two weeks. The next generation adults emerged after one month under these conditions.

2-2. Fungi

C. cladosporioides (NBRC6348) and *P. citrinum* (NBRC 6352) were obtained from the Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan. *P. decumbens* was collected from above the ceiling of the laboratory where *D. argus* was collected. An open plate method was used in which the airborne particles were passively collected and preserved on PDA in a petri dish that was uncovered for 30 min. Then, the Petri dishes were kept under 25 ℃, and fungal colonies were isolated. The primary culture and subculture of these fungi were prepared on PDA in 90-mm plastic Petri dishes for 3–6 weeks at 25 ℃. Spore-formed cultures were dried under room conditions (23–28 ℃ and 40–70 % RH) and provided to the insects.

2-3. Developmental parameters

The developmental periods from egg to adult were examined on the three fungi *C. cladosporioides, P. citrinum*, and *P. decumbens*. Ten mating pairs were placed on the dried fungi in the Petri dishes. The Petri dishes were enclosed in 11.5 cm × 19.5 cm × 7.0 cm plastic containers with saturated NaCl solution in a 2.5 cm ϕ × 5 cm cup to maintain humidity at 70–75 % RH and kept in a 28 ℃ chamber. After 16–20 h, the adults were removed and the containers were transferred to chambers set at 16, 20, 24, 28, 32 ℃ under dark conditions. Two replications were carried out for each fungus and temperature. Actual temperatures in the containers were recorded at one hour intervals by the thermo recorder. Adult emergence was checked at 1–3 d intervals. The timing of eclosion was assumed to occur during the observation intervals that were in the middle of the study period. The low developmental threshold temperature (T_0) and thermal constant (K) were obtained from the regression lines of $1/D$ against T, where D is the developmental period in days from egg to adult, and T is temperature in ℃ (Kiritani 2012).

3. Results

Egg to adult development occurred for all fungi tested, and the adults that emerged were both viable and fertile. Table 1 shows the developmental periods under different temperature conditions, and the values of T_0 and K calculated for the three fungi. Development periods were slightly different among the fungi. The period was delayed by one week to ten days for complete growth on *C. cladosporioides* compared to the periods for *Penicillium* spp.

Tab. 1 Developmental periods at five temperatures and developmental parameters for *Dienerella argus* on three fungi.

Temperature, ℃	Developmental period in days, mean ± SD (Number of adults emerged)		
	Cladosporium cladosporioides	*Penicillium citrinum*	*Penicillium decumbens*
15.4	95.5 ± 4.3	85.0 ± 5.1	88.4 ± 3.0
19.4	54.2 ± 2.5	46.0 ± 3.7	45.8 ± 3.1
23.3	40.3 ± 2.6	31.3 ± 0.8	29.3 ± 0.8
27.3	31.5 ± 8.5	26.8 ± 2.9	21.1 ± 2.3
31.7	25.4 ± 3.9	22.2 ± 3.1	18.9 ± 0.4
Low developmental threshold	10.5 ℃	9.0 ℃	10.9 ℃
Thermal constant	526 DD	500 DD	370 DD
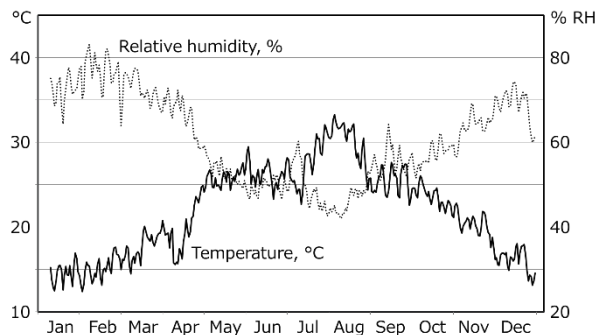
Fig. 1 Temperature and humidity above the ceiling panel of the laboratory where the outbreak of *Dienerella argus* occurred. Solid and dotted lines represent daily mean temperature and relative humidity, respectively.

Discussion

Although the developmental parameters for Latridiid beetles have not been elucidated, Hinton (1941) described the life histories of several species on cultures of *Penicillium glaucum* and *Mucor mucedo* on bread and cheese. The durations of the time the egg was laid to adult emergence were 27–32 d (egg to the second instar larval stage was at 15.6 °C and the third instar to pupal stage was at 20 °C) for *Cartodere nodifer* (Westwood), 36 d at 23.9 °C for *Dienerella filum* (Aubé), 51–52 d (egg at 17.2 °C and larva to pupa at 19.4 °C) for *D. filiformis* (Gyllenhal), and 40 d at 18.3 °C for *Corticaria fulva* (Comolli) (Hinton 1941). The period from egg to adult observed for *D. argus* in the present study was longer than the periods observed for *Cartodere nodifer* and *Corticaria fulva*, and was the same as the periods observed for the two *Dienerella* species at comparable temperatures. Based on the systematic review by Kiritani (2012), the average T_0 and K values from egg to adult for 31 coleopteran species are 10.9 ± 2.5 °C and 415 ± 239 DD (mean ± SD), respectively. The T_0 and K values for *D. argus* were 10.5 °C and 526 DD on *C. cladosporioides*, 9.0 °C and 500 DD on *P. citrinum*, and 10.9 °C and 370 DD on *P. decumbens*, all of which are average values for coleopterans.

The daily mean temperatures of the garret of the laboratory, the original location of the test insects, fluctuated within the range of 12–17 °C in the winter, constantly surpassing T_0 (= 9.0–10. 9 °C) (Fig. 1). This result strongly suggests that *D. argus* had bred there year-round. In fact, the adult beetles were caught irrespective of the season. Because the female adults require at least one week before starting oviposition at 25–30 °C, the thermal constant K for one generation on *P. decumbens* was assumed to be 470–500 DD (370 DD for egg to adult emergence + 100–130 DD for the pre-oviposition period). The accumulated daily mean temperature above 10.9 °C was calculated at 3974 DD in the garret of laboratory, and the number of generations per year was estimated at eight.

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Comparison of mandible morphology of two stored product bostrichid beetles, *Rhyzopertha dominica* and *Prostephanus truncatus*

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Abstract

Insect mandibles are most frequently encountered fragments in processed foods. Thanks to their sclerotised and darkly pigmented nature, they usually remain intact in foods and are relatively easily detectable. Moreover, because of their complexity and variety of shapes, stored product beetle mandibles may be useful in species determination. The present work deals with a comparative morphology of two stored product bostrichid beetles, *Rhyzopertha dominica* and *Prostephanus truncatus*. The mandibles were studied using by light and scanning electron microscopy and their morphological details, overall appearance and size are provided.

Keywords: mandibles, stored product pests, Bostrichidae, *Rhyzopertha dominica*, *Prostephanus truncatus*

1. Introduction

Beetle mandibles serve as effective tools for both intake and processing of food. From the stored product perspective, they are used for overcoming barriers imposed by a manufacturer thus enabling to infest a commodity (Stejskal et al., 2017). For this reason, external morphology of mandibles can provide information about mechanism of feeding and infesting potential of the particular insect species. Lesser grain borer *Rhyzopertha dominica* and larger grain borer *Prostephanus truncatus* are serious pests of stored grain in many regions worldwide (Stejskal et al., 2015). As both species are internal grain feeders with relatively inconspicuous adult way of life (Edde, 2012), early detection of the infestation is problematic. Nevertheless, thanks to their microscopic size and highly sclerotised nature, mandibles are most numerous fragments in the processed foods (Trematerra et al., 2011) and may thus serve as an indicator of level of a product contamination and for species identification.

2. Materials and Methods

Both species were reared at 27 °C and 75% relative humidity on wheat (*R. dominica*) or maize grains (*P. truncatus*). Only newly emerged, 1 – 7 days old individuals were used. The mandible measurements were taken using stereomicroscope Olympus SZX10 equipped with a Canon 1300D digital camera and analysed by QuickPHOTO INDUSTRIAL 3.1 software. Before examining with the JEOL 6380 scanning electron microscope, the mandibles were cleaned in 20% lactic acid for 24 hours, dried with critical point drying and mounted on aluminium plates.

3. Results

For both species, interspecific differences in size, shape, as well as morphological details were indentified. The mandibles of *P. truncatus* were described for the first time. The morphological characteristics and the most important differences are summarized below:

3.1 Rhyzopertha dominica (Fig. 1A)

Mandible length 270 - 310 µm. Shape of incisive part: incisor lobe relatively short and blunt. A setal tuft relatively small, containing only 10 – 20 long branched setae. Mola quadrate from the dorsal aspect, smooth, without any apparent structure, only with shallow groove approximately in the middle. Setae along the molar area in dorsal part uniform. Lateral margin without any lateral protuberance.

3.2 Prostephanus truncatus (Fig. 1B)

Mandible length 390 - 440 µm. Incisor long, with blunt mesal protuberance in proximal part, primary incisor pointed. Wide setal brush containing > 100 short setae. Mola rounded, chewing area concave with coarse surface. Setae along the molar area of two types. Lateral margin with two large protuberances on lateroventral and laterodorsal aspect.

Fig.1 SEM photographs of mandibles of (A) *Rhyzopertha dominica* and (B) *Prostephanus truncatus*.

4. Discussion
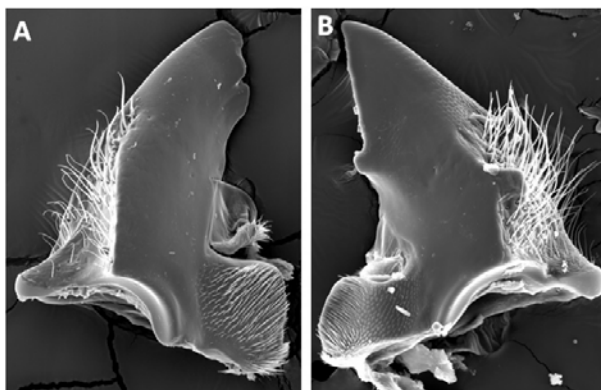
Despite great variability of insect mandibles, there exist generalities in their morphology according to feeding habits of the species (e.g. Samways et al., 1997; Smith and Capinera, 2005). For example, pointed bifid or unidentate apex of mandibles serves as a piercer and is mainly present in predatory species. Similarly, highly developed mola is used for trituration of a dense material and is thus present in species feeding on hard material. The relative size and shape of mola and molar surface are different in both studied species and probably reflect their slightly different food source. Nevertheless, the well developed mola in both species is probably linked with presence of a dust in the infested commodities by these species (Kumar, 2002). Also, the incisors are adapted for scraping and play a role in removal of a food as well as in penetration of a hard material (e.g. wood, seed surface, or, secondary, food packages). Thus, the length and robustness of the incisive part may be a reason of a great penetration ability of the studied species (Stejskal et al., 2017).

In this work, we described mandible morphology in two stored product bostrichid beetles, *R. dominica* and *P. truncatus*. The mandibles of *P. truncatus* are described for the first time. We identified differences between the two species in size, shape as well as morphological details. We conclude that the two species can be easily identified based on their mandibles and that the species determination is possible at low magnifications by light microscopy.

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Behavioural responses of Callosobruchus maculatus to volatiles organic compounds found in the headspace of dried green pea seeds

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There is growing evidence that insects rely on chemical cues to locate food, hosts, predators, and potential mates. The pulse beetle Callosobruchus maculatus has been recognised for decades as the major post-harvest insect pest of legume seeds. In a previous study, we identified five volatile compounds in the headspace of dried green pea seeds as electroantennographically active in C. maculatus antennae: 1-pentanol, 1-octen-3-ol, (E)-2-octenal, nonanal and 3-carene. Volatile compounds are generally perceived by insects as blends, we hypothesized that *C. maculatus* might particularly show attraction to different mixtures of the aforementioned compounds. To test this we examined the behavioural response of *C. maculatus* towards volatile mixtures in a dual choice Y-tube olfactometer. The results showed that females were attracted to five mixtures while males were attracted only to two binary mixtures consisting exclusively of aldehydes. The other mixtures caused *C. maculatus* to move away. Further investigations with the attractive mixtures should be done in real storage conditions with the aim of developing a trap for the pulse beetle, *C. maculatus*.

Investigation on the Species and Distribution of Stored Grain Insects in Northwest China

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Abstract

To understand the diversity of stored grain insects in northwest China, we have fulfilled insect collection in 56 grain storage enterprises, 60 grain, oil and feed processing plants and 65 farmers situated in 26 cities of five provinces (Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang) in northwest China from 2016 to 2017. After systematical identification, totally 83 species of stored grain insects have been found in this investigation, belonging to five orders, namely Class Insecta Order Zygentoma, Order Coleoptera, Order Lepidoptera and Order Hymenoptera, as well as Class Arachnida Order Chelonethida, in which Order Coleoptera owns 74 species in 22 families, Order Lepidoptera owns six species in four families, Order Zygentoma and Order Hymenoptera own one species in one family respectively, and Class Arachnida Order Chelonethida has one species in one family. After the statistics of four insect investigations in northwest China during 1955-2017, this paper has analyzed the results of four insect investigations and the representative stored grain insects in northwest China.

Key Words: northwest China, stored grain insects, species, distribution, investigation

1. Introduction

Located in the hinterland of the Eurasian continent, the northwest region covers the first (high-cold and dry stored grain region), second (low temperature and dry stored grain region) and fourth

(medium-temperature and dry stored grain region) of China's seven stored grain ecological regions, and consists of Shaanxi, Gansu, Qinghai, Ningxia Hui Autonomous Region, and Xinjiang Uygur Autonomous Region. Wide area with scarce rainfall, the northwest region, as a traditional grain deficit area, has the most serious soil erosion, land drought and desertification problems in China. Main grains planted and stored in northwest region include wheat, corn, rice, highland barley, soybean, buckwheat, pea, naked oat, proso millet, flax and other small grains.

Since the founding of the people's Republic of China, there have been seven national insect investigations related to the grain system, in which Gansu, Ningxia, Xinjiang and other northwest provinces were not included in the first and second investigations (Qizong Chen, 1994; Xiaoping Yan, et al., 2008). Besides the national insect investigations, some provinces have carried out their own investigation independently. The coleoptera pest investigation in Shaanxi commercial warehouses carried out by He Jinyan, et al. in 1983-1984 discovered 30 species of insects in eight families, Coleoptera Order (Jinyan He, et al. , 1993), Gao Duping reported the main stored grain pests in Pingliang, Gansu, composed of 19 species in 10 families, three orders (Jinyan He, et al. , 1993), the stored grain pest investigation in Ningxia Hui Autonomous Region carried out by Zhu Desheng, et al. in 1983-1984 found 47 species of pests in 19 families, two orders (Desheng Zhu,1987); the stored grain pest investigation in Changji Hui Autonomous Prefecture, Xinjiang carried out by Li Mingshan, et al. in 1981 found 39 species of pests in 22 families, seven orders (Mingshan Li, et al.,1994); and the stored grain insect investigation in Tibet Autonomous Region carried out by Chen Qizong, et al. in 1987 found 73 species of stored product insects (Qizong Chen,1990) Over the past 20 years, the farming mode and storage environment in northwest region have changed greatly. Therefore, regular investigation and research on pest species, location, distribution area and object should be carried out for effective stored grain pest control. Considering 2015 Grain Public Welfare Industry Research Project and *Notice of State Administration of Grain on the Seventh National Stored Grain Insect and Mite Investigation* [GLBC (2016) No.95], under the great support of grain administrations and local grain bureaus of Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang as well as relevant enterprises, an investigation on the species and distribution of stored grain insects has been carried out in grain storage enterprises, grain, oil and feed processing plants, farmers and other relevant places within three stored grain ecological regions, namely high-cold and dry stored grain region (the first region), low-temperature and dry stored grain region (the second region), and medium-temperature and dry stored grain region (the fourth region), for a better understanding of the stored grain insect diversity in the northwest region and pest control.

2. 2. Investigation Method and Scope

2.1 Sampling Site

Within the scope of five northwest provinces (Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang), besides some representative cities selected in the light of each stored grain ecological region, some relevant enterprises in the east, west, south, north and middle of each province were also selected as sampling sites. Totally, 56 grain storage enterprises, 68 grain, oil and feed processing plants, and 65 farmers from 26 cities in the first, second and fourth stored grain ecological regions, participated in this investigation. (See Table 1)

Table 1 Number of Cities, Relevant Enterprises and Farmers in Five Provinces.

Stored Grain Ecological Region	Province	Number			
		City	Grain Storage Ent.	Processing Plant	Farmer
First: High-cold and Dry	Qinghai	4	7	7	4
Second: Low-temp. and Dry	Xinjiang	6	10	14	1
	Gansu (partial)	4	14	6	19
	Ningxia	3	3	8	1
Fourth: Med.-temp. and Dry	Gansu (partial)	3	12	5	15
	Shaanxi	6	10	20	25

2.2 Sampling Method

Field sampling and screening were adopted by this investigation, and the durations from July to September, both 2016 and 2017, were selected as its sampling time. Collected samples were preliminarily classified and processed on site with original information registration, such as host of pest, sampling time, sampling site, etc. After then, a series of follow-up processes were carried out at the laboratory, including further separation, processing, preliminary species naming, classification and preservation, as well as specimen preparation.

2.3 Insect Identification Method

In terms of traditional morphological characteristics of insects, the species of each sample was identified, named and then reviewed by an expert team composed of researcher Zhang Shengfang from China Academy of Inspection and Quarantine, Professor Bai Xuguang and Professor Zhou Yuxiang from Henan University of Technology, in case of any preliminary naming error.

3. 3. Investigation on Insect Species and Distribution

3.1 Catalogue of Stored Grain Insects of Five Provinces in Northwest China

Through this investigation on stored grain insects in the northwest region, 83 species of stored grain insects were identified, respectively belonging to 29 families in five orders, two classes (Class Insecta: 74 species in 22 families of Order Coleoptera, six species in four families of Order Lepidoptera, and one species in one family of Order Zygentoma and Order Hymenoptera respectively; and Class Arachnida: one species in one family of Order Chelonethida). There were 16 species of undetermined species, in which seven were natural enemies of stored grain pests.

3.2 Insect Distribution Difference in Different Grain Storage Environments

Main pests found in this insect investigation in northwest China totaled eight species, including *Sitophilus zeamais* (Motschulsky), *Sitophilus oryzae* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Tenebroides mauritanicus* (Linnaeus), *Bruchus rufimanus* (Boheman), *Araecerus fasciculatus* (Degeer), *Sitotroga cerealella* (Olivier) and *Plodia interpunctella* (Hübner). It is reported that *Sitophilus zeamais* (Motschulsky), *Sitophilus oryzae* (Linnaeus) and *Rhyzopertha dominica* (Fabricius) are the main wheat, corn and rice pests in many temperate and tropical countries [8, 9], while *Rhyzopertha dominica* (Fabricius) is more common in warm and dry wheat producing areas of China, Australia, India and Pakistan[10].

There exist obvious differences in species numbers collected in different storage environments. Grain, oil and feed processing plants usually have suitable temperature and humidity and difficulty in complete cleaning [11], especially the small flour and rice mills in rural area without any pest control measures, where more (72 in total) species of stored grain insects were found. As for grain storage enterprises, 50 species of stored grain insects were found, belonging to 24 families, four orders, in which 25 species were found in Sinograin depots, and 43 species were found in local grain depots. Relying on less stored grain types, standard management, regular fumigation and better storage conditions, the species number of pests founded in grain depots is less than that of processing plants. In recent years, few farmers store grains by themselves, and their grain storage environment is improved after the wide application of small steel barns, hence the occurrence of stored grain pests is reduced. As a result, only 26 species of stored grain insects were found in farmers' barns in northwest China. (See Table 2).

Table 2 Information of Stored Grain Insect Distribution in Different Environments.

Classification	Sinograin Depot	Local Grain Depot	Grain, Oil and Feed Processing Plant	Farmer
Order	3	4	5	4
Family	15	23	26	17

Species	25	43	72	26

3.3 Insect Distribution in Stored Grain Ecological Regions

The investigation on stored grain insects in northwest China involves three stored grain ecological regions, the first, second and fourth ones, covering five provinces. The species number of stored grain insects in each ecological area remains in a range between 46 and 60 (Tab. 3). Wang Dianxuan, et al. [12] discovered 16, 59, 34, 23 and 59 species of stored grain pests respectively in the flour mills of the third, fourth, fifth, sixth and seventh stored grain ecological regions. According to the overall data, the species numbers of stored grain pests in low-temperature and high-humidity stored grain region (the third region), medium-temperature and high-humidity stored grain region (the fifth region) and medium-temperature and low-humidity stored grain region (the sixth region) is less, which may be related to the limited numbers of sampling site, sampling point and sampling time.

Table 3 Species Numbers of Stored Grain Insects Collected in Different Stored Grain Ecological Regions (SGER).

Classification	High-cold and Dry Stored Grain Region (First)	Low-temp. and Dry Stored Grain Region (Second)	Medium-temp. and Dry Stored Grain Region (Fourth)
Order	4	5	4
Family	21	23	23
Species	53	60	46

4. Discussion

4.1 Analysis on Investigations on Stored Grain Insects in Northwest China

Totally, seven national investigations on the species and distribution of stored grain insects and stored product insects have been carried out within Chinese grain system, and because of partial overlapping, investigations in 1955, 1957 and 1956-1958 were recognized by this paper as an investigation, in which date related to investigation on stored grain insects in northwest China were sorted out in this paper. According to statistics, 199 species of stored grain insects were found in total, belonging to 40 families, eight orders, Class Insecta and Class Arachnida. Among then, Class Insecta: Order Coleoptera owns 174 species in 26 families, Order Lepidoptera owns 15 species in six families, Order Blattidae owns three species in two families, Order Hymenoptera owns two species in two families, Order Hemiptera, Thysanura, Diptera own one species in one family respectively; and Class Arachnida Order Chelonethida has two species in one family, comprehensively summarizing the stored grain insects in northwest China.

Zhao Yangchang, et al. carried out comprehensive investigation on pests in stored grain, oilseeds, livestock products, aquatic products, medicinal materials, archives, timber and other stored products in northwest China with an emphasis on stored grain pests in 1955-1960, discovering 113 species of store product insects in 33 families, six orders [13]. According to the data of the fourth national stored grain pest investigation organized by the Ministry of Commerce in 1974-1975 [14], the insect species distribution investigation group found 94 species of stored grain insects in 29 families, four orders in northwest China. The sixth national stored grain insect investigation organized by the State Grain Administration in 2004-2005 found 133 species of stored grain insects in 40 families, eight orders in the northwest region. Following the task stipulated in the 2015 Grain Public Welfare Industry Research Project – "Stored Grain Insect and Mite Region System Investigation and Pest Monitoring and Forecasting Technology Research" and the arrangement of the State Grain Administration's "Seventh National Stored Grain and Mite Investigation", a project team carried out investigation on stored grain insects in the northwest region from 2015 to 2017, indentifying and recording 83 species of stored grain insects in 29 families, five orders. According to previous investigation results, the species number of stored grain insects remained stable basically. However, due to the limitation of time, site and scope of these investigations, the species of stored

grain insects may not be comprehensive.

4.2 Representative Store Grain Insects in Northwest China

Among these four investigations in 1955-1960, 1974-1975, 2004-2005, and 2015-2017, 29 species of stored grain insects occurred in the northwest region in every investigation, basically covering the main stored grain pest species in China, namely *Cryptolestes ferrugineus* (Stephens), *Cryptolestes turcicus* (Grouvelle), *Tribolium castaneum* (Herbst), *Tenebroides mauritanicus* (Linnaeus), *Oryzaephilus surinamensis* (Linnaeus), *Ahasverus advena* (Waltl), *Stegobium paniceum* (Linnaeus), *Sitophilus granarius* (Linnaeus), *Bruchus pisorum* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Lyctus sinensis* (Lesne), *Ptinus japonicus* (Reitter), *Trogoderma variabile* (Ballion), *Cryptophilus integer* (Heer), *Carcinops pumilio* (Erichson), *Palorus ratzeburgi* (Wissmann), *Alphitophagus bifasciatus* (Say), *Alphitobius laevigatus* (Fabricius), *Tenebrio obscurus* (Fabricius), *Typhaea stercorea* (Linnaeus), *Migneauxia orientalis* (Reitter), *Thes bergrothi* (Reitter), *Holoparamecus ellipticus* (Wollaston), *Sitotroga cerealella* (Olivier), *Pyralis farinalis* (Linnaeus), *Ephestia cautella* (Walker), *Plodia interpunctella* (Hübner), *Tinea tugurialis* (Meyrick), and *Ctenolepisma villosa* Fabricius. Due to lack of data, poor test conditions, and other factors, difficulty in differentiating *Sitophilus zeamais* (Motschulsky) from *Sitophilus oryzae* (Linnaeus) may lead to an error in the 1955-1960 investigation. After combination of external genitalia anatomy and morphology was introduced into identification in 1975, differentiation between *Sitophilus zeamais* (Motschulsky) and *Sitophilus oryzae* (Linnaeus) and other allied species was finally achieved [1]. Therefore, all of the following three investigations in 1974-1975, 2004-2005 and 2015-2017 found *Sitophilus oryzae* (Linnaeus) in the northwest region, while *Sitophilus oryzae* (Linnaeus) found in the 1955-1960 investigation may be mistakenly identified as *Sitophilus zeamais* (Motschulsky).

The representative stored grain insects in the northwest region (incl. the first, second and fourth stored grain ecological regions) listed in the *Technical Specification for Grain and Oil Storage* (GB/T 29890-2013) total 13 species, namely *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hübner), *Oryzaephilus surinamensis* (Linnaeus), *Tenebroides mauritanicus* (Linnaeus), *Tribolium castaneum* (Herbst), *Tribolium madens* (Charpentier), *Attagenus augustatus gobicola* (Frivaldszky), *Trogoderma variabile* (Ballion), *Niptus hololeucus* (Faldermann), *Gibbium psylloides* (Czenpinski), *Ptinus japonicus* (Reitter), and *Sitophilus granarius* (Linnaeus) (Xinjiang) [15]. In this investigation, *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hübner), *Oryzaephilus surinamensis* (Linnaeus), *Tenebroides mauritanicus* (Linnaeus), *Tribolium castaneum* (Herbst), *Attagenus augustatus gobicola* (Frivaldszky), *Trogoderma variabile* (Ballion), *Niptus hololeucus* (Faldermann) and *Sitophilus granarius* (Linnaeus) were found, but *Tribolium madens* (Charpentier) *Gibbium psylloides* (Czenpinski), and *Ptinus japonicus* (Reitter) were not found. According to the newest *Stored Product Beetle*, *Tribolium madens* (Charpentier) *Gibbium psylloides* (Czenpinski), and *Ptinus japonicus* (Reitter) never occurs in China [16]. Therefore, the previous records may be naming errors. *Gibbium aequinoctiale* (Boieldieu) has been mistakenly recognized as the allied species of *Ptinus japonicus* (Reitter) in some domestic references, which coincides with the fact of discovery of *Gibbium aequinoctiale* (Boieldieu) rather than *Ptinus japonicus* (Reitter) in this investigation.

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Session 3
Detection and Monitoring

Stored Product Insects at a Rice Mill: Temporal and Spatial Patterns and Implications for Pest Management

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Abstract

Monitoring is fundamental to integrated pest management programs since it provides feedback on effectiveness of prevention programs and helps with targeting interventions as needed and evaluating their effectiveness. Rice mills are spatially complex facilities that have a combination of rough rice storage bins, buildings where rice is milled and processed, and warehouses and bulk storage bins where finished product is held before shipment. Each of these structures can have its own suite of insect species, different levels of risk, as well as different suites of management tools available. At a large rice mill in Brazil, stored product insect activity was monitored using food bait traps placed around rough rice receiving areas and storage bins; inside building containing white rice mill, rice flour mill, and packaging; and inside building for processing parboiled rice. The facility was monitored from 2010 to 2018 with 100 traps. Major pest species recovered at the facility included *Sitophilus oryzae, Sitophilus zeamais, Rhyzopertha dominica, Tribolium castaneum, Cryptolestes ferrugineus, Ahasverus advena*, *Oryzaephilus surinamensis*, *Typhaea stercorea, Anthicus floralis*, and Nitidulidae species. Temporal and spatial patterns in abundance were evaluated for each of the major species and for major functional groups (primary feeders, secondary feeders, and fungal feeders). Monitoring data generated was used to guide pest management programs and also provided the information needed to develop management thresholds.

Keywords: rice, monitoring, *Sitophilus* spp., *Rhyzopertha dominica*, *Tribolium castaneum*, spatial distribution.

Introduction

Rice is one of the three major food crops of the world, along with corn and wheat. After harvest, rice is vulnerable to infestation by a suite of stored product insect species as it is stored and processed. Rice mills consist of a complex of structures, including structures such as metal bins or concrete elevators where rough rice is stored in bulk, the mill building where the hull and outer bran layer is removed, milled rice storage structures which are typically warehouses for packaged rice, and storage areas for waste material such as rice hulls. Some facilities also have other structures or areas where additional processing occurs such as parboiling or milling into rice flour. These different areas of a rice mill complex are all vulnerable to stored product insect infestation, although the distribution of species and their inherent risk varies with area. Integrated Pest Management (IPM) for rice mills relies on a range of tactics to deal with insect infestation issues, including fumigation of rough rice and packaged rice with phosphine, treatment of mill building and warehouses with structural treatments such as fumigants or heat or aerosol insecticides, spot and surface insecticide treatments. However, the major focus of IPM for food facilities needs to be on prevention of insects entering storage and processing areas, and ultimately into the finished product. Stored product insects can be captured in large numbers outside of rice mills (McKay et al. 2017), so understanding patterns of activity outside of rice mills and the impacts of management tactics to target these outside populations is critical.

Brazil is a top 10 worldwide rice producer and the state of Rio Grande do Sul is the largest rice producing region in Brazil. The objective of this study was to monitor insect activity in and around a large rice facility in this region of Brazil. A high density of traps in place across multiple years was

used to determine the community of insect species that are active at the mill, seasonal patterns of activity, and the spatial patterns of distribution. This information is useful in determining times and areas at greatest risk and also for providing information to guide pest management programs and evaluate their success.

Materials and Methods

Stored-product insect abundance was monitored at a large rice facility located in southern Brazil. The rice facility included rice receiving areas, drying facilities, metal storage bins for holding rough rice, a structure for white rice and rice flour milling, and packaging/warehouse, and a structure for parboil rice manufacturing. Insects were monitored using 100 food-baited cage traps [based on Throne & Cline (1991) and adapted by Pereira (1999)] placed outside around the bins and inside the white rice and parboil rice plants. The bait used in the traps consisted of 150 g of whole corn kernels, broken corn kernels, whole rice, broken rice, whole wheat, and wheat germ that had been previously sifted and frozen for 7 days at -18°C to kill any insect infesting the raw material. Personnel at the rice facility placed traps out for 15-day periods once a month and returned the traps so that the captured insects could be identified and counted. However, given the range of factors that arise from working with commercial operations, not all traps were returned for each monitoring period, there were gaps in the data collected, and for some of the early monitoring periods traps were out a couple times a month but only one of the 15-day intervals is presented here. Data are presented as the number of insects captured per 15-day period. Monitoring started in Jan 2010 and continued until January 2018.

Results

Across the total duration of the monitoring program, *Rhyzopertha dominica* was the most abundant species recovered, accounting for 47% of the stored-product species captured. Other pest species captured included *T. stercorea* (11%), *Sitophilus* spp. (8%), *Cryptolestes ferrugineus* (7%), *A. advena* (3%), *T. castaneum* (1%), and low numbers of *O. surinamensis* and *L. serricorne* (<1%, respectively). Sap beetles in the family Nitidulidae were the second most abundant group of insects in the samples, accounting for 22% of the species captured. Although was considerable variation in captures among years, for *R. dominica* there was a temporal pattern of greater captures during the summer months, between November and February (Fig. 1). This seasonal pattern also appeared to apply to *C. ferrugineus*, but captures of *T. stercorea* and *Sitophilus* spp. did not exhibit as strong a seasonal trend (Fig. 1). There was a significant relationship between average monthly temperature and total insect captures, with captures low and stable at average temperatures below 22°C and with peak captures around 26°C (Fig. 2).

Insect captures were greatest in the traps near the rough rice storage bins, followed by captures in traps in the receiving/drying area, with the least captures inside the rice mill and the parboil facility (Fig. 3). In the rough rice area, *R. dominica* (640±150 total adults/trap), Nitidulidae (262±106 total adults/trap), *Sitophilus* spp. (102±22 total adults/trap), *T. stercorea* (99±14 total adults/trap) and *C. ferrugineus* (75±22 total adults/trap) were the five most commonly captured species. In the receiving/drying area, *R. dominica* (273±103 total adults/trap), Nitidulidae (206±56 total adults/trap), *T. stercorea* (113±25 total adults/trap), *C. ferrugineus* (65±14 total adults/trap), and *Sitophilus* spp. (43±8 total adults/trap) were the five most commonly captured species. Inside the rice mill, *R. dominica* (34±15 total adults/trap), *T. stercorea* (29±14 total adults/trap), Nitidulidae (18±8 total adults/trap), *Sitophilus* spp. (15±2 total adults/trap, and *T. castaneum* (9±3 total adults/trap) were the five most commonly captured species. And inside the parboil facility, *R. dominica* (62±6 total adults/trap), Nitidulidae (61±24 total adults/trap), *Sitophilus* spp. (21±6 total adults/trap, *T. stercorea* (9±3 total adults/trap), and *T. castaneum* (5±2 total adults/trap) were the five most commonly captured species.
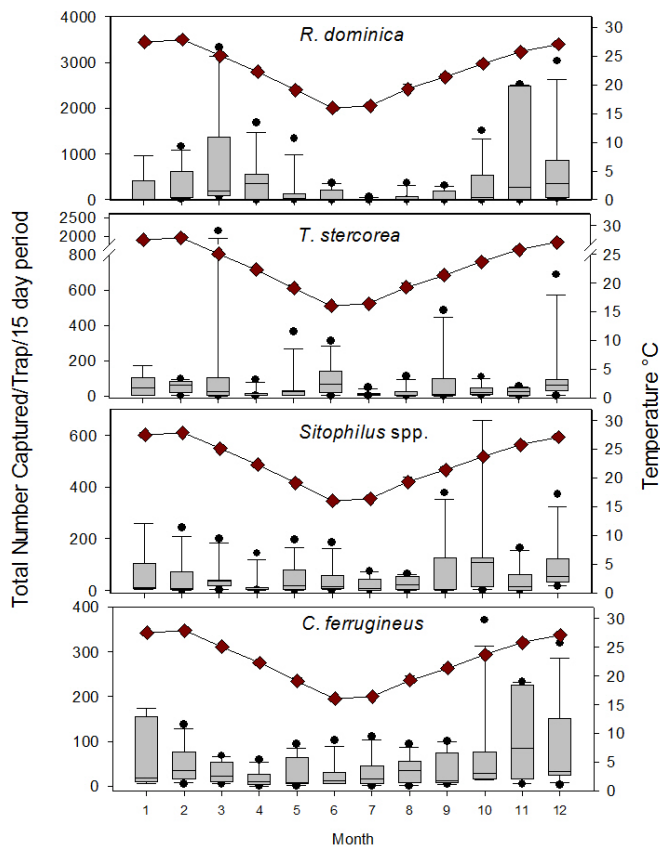
Fig. 1 The total number of each species captured per month across the eight years study shown as box plots, with 50% of data in the box, 95% in the whiskers, and outliers shown black circles. Average monthly temperatures obtained from a nearby weather station are shown as diamonds.
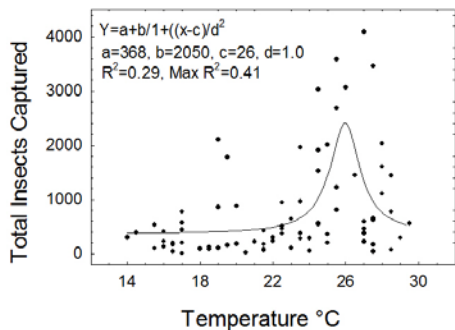
Fig. 2 Relationship between total number of insects captured and average monthly temperatures obtained from a nearby weather station.
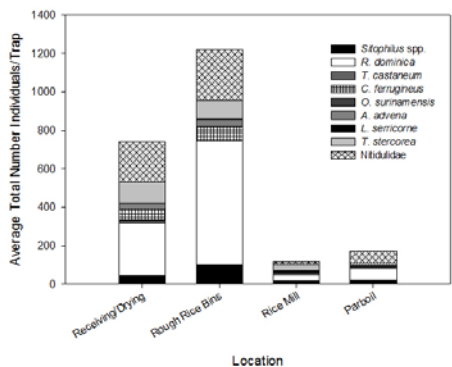
Fig. 3 Total number of individuals of each species captured over the course of the study in four areas of the rice mill facility.

Discussion

There were high levels of stored product insect activity throughout the rice mill facility, especially outside in the areas that handled rough rice – both the receiving and drying areas and the storage bins. Some of the major primary pest species were recovered in these areas, including *R. dominica* and *Sitophilus* spp. (*S. oryzae* or *S. zeamais*). These species were monitored outside of the rough rice bins, so it is not known how these activity levels relate to levels of infestation in the rice within the bin. Insects captured outside could originate from within the bins, from grain spillage accumulations onsite, or immigrate from offsite locations. However, these activity levels indicate the potential for insect movement into and out of the bins and potential for movement into the rice processing structures and ultimately into finished products (Campbell and Arbogast 2004; Campbell and Mullen 2004; Toews et al. 2006)

Stored product insect monitoring at other rice mill locations have indicated difference in relative species abundance, but these differences might be due to a combination of geographic location and monitoring method. In this study, food baited traps were used, but in other studies pheromone traps were used for monitoring. In Portugal, Carvalho et al. (2013), using pitfall traps with food and pheromone attractants inside a rice mill, found that *Sitophilus* spp. and *T. castaneum* were the most abundant species captured. In the USA, McKay et al. (2017) used pheromone-baited flight traps outside a rice mill and found that *Trogoderma variabile* was the most abundant species, although this species was not recovered at this rice mill in Brazil. High numbers of *Plodia interpunctella* were also captured in the McKay et al. study, and they were also present at this Brazil rice mill location, but were primarily captured in light traps and not in the bait traps. At the USA rice mill, *R. dominica* was captured in high numbers, but few *Sitophilus* spp. were captured, probably due to the monitoring method. Interestingly, at this Brazil rice mill the insect community inside the rice mill and parboil structures was similar to that in the rough rice areas, although overall numbers were much lower. In other studies, *Tribolium castaneum* is one of the most abundant and economically important pest species inside mills (Buckman et al. 2013).

Activity of stored product insects in this current study and in others has tended to show seasonal patterns. Temperatures inside rice mills tend to track those outside the mills and to be associated with levels of insect activity inside mill (Buckman et al. 2013). Captures of insects at this Brazil rice mill was associated with temperature, but rather than a linear or increasing relationship there appeared to be a threshold below which there was lower insect captures and above which there tended to be a peak in captures around 26°C. It is often difficult to determine consistent relationships with temperature (e.g., Carvalho et al. 2013; McKay et al. 2017) most likely due to other variables such as movement of grain and treatment activity having strong influences on abundance. Outside temperatures and captures of stored product insects in flight traps were positively related at a USA rice mill, but only at temperatures above 15°C and the nature of the relationship varied with species and year (McKay et al. 2017). Given the multi-year duration of this current monitoring study, it provides the opportunity to detect patterns that might otherwise be missed.

Understanding the stored product insect community at a location and its temporal and spatial patterns of distribution provides the foundation for IPM programs. Given the variation among locations this information is important in developing site-specific programs and for the continual evaluation of program success. The data from this study can be further evaluated to relate activity to specific locations and with management tactics implemented during the study. Understanding outside and inside insect activity can provide important insights into the sources of insect infestation and help more effectively target pest management.

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From stored-product psocids to the other pests: the developments, problems and prospects on research and application of molecular identification

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Abstract

Psocids, beetles, moths and mites are regarded as the common kinds of stored-product pests in the world. The rapid and correct identification of stored-product pests is significant for quarantine, monitoring and control purposes. Molecular methods and techniques have been studied and applied for stored-product pest identification. Based on collection and analysis of literature in the last decade, this paper reviews the developments, questions and prospects for molecular identification of stored-product pests. As a representative model, the molecular methods and techniques for species identification of stored-product psocid pests were developed and applied systematically based on international collaboration involving China, Czech Republic, the United States and other countries. More than 10 studies on stored-product psocids related to RFLP, DNA barcoding, PCR, real-time PCR and gene chip have been published during this decade. Subsequently, DNA barcoding, PCR and real-time PCR techniques for the identification of common species of *Tribolium* and *Cryptolestes* pests of stored products have been reported by the same international team. Recently, a web system called Grain Pests DNA Barcode Identification System (GPDBIS) has been established in China using SOL SERVER and C#. Like a marathon that requires persistence, we should do our best to continue to promote research and application of molecular identification of stored-product pests with more international collaboration.

Keywords: stored-product pests, molecular identification, review, research, application

Globally, stored-product arthropod pests include a large number of species. The rapid and correct identification of stored-product pests is significant for quarantine, monitoring and control purposes. In recent decades, molecular methods and techniques have been studied and applied for stored-product pest identification. There is quite a substantial amount of literature related to stored-product pests and their molecular identification. In this work, literature from 1900 to 2017 was collected and analyzed using Web of Science (http://apps.webofknowledge.com/). The total count of articles on stored-product pests was found to be 32,123 whereas the total count of articles on molecular identification of stored-product pests was 179. The years with the highest counts for these two categories were 2015 and 2012, respectively. In decreasing order, countries with the most contributions to literature on stored-product insect pests were USA, China, UK and India (Figure 1).
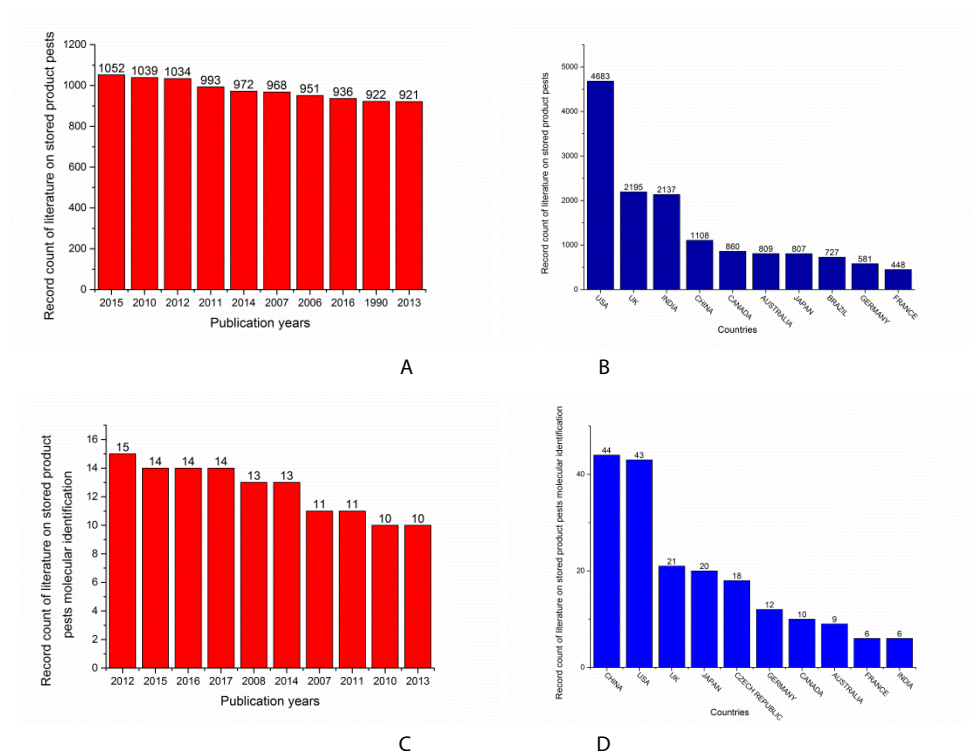
Fig. 1 The top 10 contributors to literature on stored-product pests and molecular identification. A: top 10 years of stored-product pest literature, B: top 10 countries contributing to stored-product pest literature, C: top 10 years of literature on molecular identification of stored-product pests, D: top 10 countries contributing to literature on molecular identification of stored- product pests.

Psocids, beetles, moths and mites are the common kinds of stored-product pests. From the number of articles on molecular identification of stored-product pests, most of the research and application have obviously been in the last 10 years (Table 1). As a representative model, the molecular methods and techniques for species identification of stored-product psocid pests were developed and applied systematically based on international collaboration involving China, Czech Republic, the United States and other countries. More than 10 articles, theses and dissertations on molecular identification of stored-product psocids have been published between 2008 and 2017; methods used including RFLP (Qin et al. 2008; Qin 2009), DNA barcoding (Li et al. 2011; Yang et al. 2012; Cui 2013; Yang et al. 2013b; Yang 2014), PCR (Arif et al. 2012; Yang et al. 2013a; Zhao et al. 2016), real-time PCR (Pang 2017), and gene chip (Liu et al. 2017). Recently, this team discovered the highly divergent mitochondrial genomes and indicated that *Liposcelis bostrychophila* was a cryptic species, which provided a taxonomic basis for species identification of stored-product psocids (Feng et al., 2018). Subsequently, the techniques such as DNA barcoding, PCR and real-time PCR have been reported for the identification of common species of *Tribolium* (Wang 2015; Zhang et al. 2016; Zhang 2017), *Cryptolestes* (Wang et al. 2014; Varadínová et al. 2015; Chen, 2018) and predatory mites (Wu et al. 2016) by the same international team. For more application of DNA barcoding, a web system which was entitled as Grain Pests DNA Barcode Identification System (GPDBIS) has been established in China using SOL SERVER and C# (Figure 2) (Li 2016; Wu et al. 2017).

Tab. 1 Number of articles on molecular identification of common stored-product pests during the period 2008–2017

Arthropods	2017	2016	2015	2014	2013	2012	2011	2010	2009	2008	Total
Beetles	9	7	10	8	7	8	3	4	2	8	66
Moths	3	2	1	1	2	2	0	2	0	0	13
Psocids	1	1	1	1	2	3	4	3	0	2	18
Mites	2	3	0	1	0	0	1	1	0	0	8
Total	15	13	12	11	11	13	8	10	2	10	105
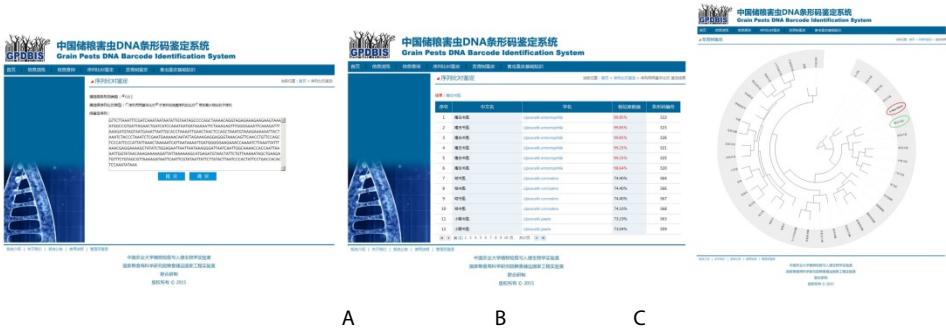
Fig. 2 The main pages of molecular identification in GPDBIS. A: page of sequence input, B: page of sequence similarity, C: page of phylogenetic tree

Globalization accelerates the spread of stored-product pests among different countries and regions. What are the related questions and prospects for research and application on molecular identification of stored-product pests? Apparently, there is more need for molecular identification and common action for the prevention and control of stored-product pests. There is still a gap between the research and application. Like a marathon that requires persistence, we should do our best to continue to promote research and application of molecular identification of stored-product pests with more international collaborations that involve the sharing of more representative samples, development of more practical techniques, and establishment of a more common platform through further research, training and application.

Acknowledgement

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Enhancing surveillance for exotic stored pests in the Australian grains industry using a partnership approach with industry and government.

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Abstract

Verifying freedom from exotic pests such as Khapra beetle (*Trogoderma granarium*) & Karnal Bunt (*Tilletia indica*) is critical to supporting & maintaining access for Australian grain producers to international markets. Despite Australia's geographical isolation & strong quarantine systems, increasing levels of travel & trade continues to place pressure on our biosecurity systems, emphasising the need for improving our regional efforts in prevention, preparedness & surveillance to mitigate risks. The Australian Grains Farm Biosecurity Program (GFBP) is a national initiative to assist in the development & implementation of improved biosecurity practice, playing a vital role in the education of exotic pests & the role of surveillance by industry. The GFBP has undertaken a targeted surveillance program for stored product pests, with Khapra beetle as the main focus. A range of sites based on potential risk groups & pathways (e.g. farming enterprises, seed distributors & agricultural stores) were targeted, with different approaches used across the three grain growing regions of Australia depending on State

activities & pre-existing collaborators. All regions used a combination of pheromone traps & other sampling methods appropriate for host materials & environment. The surveillance is aimed at strengthening evidence of absence, building industry knowledge & participation in grain storage surveillance & promoting improved management practices around storage. These regionally specific engagement methods & surveillance efforts are discussed. Australia remains free of Khapra beetle.

Keywords: grains biosecurity, exotic pest surveillance, on-farm storage and hygiene, risk mitigation practices

Introduction

Exotic plant pests threaten production, market access and sustainability of Australian plant production systems. For the Australian grains industry, over 600 exotic pests have been identified of which 54 are considered high priority pests (HPPs), posing a significant threat. Despite Australia's geographical isolation and strong quarantine systems, increasing levels of travel and trade continues to place pressure on our biosecurity systems, emphasising the need for improving our regional efforts in prevention, preparedness and surveillance to mitigate risks. Verifying freedom from HPPs such as Khapra beetle (*Trogoderma granarium*) and Karnal Bunt (*Tilletia indica*) is critical to supporting and maintaining continued access for Australian grain producers to domestic and international markets (including biosecurity, food safety and quality assurance aspects).

Currently many surveillance activities are done through crop monitoring in the field and sample assessment through the bulk handling system, but little useful data is captured at the national level with regards to exotic stored grain product pests. This type of data is limited, and gaps exist particularly on-farm, where on-farm storage of grain is increasing and becoming common practice, particularly in eastern Australia. Thus, expanding surveillance efforts regionally on-farm to capture more evidence of absence is required.

The national Grains Farm Biosecurity Program

Within Australia, the Grains Farm Biosecurity Program (GFBP), a national initiative to assist in the development and implementation of improved biosecurity practice, plays an instrumental role in awareness and education about exotic pests (Taylor-Hukins et al, 2015). As Australia's flagship biosecurity extension program, the GFBP contributes to the Australian grains industry's risk mitigation activities (under the formal signing of government / industry agreements around biosecurity and emergency response (PHA, 2016) and has now been running for 10 years. The GFBP was acknowledged with a national biosecurity award in 2018 for its contribution to biosecurity and promoting a partnership approach involving government, industry and community (http://www.agriculture.gov.au/biosecurity/australia/public-awareness/aba#australian-biosecurity-award--government).

The GFBP emphasises the importance of surveillance and reporting by industry stakeholders to support and maintain market access and to detect an incursion early, increasing the likelihood of early detection and facilitating the eradication or containment thus reducing its impact on industry and community. A strength of the program is the ability to build collaborative networks for a wide range of activities at national, state, regional and local levels (Bellati et al 2010). This strength has been used to encourage general surveillance and the collection of data for key exotic grain pests through a variety of industry reporting avenues: e.g., National variety trails, state diagnostic laboratories, bulk handlers, researchers, industry consultants and grower groups. Data has been captured from over 90 surveillance programs from a range of broad acre crop types.

Whilst surveillance has been one of the GFBP key activities, it has also been one of the most challenging to execute and maintain, as its voluntary and relies on the 'good will' of those contributors.

The Project (Objectives)

The GFBP recently piloted a targeted surveillance monitoring program for stored product pests with the main target being Khapra beetle (*Trogoderma granarium*), one of the highest ranked exotic pests

for grains which is also listed as a prohibited or invasive species for many of Australia's export trading partners.

The key aims of the project were to strengthen evidence of absence data for Khapra beetle and to build industry awareness, knowledge and participation in grain storage surveillance regionally within the grain-growing regions of Australia. The program also aimed to identify and promote industry advocates and to assist industry in promoting and improving management practices around grain storage, especially in hygiene, and improving the use and efficacy of phosphine application in on-farm storage systems.

The Approach (Methods)

For the pilot surveillance program to be successful, it was imperative to use a partnership approach with reputable programs, networks and alliances for effective industry engagement and uptake. Benefits to using a partnership approach also allowed for a wider coverage of locations, took advantage of cost-sharing for required resources and ensured we were value-adding to contributors.

Different approaches for implementation were used across the three grain regions of Australia (southern, western and northern zones), depending on types of linkages and pre-existing collaborators, state government surveillance activities and industry networks and alliances that could assist and were willing to participate.

A range of sites based on potential risk groups and pathways were targeted. These included privately owned farming enterprises (grain, mixed production and intensive animal production systems), milling, processing and bulk handler establishments, importers of high risk materials, seed distributors, grain/ stock and/or feed producers/ wholesalers and regional agricultural re-sellers.

Host materials and target environments included: older silo systems; products with slow turnover/ minimal fumigation routines; longer term storage, containers and bulker bags (feed /seed, fertiliser/ baits, by-products, other); stockfeed and other dry food stuffs; packing/ bagging materials; cracks/construction joints in cement walling near product storage; areas of low hygiene and inadequate sanitation (within sheds, barns and around machinery and product storage areas) and; dark, dry and low movement corners spaces in processing and production areas.

A range of complimentary sampling techniques appropriate for host materials and favourable environments, for Dermestidae species, were used. These included vacuuming and visual inspection of grain and other host materials, and pheromone specific traps which improved participation and industry engagement due to its novelty.

Access to expert diagnostic support for identification of Dermestidae species was a critical component to the program and states had access to a service (paid or provided in-kind).

Regional specific (State) focus and development of surveillance efforts in 2016-2017 included:

- *Queensland (Qld):* Growers (grower groups) targeted - surveillance and monitoring is occurring, but not formally recorded; potential to develop a storage best management practice / accreditation based around storage and monitoring in conjunction with Qld grains storage research and extension team.

- *New South Wales (NSW):* Targets included privately owned farms, warehouses importing high risk products (e.g. rice, pulses, seed and spices), feedlots and stock-feed manufacturers and wholesalers. Partnered with regionally based NSW Local Land Services (NSW Govt.) that provided staffing to service the traps.

- *Victoria (Vic):* Intensive farming enterprises (e.g. poultry, feed lots, dairy) were targeted due to their tendency of having poorer hygiene practices around grain storages. Also, targeted grain mix and stock-feed manufacturers. The CropSafe program is being used for diagnostics support (http://agriculture.vic.gov.au/agriculture/grains-and-other-crops/cropsafe-program).

- *South Australia (SA):* Significant State government support provided an extensive extension of the program that allowed for a wide coverage of locations and a wider range of target groups surveyed compared to other states which included producers, milling/ processing, stock feeders, bulk handler/ seed distributors, agri-suppliers, regional high school, and a regional research centre. Program was also promoted across the supply chain through a State campaign (http://www.pir.sa.gov.au/primary_industry/crops_and_pastures/clean_grain).
- *Western Australia (WA):* Commercial agronomists targeted, and value added to the existing sentinel merchants and agronomist activities under the Biosecurity eSurveillance projects in WA, which was modelled on the successful Pantry Blitz campaign (an externally funded 'citizen science' project that demonstrated absence of Khapra beetle in WA with 2,252 reports (pers comm. L. Fagan, Department of Primary Industries and Regional Development, WA).

Outcomes (Results)

Over 100 target sites were surveyed and over 1000 'zeros' scored against Khapra beetle in 2016-17. The surveillance data captured is compliant with the Australian national minimum dataset specification for plant health surveillance and was entered into AUSPestCheck, a national database for plant pest surveillance (http://www.planthealthaustralia.com.au/resources/auspestcheck/).

As the program is currently on-going the large sample size being generated allows for comparisons and evaluation, in terms of target group risk profiles, suitability and effectiveness of trap types and where closely related Dermestidae species are found on-farm and within the farming environment regions.

The challenges and considerations identified to date included:

- trap positioning and suitability on farm; every place is different; trial and error due to other factors (e.g. abiotic and biotic factors)
- time length the traps and lures stayed out in the environment (dependent on remoteness of location and who could assist to service traps)
- surveillance program rigor, uniformity and geographical coverage across the regions
- finding voluntary participants and concerns of confidentiality
- reliability of contributors and their ability (skills and training requirements)
- processing and pre-sorting of multiple samples (stored grain insect identification training required)
- value of by-catches (non-targets) to producers and others (e.g., researchers)
- new technologies to assist / trial and to improve automation for data collection
- use of postal services (for sending lures to participants) to help reduce travel costs associated with servicing traps

Discussion

Over 200 industry advocates were identified during the surveillance activities in 2016/17. While there were mixed results within the regions in terms of industry engagement, in general the benefits of the program were positive overall and provided valuable insights.

Anecdotal evidence shows a higher level of learning and training is being sought by producers, with extension moving from simple awareness to more technical and specific information for their farming enterprise.

There was value in the by-catches for grower engagement as it provided insights into species composition within their own farming environments. Producers known to have a closely related Dermestidae species present in their farming system or operations, will hopefully help them to implement improved management practices and encourage extra vigilance in their operations.

Practice change especially around improvements to hygiene of grain storage was observed in many participants throughout the surveillance program.

In South Australia (SA), the programs significance was also acknowledged through additional industry funding (in the form of a SA grains industry trust grant to state diagnostics) as the extension of the program provided a unique opportunity to investigate the by-catches and the related native species composition in SA. The grant has allowed for further analysis, curation and permanent lodgement of reference material into a nationally recognised collection (Waite insect and Nematode Collection).

Biosecurity strategies emphasis the need for industry and community participation. Clearly this type of biosecurity surveillance program is a lot of work, expensive and time consuming, but has made a beneficial contribution in the collection of proof of absence data and industry awareness and education. Future engagement, cost effective resourcing, collaboration and value adding are required along with evaluating the real value of this type and source of surveillance data.

Acknowledgments

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Testing Wheat for Internal Infesting Insects with an Electrically Conductive Roller Mill

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Abstract

Although grain is always inspected for adult insects and insect damaged kernels upon shipping and receiving, immature insects living inside the kernels of grain cannot be readily detected. A laboratory roller mill was modified to measure and analyze the electrical conductance of wheat as it was crushed. The electrical conductance of normal wheat kernels is low and fairly constant. In contrast, the electrical conductance of infested wheat kernels produces a sudden change in the electrical signal. The peak height of the electrical spike depends on the size of the larvae and the resulting contact of the crushed larvae between the rolls. This instrument was designed to test wheat with moisture content of 13.5% or less. The laboratory mill can test a kilogram of wheat in less than 2 min. Hard red winter and soft red winter wheat samples were used in experiments. Known numbers of infested kernels were added to the wheat samples. The infested kernels contained larvae of rice weevils and lesser grain borers sorted into large, medium, and small size groups. The instrument detected ~8 of 10 infested kernels per 100 g of wheat with large-larvae (fourth instar or pupae). It detected ~7 of 10 infested kernels with medium-larvae (second or third instar) and ~5 of 10 infested kernels infested with the small-larvae (first or second instar). Under

reasonable grain moisture and careful sample handling, there were no non-infested kernels classified as insect infested. The mill can lead to rapid and automated detection of infested wheat.

Keywords: rice weevil, lesser grain borer, x-ray, insect fragment test

Introduction

Grain is commonly inspected for insect contamination using visual indicators such as sieving for adult insects or inspection of a 100-g sub-sample for insect-damaged kernels (GIPSA, 2009). However, internal infestations by insects such as *Rhyzopertha dominica*, lesser grain borer, and *Sitophilus* spp. are not easily detected with visual methods alone. With subsequent storage, these hidden infestations can lead to increased pest populations that require treatment such as fumigation and potentially contaminate resulting flour with insect fragments. Many methods of detecting infested wheat have been developed and are available, but all are relatively expensive and/or time consuming. Some of these methods include staining the wheat to detect weevil egg plugs (Milner et al. 1950), microphones for listening to insects feeding (Hagstrum et al. 1990), single kernel compression testing (Pearson et al. 2003), single kernel NIR measurements (Dowell et al. 1998, Perez-Mendoza et al. 2003, 2005), and x-ray imaging (Karunakaran et al. 2004; Haff and Slaughter 2004, Fornal et al. 2006).

X-ray images provide accurate determinations of infested seeds and larvae stages and number of internally infested kernels. However, x-ray systems are expensive and are only able to test a single layer of wheat and small sample sizes. NIR systems were able to correlate actual and predicted fragment levels over a range of 0 to 300 fragments. However, measurements below 100 fragments contained too much variability to clearly determine whether the flour is above the FDA (1988) defect level of 75 fragments from an average of six 50-g flour samples. Fragments in flour are estimated using a chemical method, AOAC 972.32.

The laboratory mill developed by Pearson and Brabec (2007) monitors electrical conductance through crushed wheat. The conductance mill can detect over 70% of the kernels infested with medium and large larvae and pupae, and is able to test 1 kg of wheat in about two minutes. If infested grain is detected, management could react by rejecting the lot, fumigating, storing the lot separately, or quickly milling the grain before insects have time to multiply. The objective of this study was to investigate the ability of the conductance mill to detect different size larvae in infested kernels (experiment 1) and to determine relationship between insect detections and subsequent insect fragment counts in milled flour (experiment 2).

Materials and Methods

A laboratory roller mill was fabicated and consisted of two, 8 cm diameter by 10 cm wide rolls which were mounted on a 2.5 cm diameter shaft. One mill-roll was electrically grounded through the gear motor. The slave roll was mounted into delrin bearings which made the roll electrically isolated. A 5 Vdc supply was electrically connected via a motor brush and contacted this roll. A schematic diagram of the system is shown in Figure 1.

Hard red winter wheat was obtained from a farm in central Kansas at time of harvest and stored in small barrels in a large refrigerator. This wheat was considered non-infested. The grain was cleaned by passing it through a Carter Dockage tester (Carter-Day, Minneapolis, MN) using the dockage configuration for wheat. The moisture content of the wheat was 12.0%. For experiments, two moisture contents were created: 11% and 13%.

Experiment 1. Approximately 250 *R. dominica* or *Sitophilus oryzae*, rice weevil, adults were added to ~500g of wheat which was tempered to 13% moisture and stored at 27°C for 4-5 weeks. This infested wheat was x-ray imaged (MX20-dc44, Faxitron X-ray Corp., Wheeling, Il.) and infested kernels with large, medium, and small larvae were selected based on the x-ray images (Fig. 2).
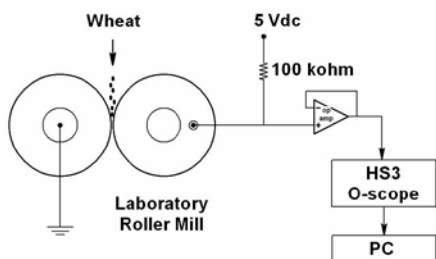
Fig. 1 Schematic diagram of the electrically conductive wheat mill and the associated circuit and basic data acquisition.
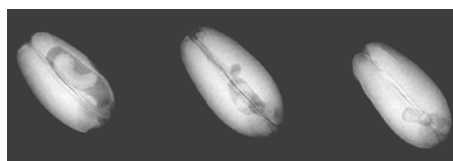
Fig. 2 X-ray image of infested kernels showing the large, medium, and small sized larvae from rice weevil.

Then 10 infested kernels of a given larvae size were added to 100g of sound wheat and crushed in the conductance mill. A micro-controller (Model EL, Tern Inc. Davis, CA) collected and processed the derivative of the conductance signal. The insect counts for a wheat sample were intermittent signal spikes above the baseline of the derivative signal (Fig. 3). The number of detects were recorded. Experimental variables were 11% and 13% moisture content wheat, rice weevil and lesser grain borer infestations, and three larval size categories.
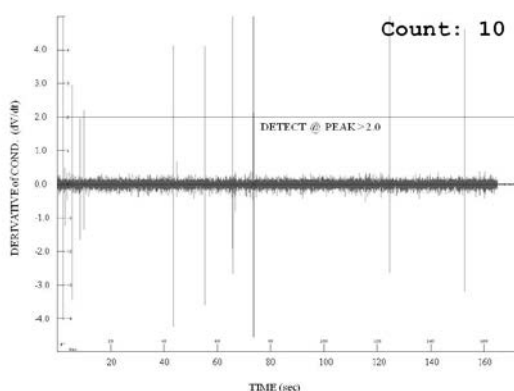
Fig. 3 Example of the software output and the derivative signal collected during the conductance milling of a 1 kg wheat sample. The signal spikes are from infested kernels containg large lesser grain borer larvae as they were compressed between the mill-rolls.

Experiment 2. To study the relationship between conductance detects and insect fragments in flour, infested kernels with lesser grain borers were prepared and added to 1 kg of sound wheat. A spoon of infested grain (~7.0 g) was taken from insect colonies and placed in small plastic bags, and x-rayed. The x-ray images were inventoried for infested kernels. For experiment #2, the initial infested kernels in the plastic bags had size distributions of ~60% large larvae and ~40% medium and small larvae. The original colonies were prepared and adults were removed after 3 weeks causing a bias in the larvae size distribution. The bags of infested kernels were added to the 1 kg of sound grain at three levels of infestation: 11-13 infested kernels (low), 23-25 infested kernels (medium), or 47-49 infested kernels (high) (Brabec et al. 2010). The infested grain samples were evaluated using the conductance mill at two times; day 0 and after six weeks of storage. After six weeks, any emerged adults were removed by sifting and the remaining grain was passed through the conductance mill.

Before each conductance test, a 300 g portion of clean wheat was passed through the conductance mill and the pre-sample was discarded. Then, the 1 kg test sample was passed through the conductance mill. The crushed wheat samples were bagged and stored at 7 °C until they were milled into flour for fragment testing.

Crushed samples were further milled using the Quadramat Jr. milling system (Quad Jr.) and AACC Experimental Milling method 26-50. Flour samples were sent to two U.S. cereal chemistry laboratories for insect fragment analysis. Both laboratories used acid hydrolysis methods. However, laboratory #1 performed the AOAC protocol (1996) using a five minute heating cycle in an autoclave at 121°C and 103 kPa. Laboratory #2 performed the AACC method 28-41b, using a 15 minute heating cycle in the autoclave. A single technician from each laboratory performed the wet chemistry and counted the fragments on filter paper using microscopy techniques.

Results

Experiment 1. The conductance mill is able to detect internal insects, but its ablility to detect varies with the size of the internal larvae. Small larvae (1st-2nd instar) were only detected on average ~50% of the time. The standard error of estimate for the small larvae was 1.5, thus for some samples with small larvae, only 2-3 infested kernels were detected. The large larve and pupae were detected ~80% of the time (Tab. 1).

Tab 1 Detection levels of the 10 infested wheat kernels within 100 g of wheat using the conductance mill for three different size classes of internally infesting larvae of *Rhyzopertha dominica* and *Sitophilus oryzae*.

	Number (+/-SE) of Infested Kernels Detected	
Larvae	*R. dominica*	*S. oryzae*
large	7.9 (1.4)	8.6 (1.1)
medium	7.1 (1.6)	7.7 (1.2)
small	5.5 (1.5)	6.3 (1.5)

Experiment 2. For the 1 kg samples prepared for the insect fragment testing, the infested kernels were a mixed population. At week 0, the lowest density infested samples had detection of ~75% of the infested kernels while the high density infested sample had detection of ~56% of the infested kernels. While accuracy was lower, detection of 28 infested seeds in a kilogram of wheat is already above the level that should raise concerns and therefore the reduced count accurary may be less of an issue. After samples were incubated for 6 weeks, the lowest infested sample went to 67 detects while the highly infested sample went to 120 detect. The insect fragment counts were significantly different between the two commercial laboratories. For laboratory #2, the week 0 samples all had insect fragments below the FDA threshold of 75. For laboratory #1, the fragment counts tended to be higher, even the control samples had fragment counts averaging over 15 counts.

Tab 2 Detection of infested seeds with the conductance mill for mixed infestions in a 1 kg sample of wheat. After the conductance milling, the crushed material was milled for flour and tested for insect fragments.

	Number (+/-SE) Detections	
Infested kernels	0 wks	6 wks
Control	0 (1)	2 (1)
Low 12	9 (1)	67 (11)
Med 25	16 (2)	88 (16)
High 50	28 (2)	120 (20)
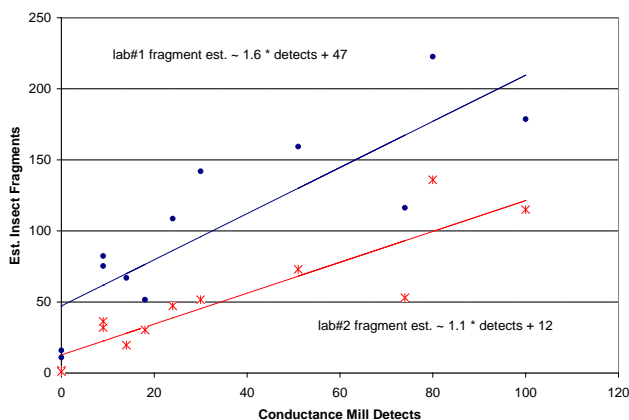
Fig. 4 Estimated insect fragment count verses the conductance mill detects. Two commercial cereal chemistry laboratories analyzed the samples; lab#1 and lab#2.

Discussion

The biggest challenge for sampling wheat for internally infesting insects is that detection is time consuming and that large amounts of wheat need to be sampled to detect low infestation levels. The conductance mill can process 1 kg in a couple minutes thus it is possible to quickly test wheat as grain is received from trucks or rail. Storey et al. (1982) studied over 2,000 wheat samples from many U.S. export grain terminals and found ~8% contained rice weevils and ~6% contained lesser grain borers after incubation, although less than 1% of their samples were graded as "weevilly". Perez-Mendoza et al. (2004) studied grain samples from eight rail cars, or 24 rail car compartments, at a grain processing facility. The study found that 20 of the 24 rail car compartments averaged less than one insect per kg of wheat. However, four compartments averaged 2, 6, 17, and 19 internal insects per 3 kg sample. Probing railcars and inspecting samples and storing samples for later insect emergence requires significant time and effort. Also, the visual sample obtained during inspection often did not match the internal infestation samples in terms of insect density. The conductance mill works well at detecting samples with lower infestation levels that are more realistitic in terms of what the industry needs to be able to detect. And the conductance mill can handle 15-20 kg of samples per hour as might be required while unloading railcars or truck.

There are different factors that can impact the accuracy of detection. False positive counts were caused by small clods of dirt in the wheat, so cleaning the wheat before processing by passing over some sieves is recommended. Also, any external moisture, such as rain or snow, could add signal noise, but usually this is not detected. Additionally, the conductance mill cannot detect internal infestations if the insects have died and are dried up, such as occurs after a fumigation and this will effect estimations of insect fragment levels in flour but will not be a factor in terms of estimating risk of insect population growth in a bin.

The conductance mill has also been test with rice and popcorn (Brabec et al., 2012, 2017). Rice is smaller than wheat and popcorn is larger than wheat, so each grain size needs appropriate mill gaps for the material to grind smoothly. For rice, the mill design included differential gearing during milling. Early test using 1:1 gearing and laboratory mill gaps of 0.018" and 0.028" show that detection decreased as roll gap increased, particularly from the small larvae (Pearson and Brabec 2007). Detection sensitivity was improved with the shearing action from differential rolls.

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analysis. This paper reports the results of research only. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The US Department of Agriculture is an equal opportunity provider and employer.

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Survey of *Trogoderma* species (Coleoptera: Dermestidae) Associated with International Trade of Dried Distiller's Grains and Solubles in the USA

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Abstract

Dried distiller's grains and solubles, DDGS, is a valuable commodity with substantial international trade. Vietnam discovered an infestation of *Trogoderma inclusum*, an actionable quarantine pest, in DDGS from the USA in 2012.

All subsequent shipments to Vietnam were required to be fumigated. A shipment to Vietnam from the USA 2015 was then discovered with *T. variaibile*. We surveyed the presence and activity of *T. inclusum* and *T. variabile* at locations in the USA that provide DDGSs for shipment to Vietnam. Seven facilities in four states that either produced DDGSs or that facilitated bulk shipments were studied. Pheromone traps were deployed at each location and monitored for several weeks. *T. variabile* was trapped at all seven sites while *T. inclusum* was trapped at just five of these. *T. variabile* were captured in nearly every trapping period and at higher numbers than *T. inclusum* at five locations, while two locations captured more *T. inclusum* than *T. variabile*. Spatial variation seemed to occur within each site, but there was no common pattern among facilities. Substantial numbers of beetles were caught in the outdoor sticky flight traps for most locations, except for relatively low flight trap numbers at locations 1, 4 and 6. The results show that *T. variabile* and *T. inclusum* are commonly associated with DDGSs produced in the USA, that these beetles could infest product being shipped overseas, and provide information that can be used to develop risk assessment and pest management programs for the future.

Keywords: Coleoptera, Dermestidae, DDGS, Vietnam, quarantine.

Introduction

The United States Grains Council (USGC) learned in late 2012 that the Vietnamese government's Plant Protection Department (PPD) had discovered an infestation of the larger cabinet beetle, *Trogoderma inclusum*, an "actionable" quarantine pest for Vietnam, in a shipment of Dried Distillers Grains and Solubles, DDGS, from the US (USGC 2012). The Vietnam PPD required the infested shipment be fumigated and then re-exported. The PPD also required that all DDGS shipments from the US to Vietnam be fumigated before delivery from that time forward. The US DDGS industry complied with the required fumigation on all subsequent shipments. No infested shipments were reported in the subsequent three years, until a shipment of 12 containers of DDGS from Norfolk, VA on September 17, 2015 was inspected in Vietnam at arrival on October 27, 2015 and found to be infested with live warehouse beetles, *Trogoderma variabile*, a close relative to *T. inclusum*. It is presumed that this shipment had been fumigated at the time of export, as required by agreement. Assuming that fumigation was performed on the commodity before leaving the US, the infestation could have occurred via one of two ways: the fumigation was not entirely effective in completely disinfesting the shipment, or that infestation occurred after the Norfolk fumigation, but before the delivery in Vietnam six weeks later.

Both *T. variabile* and *T. inclusum* are stored grain insect pests that are commonly found in the US and around the world as part of a complex of many pest species that infest post-harvest agriculture products (Aitken 1975). Commodities infested by these species incude cereal grains, ground or milled grain products, nuts, dried fruits and numerous value-added food products (Hagstrum and Subramanyam 2009). *T. variabile*, the more common of the two, is reported in the scientific literature to occur in Vietnam. To our knowledge, *T. inclusum* has not been reported to exist in Vietnam, though it is reported in the entomology literature as occurring in Thailand. The Vietnam PPD considers *T. inclusum* to be an exotic pest subject to quarantine regulations that would involve inspection followed by some action if discovered. Quarantine action for introduction of *T. inclusum could include* disinfestation of arriving shipments via fumigation, return of infested commodity to the source country, or destruction of an infested shipment. All life stages (egg, larva, pupa and adult) of both beetle species can be effectively killed by properly fumigating with an effective gas such as phosphine or methyl bromide. Both species occur in the US, and it is likely that these species could feed on and reproduce in DDGS, but we have not found published reports of these species infesting DDGS. In any case, we know that both species are common in the US and in many other countries, and that these pests can probably infest DDGS and travel with shipments from the US to any of our trading partners.

We were contracted by the USGC in mid-2016 to assess the presence of *T. inclusum* and *T. variabile* in representative supply-chain contexts of DDGS production and commerce in the midwestern USA. Information on the occurrence and relative abundance of the target insects can be used to estimate the risk of infestation at DDGS facilities and then infer how that risk could lead to these pests being carried in shipments to Vietnam. It is hoped that the USGC and other trade or agricultural product

organizations could use such insect risk information to develop better pest prevention and mitigation practices for the DDGS industry. Specific objectives for us were:

- Select and engage DDGS companies in the north-central Midwest of the USA, including both ethanol plants and trans-loading facilities, to participate in the project.
- Make site visits to each of the cooperating companies to conduct a thorough inspection, interview key personnel, deploy insect traps for *T. variabile* and *T. inclusum*, and develop plans for continued trapping.
- Analyze all traps from each facility for the presence and numbers of the target species, with specific attention to relative numbers of insects trapped over time throughout the trapping season, and among specific trapping sites at each company.

Materials and Methods

Participating companies were in our geographic area of interest, whcih was the corn-growing region of the US at sites located in the states of Illinois, Indiana, Iowa and Missouri. These sites included five ethanol plants, numbered 1-5 in Table 1 below, and two trans-loading facilities, numbers 6 and 7. On-site visits were made to participating facilities during May, June, July and August of 2016. All facilities we studied were using corn as the grain to be distilled into ethanol and the manufacturing procedures at the ethanol plants were similar. Briefly, grain was delivered, stored, mashed with water, yeast and additives for fermentation, the ethanol separated and purified form the fermentation product distillation after which ethanol was prepared for delivery and the DDGS were dried, cooled and loaded for delivery. DDGS trans-loading facilities had a simpler lay-out compared to ethanol plants. The only activities at trans-loaders was to receive recently processed DDGS from ethanol plants and then load shipping containers for movement across the US, including to export terminals for shipment overseas.

Traps were deployed at four indoor locations and two outdoor locations at each of the ethanol and trans-load facilities in this study. We used traps baited with the synthetic pheromone attractant that is used by both *T. variabile* and *T. inclusum*. The lure is synthetic mimic of the female-produced sex pheromone that attracts males in nature. Two different traps were used: one known as the "Dome Trap" (Figure 1) for walking insects, and the other a "Storgard II" sticky trap (Figure 2) for flying insects. A single Dome trap was placed at each of four indoor locations such as fermentation, distillation, loading and one or more spots in the flat storage. A sticky trap was hung at about 2 m off the ground outdoors at the farthest east and west borders of each facility. Traps were deployed during the initial site visit to each of the cooperating facilities. One individual at each company was then responsible for collecting the traps after a two-week period, shipping the traps back to us at KSU, and then deploy a new set of traps sent by us for use at the same locations for another two weeks. Our trapping system therefore allowed for detection of the target species of beetles at four indoor and two outdoor locations at each of our study sites, and we had two or more trapping periods throughout the season to assess any change in insect populations or activity over time.
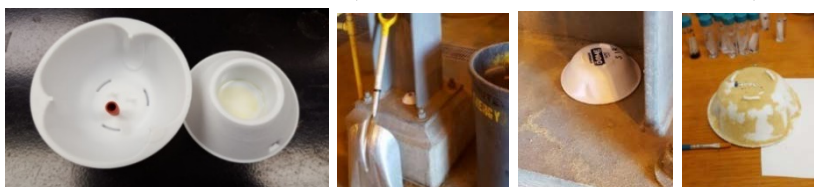
Fig. 1 The Dome trap (left) used for trapping crawling *Trogoderma* adults inside ethanol and trans-load facilities. Dome traps were placed at the bottoms of pillars or at floor-wall junctions at four places indoors (second and third from left) and then returned to the laboratory for processing (right).

Fig. 2 Storgard II sticky trap hung on a fence near the periphery of a research site (left). Beetles fly to the red rubber stopper that is slowly releasing the synthetic female sex pheromone, and then are stuck on the sticky trapping surface inside the trap (second from left). Adult *Trogoderma* are found in the trap (second from right), removed and cleaned in solvent prior to being identified to species and counted (right).

Results

There was a range of trapping periods across cooperators based on the dates we began trapping at a given facility and also due to time availability of cooperators to help with the project. Therefore, the number of trapping periods ranged from 2 trapping periods at facility 6, to 7 trapping periods at both locations 1 and 2.

Initial trap captures revealed numbers of beetles in traps ranging from no beetles upwards to over 100 in a two-week period. We soon realized that there were more than two species represented in traps at all the locations. Some insects that do not use the same pheromone as the lure used in our traps may still responded to the trap and be captured. Once we separated all members of the genus *Trogoderma* from others, we then gave special attention to accurate identification methods published by earlier researchers for these species to become proficient in the identification. Characters related to color of the elytra and diagnostic morphological features of the eyes, were critical for identification (Figure 3).
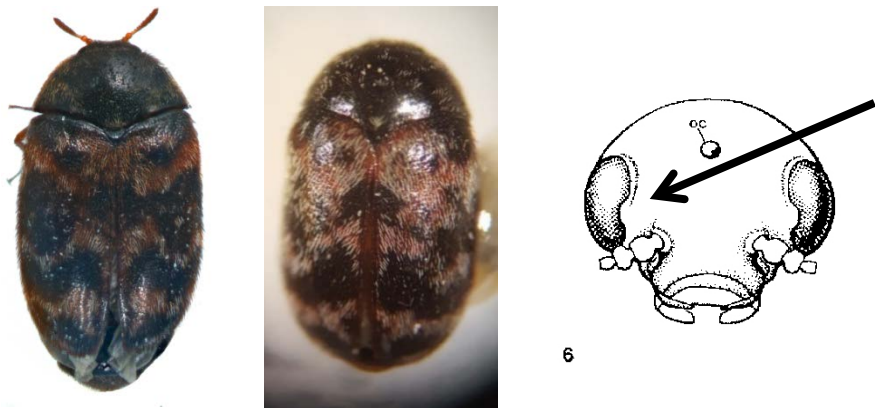
Fig. 3 Specimens of adult *Trogoderma variabile* and *Trogoderma inclusum* showing the dorsal sides. The species are very similar, but are separated by the black and brown bi-color appearance of the wing covers over which thin hairs were distributed in *T. variaible* (left) vs *T. inclusum* (middle) with just a black color under the white hairs. The best diagnostic character is the "notch" on the interior margin of the eye in *T. inclusum* shown at the arrow, and the lack of that notch, so that the margin of the eye is uniform and complete, in *T. variabile*. See ISPM (2016) for details.

Table 1 reports the numbers of adult beetles of both target species trapped at the indoor and outdoor locations for each of our seven study sites during the summer and fall of 2016. We have combined the numbers of beetles captured in the four indoor traps at each location, and these numbers are present in bold text in Table 1. Dome traps at all of our seven sites captured T. variaible,

while *T. inclusum* was trapped at five out of the seven sites, with none trapped at sites 4 and 7. Interestingly there were no *Trogoderma* trapped in our outdoor traps at locations 4 and 7, which suggests that these two species were in low or undetectable population levels at these places. *T. variabile* were captured during nearly every trapping period and at higher numbers than was *T. inclusum* at locations 1, 2, 4, 6 and 7. Traps at locations 3 and 5 captured more *T. inclusum* than *T. variabile*. Although Table 1 reports the sum of beetles from the four indoor traps in each time period, we found some trap to trap variation within and between facilities. For example, the trap in the fermentation area of site 1 consistently caught more *T. variabile* than did other locations at that facility. For the two trans-load sites that had Dome traps near the four corners of the flat storage, one corner seemed to consistently capture more beetles than any other. Spatial variation seemed to occur within each site, but there was no clear similarity between companies regarding which part of a facility had more beetles than another. Substantial numbers of beetles were caught in the outdoor sticky flight traps for most locations, except for relatively low flight trap numbers at locations 1, 4 and 6.

Tab. 1 Average numbers of adult *T. variabile* and *T. inclusum* captured per week in the indoor dome traps (sum of four trap), Inside, and in the two outside sticky flight traps to the west and east of the buildings, Out-W and Out-E, over a given number of trapping weeks at numbered ethanol plants and trans-loading facilities during the Summer-Autumn of 2016.

| Site | Type | Weeks | T. variabile | | | T. inclusum | | |
			Out-W	Inside	Out-E	Out-W	Inside	Out-E
1	Ethanol	14	1.5	10.9	1.1	0	0.1	0
2	Ethanol	14	48.5	71.4	21.1	0	0.2	0
3	Ethanol	14	125.4	4.9	20.5	87.5	18.1	10.2
4	Ethanol	6	5.2	100.3	10.0	0	0	0
5	Ethanol	10	21.5	24.6	44.0	67.8	53.9	131.2
6	Trans-Load	4	6.8	2.3	1.0	8.8	1.0	0
7	Trans-Load	10	44.6	12.4	19.0	0	0	0

Discussion

The research reported here clearly shows that the beetles *T. variabile* and *T. inclusum*, the two species that were intercepted in Vietnam with DDGS from the US, commonly occur at ethanol plants and trans-load facilities that handle and market DDGS. This result met the expectation we had at the outset. Both species are very common in the US and previous studies have found that both can be trapped in many geographic regions of the US. Although we have data showing the occurrence of these species, we cannot report the density or absolute abundance of these species at each site. Pheromone trapping is an indirect sampling method that can only detect presence vs absence of a pest, and the relative numbers across locations and over time. Insects per unit of commodity (e.g. per bushel of grain or hundred-weight of DDGS) or per square meter of space would require more thorough and laborious methods to directly sample the pest populations. During our visits to cooperator sites we collected spilled DDGS and found no insects of any kind upon sifting these samples at our lab. Our trapping work clearly showed differences in relative captures of the two species, and also within species and across locations in a plant. It appears that numbers trapped at a given location in a facility could point to a need for sanitation or pest control to clean or disinfest areas with high trap captures. Captures of *Trogoderma* beetles at our outside traps indicate that beetles can be both outdoors and indoors, while the source location of trapped beetles is not confirmed.

Despite both beetle species being common and widely distributed, the risk of DDGS infestation by these pests and the risk that such pests may be transported with infested product, should vary in predictable ways. Trapping shows these species are common and thus could infest a suitable grain product at most times and places when weather and other environmental conditions are good for insects. However, these beetles can infest and persist in DDGS in only a few cases. Corn delivered to a site could be infested after harvest and through transport and storage periods. The longer grain

is stored, the more likely that infestation will occur. However, before becoming DDGS the corn is mashed and cooked, a practice that will kill all insects. The fermentation and distillation processes are fully insecticidal, and the temperatures during DDGS drying are extreme, over 600 F. The cooling period lasts about 24 h and during the majority of that time the DDGS would be too hot for infestation. DDGS should be susceptible to insect infestation when it is cool and handled in the flat storage for the 1-2 days prior to being loaded and shipped. Trapping has shown that beetles can be at all locations mentioned here, but access to suitable new DDGS would be only at the flat storage and also at the loading out location. Trans-loading facilities have no heating practices that can kill insects, and our trapping study shows that the target beetles can be present, but the product does not stay long before it is loaded into a container and shipped out. We were fortunate to encounter a man from the US Grain Inspection Service at one of our trans-load facilities who was taking timed samples of DDGS while they were being loaded into a container. He said that the samples were to be sifted for insects back at his office, and he told us that he had never found any insects in any samples like these he has taken in the past. Even if infestation of cooled DDGS occurs commonly, a buildup of detectable numbers would require several weeks under suitable conditions for substantial reproduction and increases in pest populations to occur. After leaving a trans-loading facility the DDGS may reach their ultimate destination within one day, or after several days or weeks for domestic rail service, or weeks to months for international ocean-going shipment. It is these time periods after drying and cooling that DDGS can be at risk for infestation.

Fumigation is the most effective and practical means to treat a potentially infested commodity to eliminate actionable quarantine pests before the commodity arrives at its destination (Myers and Hagstrum 2012). None of the seven facilties studied reported fumigating DDGS prior to any internatiaonl shipments, and all had discontinued shipping product to Vietnam at the time of our work. We interviewed one fumigation company about their practices with containers of DDGS. We were told they had fumigated containers near an export terminal with phosphine gas for 24 hours, and then the containers were ventilated and transported locally for loading onto a barge or ship destined for export. In our opinion this practice would not be the most effective to ensure a good kill of pests and quarantine security for the product (Hagstrum and Subramanyam 2009). The time after ventilation and prior to loading on a ship represents a period of susceptibility to pest invasion into the recently fumigated product. Also, the 24-hour fumigation may not give the most effective kill due to the short exposure time. Some pest species and certain lifes stages can be relatively tolerant to phosphine and a longer fumigation may be recommended. Many other variables can affect the efficacy of a 24-hour phosphine fumigation of shipping containers.

Acknowledgement

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Insect pest monitoring in museums - old and new strategies

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Abstract

Integrated Pest Management (IPM) is an important part of preventive conservation of museum objects made of wood, textiles, starch, paper, keratin and other organic materials. Long term monitoring data help us to discover new infestations and locate them in the building. Results from over 20 institutions (museums, storages, historic libraries and historic palaces) are presented and discussed how the monitoring can be improved, where active infestations were found, what treatment was done as a response and what new methods are used. What pests are the most abundant, which species are new for the indoor museum environment and when do we actually have active infestation and damage of museum objects? Monitoring and IPM in museums is also compared with the food storage industry. IPM is applied in many museum today, mainly to reduce the application of pesticides, for a long-term protection of the objects and collections and early detection of infestations. In this presentation, long term monitoring data with sticky blunder and pheromone traps for webbing clothes moth Tineola bisselliella is described. The analysis of the data show that in all museums and storages buildings with a monitoring in place different insect pest species are present, but only in few collections damage to museum objects was found. New pests like the grey silverfish Ctenolepisma longicaudata and Ctenolepisma calva - another species of Lepismatidae, are now found in many museums in Vienna, Austria. The odd beetle Thylodrias contractus was found recently in Austria, surprisingly in four different locations.

Remote monitoring of stored grain insect pests

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Abstract

A number of remote sensing methods were developed and tested in commercial grain warehouses; probe pitfall traps attached to vacuum lines, surface pit fall traps equipped with video cameras and white boards on grain surface monitored with video cameras. These methods were compared with detecting insects using grain samples. Warehouse trials by trapped methods were carried out in bins with 8520 t of wheat from 23 May until 8 August 2016. Grain temperatures were from 22.7 to 31.6℃. Pscoids, *Liposcelis bostrychophila* Badonnel, were detected by grain samples, but there were higher number of pscoids trapped with the probe pitfall traps and pitfall traps than found in grain samples. *Plodia interpunctella* (Hübener), *Sitophlius zeamais* Motchulsky and *Cryptolestes ferrugineus* (Stephens) were detected by probe pitfall trap, but not in the grain samples. *S. zeamais* was detected by the pit fall traps. Using the remote controlled video camera in the warehouse head space, we were able to distinguish and count *S. zeamais*, *C. ferrugineus* and psocids on white boards. The video from pitfall traps can be sent to mobile phones. With all these methods, data can be collected remotely, and could be analyzed by imagine analysis allowing for rapid real time monitoring of insect pests.

1. Introduction

Efficient sampling is a decisive factor for the timely and safe undertaking of measures in the management of stored products and foods (Buchelos and Athanassiou, 1999). Timely monitoring is necessary for pest management of stored grain, especially for wheat and paddy rice which can be stored for three to five years in China. The need for as many samples as possible, as frequently as possible, is a technical problem of conventional sampling methods (Subramanyam and Hagstrum, 1995). Sampling and sieving grain is a current method in stored insect monitoring as recommended in Chinese grain storage regulation. The grade of insect infestation of stored grain is decided by sampling, although this technique is effective primarily for detection of adults and some larvae.

Manual sampling of insects in stored grain is a laborious and time-consuming process (Flinn et al, 2009). Over the past few decades, many researchers have developed traps for use in store facilities as an alternative sampling method (Buchelos and Athanassiou, 1999). Probe traps, when compared to other trap types, have given satisfactory results in the trapping of many important Coleoptera and other stored product species; at the same time, they are easy to use and reliable even without the use of an attractant (Lippert and Hagstrum, 1987; Subramanyam et al., 1993; Fargo et al., 1994；Buchelos and Athanassiou, 1999). Pitfall traps were also developed for insects that are active on top, higher temperature, layer of grain bulk in summer. Automation of grain sampling and insect monitoring should help to increase the adoption of stored grain integrated pest management. A new commercial electronic grain probe trap (OPI Insector) has recently been marketed (Flinn et al, 2009). A probe pitfall traps system attached to vacuum lines had been developed ten years ago in China. The insects can be vacuumed from trap bottom through the line by remote control and then counted. Another approach is the use of a video camera in the headspace of grain warehouse which can be controlled remotely to capture insect pictures when they walk on a white board that was laid on surface of grain bulk. This method has been used in grain depots of Sino-grain. A surface pitfall trap equipped with video cameras was made and the captured pictures can be monitored by mobile phone. Here some insect monitoring results were reported for a number of remote sensing methods, including probe pitfall traps attached to vacuum lines, surface pitfall traps equipped with video cameras and white boards on grain surface monitored with video cameras. These methods were compared with sampling insects using grain samples.

2. Materials and Methods

2.1. Trial 1

The plastic probe pitfall traps, attached to vacuum lines (PPTAVL), consisted of probe with hole, on its wall were holes insects can go through, insect collecting chamber on bottom, after insect fall in, and vacuum line for sucking out the collected insects in the chamber. The vacuum line was connected with vacuum pump, insect collecting bottle, insect checking sensor, and remote controller (Fig. 1). The probe pitfall was inserted into grain mass so that the head was beneath the surface of the bulk.
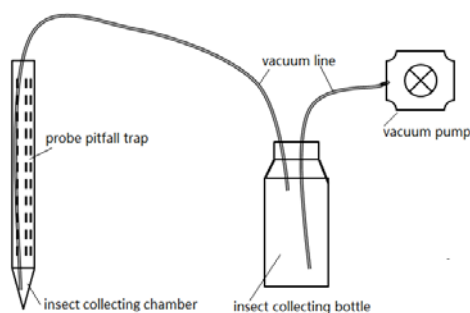
Fig. 1 Diagram of the probe pitfall trap with attached vacuum line.

The surface pit fall traps equipped with video cameras（SPTEVC）consisted of a disc contained radial channels, where insects can through, insect collecting chamber attached centrally below the disk, video camera right over the chamber, communication device with WiFi (Fig. 2). It was mostly made of plastic. Insect collecting chamber in the SPTEVC was inserted into top layer of bulk. The disk with the Insect going channels was laid on the level of bulk surface while monitoring. The insect trapped in the chamber were collected manually.
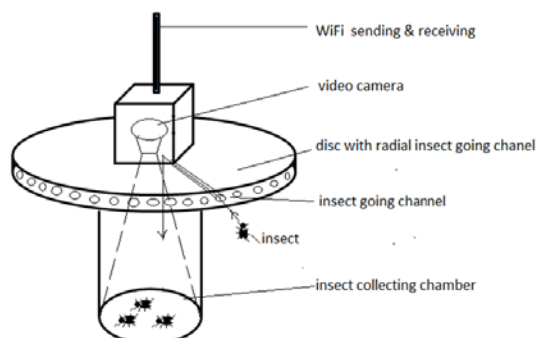
Fig. 2 Diagram of the surface pitfall trap equipped with video camera.

Sampling check was carried out using one kilogram grain samples for each monitoring time and position. The relative distance among between traps, grain sampling location, and warehouse walls, was one meter. There were five sets of traps and sampling points located in four corners and one central position in the storage which contained 8520 tons of wheat (Fig. 3). The stored wheat was loaded in June 2015 with 12.6% moisture content and 784 g/L test weight. The highest temperature average was 20.2℃ and and the lowest 0.1℃ in the winter of 2015. On the beginning of the trial, May 16th of 2016, insect density was zero per kilogram of grain by sampling method for beetle, moth and psocids.
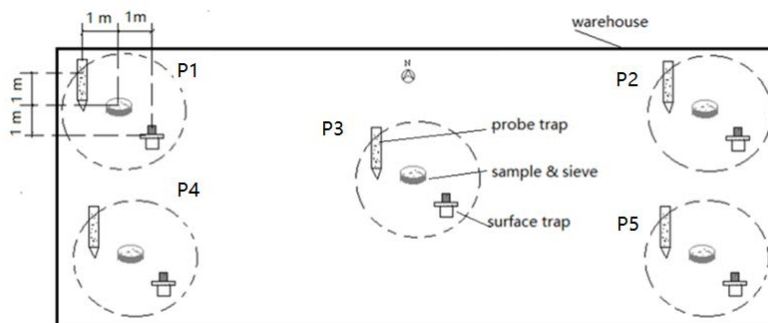
Fig. 3 Traps, sample position and five sets on grain bulk of the warehouse

2.2. Trial 2

White boards on grain surface were monitored with video cameras（WBMVC）to measure insect activity. One high definition video camera was set up in headspace of a warehouse, in which 7000 tons of wheat was stored in bulk. The video camera can scan whole surface of the bulk remotely to get clear figure of insect on grain surface, as is shown at Fig. 4. A white board with 1 cm grid pattern was laid on bulk surface. The insects that crawled on white board, even psocids, can be seen on screen of a remote control computer (Fig. 5).

Fig. 4 A picture of psocids on grain bulk surface captured by computer from high definition video camera
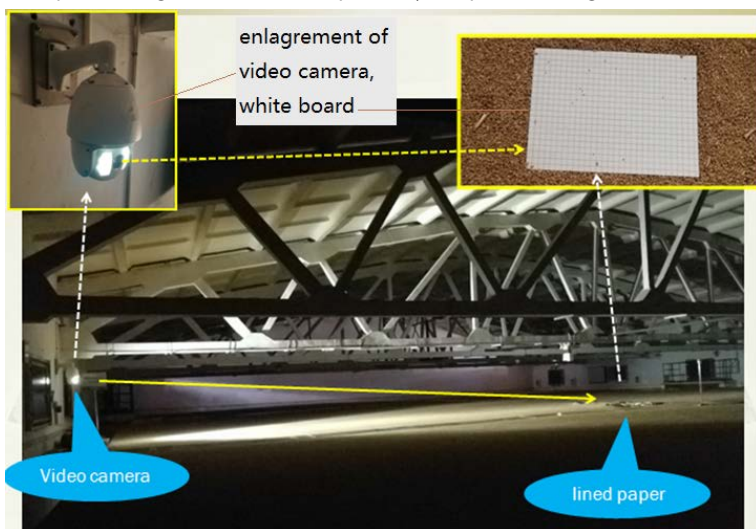
Fig. 5 The white board laid on surface and video camera in headspace of warehouse

2.3. Insect checking and handling

In trial 1, insects were monitored, and samples collected and checked, once a week. The number in traps was recorded as adults or larvae per week. The number in sample was recorded in adult per kilogram of sampled grain. All insects in traps were removed at each checking time. In trial 2, the picture was captured once a week. The dark spot in different sizes and outline shape of insects on white were checked and counted.

3. Results

3.1. The species detected by the different monitoring methods

During the monitoring time from May 16th to August 8th, *Liposcelis bostrychophila* Badonnel was trapped in PPTAVL and PTEVC and found in sieved samples. *Plodia interpunctella* (Hübener), *Sitophlius zeamais* Motchulsky and *Cryptolestes ferrugineus* (Stephens) were detected by probe pitfall trap, but not in the grain samples. *S. zeamais* was detected by the pitfall traps. The method of sample and sieve only detected the psocids even when beetles and moths were exiting in grain mass.The pitfall trap captured S. zeamais and *L. bostrychophila*, but not *P. interpunctella* and *C. ferrugineus* even though the probe pitfall trap can trap all the insects mentioned above.Due to the random distribution of insects in different monitoring locations, the probability of detection is very

different in the same monitoring method for the detected species. In these methods, however, the ability to detect insect species is obvious.

3.2. Comparison of number of captured insects using different methods

The insect number monitored by same method varied among the five positions at same checking time. The numbers at different checking times also varied for insect species and monitored methods in all trials. And the number of insects sharply varied among different methods at same positions (Table 1). For example, on May 23rd, 32 adults of *L. bostrychophila* in one week was trapped in PPTAVL which was obviously more than the 5 adults captured at the same time in PTEVC. There were eight *L. bostrychophila* adults sieved from grain sample. It means that the psocids can be checked or attracted by the three methods, but that they may be detected in greater numbers in probe pitfall trap.

The number of beetles and moths captured in two trapping methods was obviously different, although few insects were trapped in the trap trials. *P. interpunctella* number was 1-3 larvae per week detected by probe pitfall trap and zero per week captured in pitfall trap. The number of *C. ferrugineus* captured was 2-5 adults per week in probe pitfall trap and zero per week in pitfall trap. For *S. zeamais* was 1-3 adults per week in the probe pitfall trap and only one per week in pitfall trap. The pitfall trap set on surface of grain mass detected fewer beetles and moths than probe pitfall trap inserted into the bulk. Sampling and sieving method detected no beetles or moths, which indeed existed in the grain mass during June 20th to August 8th.

3.3 Insects on white board under of video camera

A picture from video camera in headspace of grain warehouse of the white board was captured on computer as shown in Fig. 6. The biggest dark spot was revealed as a *S. zeamais* adult, the middle sized dark spot was a *C. ferrugineus* adult, and the smallest dark spot indicated that *L. bostrychophila* crawled on the board. The species judging was based on dark spot size, picture outline and experienced knowledge. The picture captured by computer received from video camera can provide information about insect species and dynamic number during monitoring. Insect number or population dynamic can be known by counting the dark spots in 1 cm subsample or on whole white board at any time.
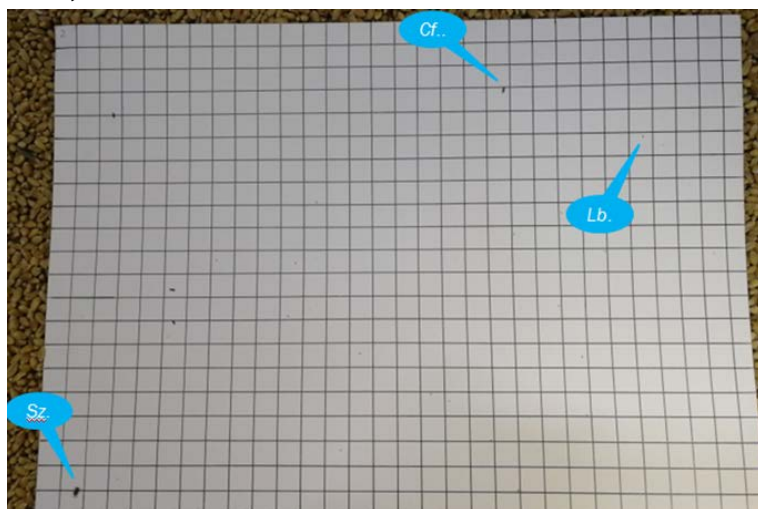
Fig. 6 Identification of insects on white board made from a image captured from a video camera (Sz for *S. zeamais*, Cf for *C. ferrugineus* and Lb for *L. bostrychophila*.

Tab. 1 Insect number captured by traps or recovered in grain sample.

Date /Month·Day	Temperature outside of warehouse /°C	Temperature in headspace /°C	Temperature of bulk /°C	L. bostrychophila in PPTAVL (adult/week)	P. interpunctella in PPTAVL (adult/week)	S. zeamais in PPTAVL (adult/week)	C. ferrugineus in PPTAVL (adult/week)	L. bostrychophila in PTEVC (adult/week)	L. bostrychophila sieved from grain (adult/kg grain)
5.16	20.0	25.0	20.8						2
5.23	26.0	28.0	22.7	32.0				5	8
5.30	24.0	28.0	21.8	80.0				10	30
6.06	22.0	27.0	23.2	100.0				15	30
6.13	26.0	31.0	23.6	180.0				15	25
6.20	27.0	35.0	25.5	190.0	1.0			10	30
6.27	24.0	32.0	28.8	130.0	1.0			5	25
7.04	24.0	34.0	28.9	85.0				3	23
7.11	28.0	33.0	29.4	67.0	3.0			5	27
7.18	25.0	32.0	28.9	76.0	2.0	1.0	2	4	33
7.25	29.0	36.0	30.3	65.0	2.0	3.0	2	6	40
8.01	29.0	38.0	32.5	5.0	2.0	3.0	5	1	2
8.08	26.0	35.0	31.6	15.0	1.0	3.0	2	8	10

4. Discussion and conclusions

Remote insect monitoring is being realized by insect sensors and remote information transfer which should be more convenient than manual methods of insect detection in grain storage. Accurate monitoring is needed and spatial analysis techniques are increasingly being used in entomological investigations (Liebhold et al., 1993; Trematerra and Sciarreta, 2004). These techniques apply specialized software to trap captures, interpolating the data from the sampled locations to generate data for a non-sampled location. All these data are subsequently represented in contour maps, from which a wide range of information can be obtained, notably the distribution of different pest species in space and time (Schotzko and O'Keeffe, 1989; Arbogast et al., 2000; Campbell et al., 2006; Trematerra and Sciarreta, 2004), their movements through facilities (Campbell and Hagstrum, 2002; Arbogast et al., 2002; Athanassiou et al., 2005). It is important to know the relationships for different species of insect between trapping and sampling & sieving under specific cases such as grain storage types, warehouse conditions, capacities of the bulk, temperature of grain mass and warehouse, quality of stored grain, status of insect infestation.

With the results in this research psocids can be found by traps and sampling. But there were higher number of psocids trapped with the probe pitfall traps and pit fall traps than found in grain samples during whole monitoring process. *P. interpunctella*, *S. zeamais* and *C. ferrugineus* were detected by probe pitfall trap, but not in the grain samples. *S. zeamais* was also detected by the pit fall traps. All detected information of insects was able to be transfered and controlled remotely. Using the remote controlled video camera in the warehouse head space in other trial, we were able to distinguish and count *S. zeamais*, *C. ferrugineus* and pscoids on white boards. All information can help us to improve pest management by indicating if it needed to kill the insect or not. It can also increase the effectiveness of treatments (Brenner et al., 1998; Blom et al., 2002; Campbell and Hagstrum, 2002) and reducing prospects for the development of resistance (Belda et al., 2011). Consequently treatments costs of insect monitoring may be reduced due to reducing on manual work.

Acknowledgement

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Can the DI-SPME gas chromatography mass spectrometer be a tool for identification of stored grain insects - fatty acids and sterols profiling

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Abstract

Identifying insect pests is essential for management, but these insects can only be reliably identified by a limited number of highly skilled taxonomists. Expert morphological determination can require dissection and slide mounting of specimens in order to examine distinguishing diagnostic features. Suspected insect pest specimens found in grain products usually consist of only the larvae or larval skins which are very difficult to identify to species, and sometimes impossible to diagnose morphologically. Adult specimens are usually scarce and more often damaged. Misidentification of species could lead to misled pest management practice.

Fatty acids (FAs) have long been recognised as biochemical markers for organism classification. The direct immersion solid phase microextraction gas chromatography-mass spectrometry (DI-SPME-GCMS) technology has been developed and validated for selectivity and accuracy by isolating fatty acids from natural fatty acid methyl esters. Seven different species of stored grain insect pests were analysed by using DI-SPME-GCMS method profiled fatty acids and sterols from insect extractions. Palmitic acid (C16:0), Stearic acid (C18:0) and Oleic acid (C18:1) were absorbed. The ratio of FAMEs/FAs (ME) were calculated and validated as a new biomarker for insect classification. Mid-

chain waxes, low boiling point semi-VOCs, and other lipid components can also be identified by the same method, which can be adopted to be an automated high-throughput method for insect classification, surveillance and quarantine purposes.

Keywords: direct immersion solid phase microextraction (DI-SPME), fatty acids & sterol lipids, biomarker, stored grain insect, insect morphology and identification.

Webbing Clothes Moth, Tineola bisselliella (Hummel) Sex Pheromone Transfer from Monitoring Lures to Textiles

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Abstract

The use of synthesized sex pheromone lures for the purpose of monitoring populations of webbing clothes moth, *Tineola bisselliella* (Hummel) in museum storage environments is typical in many museums. Questions about whether the pheromone incorporated in the dispensing lures could possibly transfer over to textiles that are in close proximity to the lures have been posed by museum conservators. Although some textiles may be naturally attractive to clothes moths, the concerns are that the textiles themselves may become even more attractive to insects due to exposure to the pheromone and that this could ultimately cause further damage to the collections. The focus of this study was to determine the degree to which textiles that have been exposed to pheromone lures absorb the pheromone and become attractive themselves. Based on the results of this study, the textiles observed here have little to no additional attraction to insect pests after focused exposure to synthetic pheromone lures over a two-week period.

Keywords: Webbing clothes moth, Tineola, sex pheromone, textile, monitoring.

1. Introduction

The webbing clothes moth, *Tineola bisselliella*, is a cosmopolitan pest that carries economic importance due to damage caused by their larvae feeding on objects that incorporate wool, feather, hair and hide (Krüger-Carstensen and Plarre 2011). Textiles that incorporate cotton, silk, linen, paper and synthetic fibers can also be damaged by *T. bisselliella* if these items have been soiled with urine, sweat, beer, milk, soft drinks, tomato juice or other substances that contain nutritional needs for the moths (Sloderbeck 2004).

Being one of the most common pests in museums in many parts of the world, this species of moth has caused severe damage to cultural heritage objects (Querner 2014). The use of synthetically produced sex pheromone monitoring lures specifically for *T. bisselliella* for the purpose of early detection and locating sources of infestation has become commonplace in some museum institutions to prevent this damage. The use of a pheromone lure within a sticky trap increases the rate of capture twenty-fold over a sticky trap with no lure (Cox et al. 1996) and is a key factor in determining increases in population density and economic thresholds (Plarre 2013).

Concern over the practice of pheromone monitoring was raised by a prominent museum conservation scientist and author who believed that the pheromone incorporated in the dispensing lures would transfer over to museum objects (Florian 1997). Following up on this, this same author made a statement in an online museum conservation listserv that suggested that the volatile fat-soluble pheromone can be adsorbed by materials of artifacts and thus make the artifacts themselves attractive to insect pests (Florian 2011). This posting suggests that even after monitoring lures are removed, the museum collections would continue to attract and draw-in damaging museum pests. The question that this study aims to answer is if pheromone transfer between the sex pheromone lures and a variety of textiles found in museum storage environments is occurring and if these pheromones are making the textiles themselves attractive to pests.

2. Materials and Methods

2.1 Exposing the Pheromone to the Textiles

In order to answer the question of whether textiles exposed to sex pheromone monitoring lures become more attractive to the insect pests themselves, it was first necessary to establish a means of exposure for the textiles so the theory could be tested. Pheromone plumes emanating from monitoring lures are typically carried by air currents out to the surrounding areas where they attract the insects back to the lure (Murlis et al., 1992). In order to ensure exposure of the textile to the pheromone in this study, a constant air current generated by electric fans was blown across the lures towards the textile at an air speed of 40 ± 1.5 meters/min for a 2-week period in controlled temperatures between 21.1° C and 22.7° C and within a relative humidity between 40% - 50%. This exposure system was set up using eight 30.48 cm long sections of 10.16 cm diameter corrugated polyethylene field drainage tile as a conduit for the air flow (Figure 1). The fans were placed 40.6 cm away from the corrugated field drainage tiles and were directed to blow air through the open center of the tiles. The airflow was calculated using a hand-held anemometer (#DCFM8906, General Tools & Instruments, Secaucus, NJ, USA). The pheromone lures used in this study were standard, commercially available webbing clothes moth Bullet® lures (Insects Limited, Westfield, IN USA). The lures used in the study had been manufactured within the previous month of the study, were frozen to ensure freshness and were then taken fresh from the package. These lures incorporate a pheromone dose of 4.5 micrograms per lure. This dose can be considered on the high end of commercially available pheromone lures for webbing clothes moth (Van Ryckeghem, 2014). The lures were suspended on the inside of the drainage tile using a flexible metal wire positioned at the opposite end from the fan. A screen mesh was placed over the open end of the drainage tile on this same side. This screen was set in place for the purpose of creating a physical barrier between the lure and the textiles being exposed, while still allowing air to flow freely across the lure and onto the textile. No direct physical contact between the pheromone lures and the textiles was made in any of our studies. The mesh screens were standard fiberglass insect screening with a 7 X 6 mesh count per cm and the fabric was 0.3 mm thick. The close-range exposure between the lures and the textiles was performed using only the screen mesh between them at a distance of 0.3 mm. A single set a data points was retrieved at the greater distance of 152 mm between the lure and modern synthetic pile carpet to give data that represents a distance that is more commonly found in a museum setting.

The five textiles that were chosen to be exposed in this study were selected as being textiles commonly found in museum storage settings. These textiles include:

- Antique Wool Pile Carpet (mid to late 19th century)
- Modern Synthetic Pile Carpet (late 20th century)
- Modern Synthetic Plain Weave (early 21st century)
- Antique Wool Plain Weave (mid to late 19th century)
- Antique Wool Flannel (late 19th century)

Relatively larger 30 cm^2 sheets of the various textiles were cut into smaller 50 mm X 50 mm squares for use in the exposure study. The textile squares were secured to the screen mesh using metal paper clips and were placed directly on the opposite side of the screen from the lures to ensure exposure to the pheromone. The textiles were handled only while the technician was wearing latex gloves to prevent any exchange of pheromone from person to textile. After an exposure period of two weeks to allow the sex pheromone to blow directly across the lures onto the textiles, the textiles were immediately taken and placed into 10.16 cm X 15.24 cm, 4 mil Metalized PET (Polyethylene terephthalate) Zipper Pouches. The zipper pouches were then sealed and placed into a standard upright freezer (-20°C) until they were used in the insect portion of the study. The PET is considered a barrier film for oxygen (Frounchi and Dourbash 2009). Since pheromones are larger molecules than oxygen, the PET pouches can also be considered a barrier for the pheromone that will retain

any pheromone absorbed onto the textile. Freezing the samples also slows molecular movement (Debenedetti and Stillinger 2001) and thus should slow any loss of pheromone out of the textile pouches and into the environment prior to use in the study.

After pheromone exposure of the textiles was performed, the second portion of this study was to determine if the adult clothes moths prefer pheromone-exposed textiles over non-exposed textiles of the same material. This determination was made with a choice test that included 4 different options for the adult moths to choose. The four options are:

1. Sticky trap containing a 50 mm X 50 mm square of textile that has been exposed to the pheromone and placed into the center of the base of the trap.

2. Sticky trap containing a 50 mm X 50 mm square of the same textile as above that has not been exposed to the pheromone and placed into the center of the base of the trap.

3. Sticky trap containing a pheromone Bullet lure specific for webbing clothes moths placed into the center of the base of the trap as a positive control.

4. Sticky trap with no textile or attractant inside, used as a control.

The pheromone lure option and the empty trap option were added as controls to the choice test to give comparative trap capture numbers: source moths are known to be attracted to (pheromone lure) and source that should have no attraction (empty trap).

The test arena that was used in the choice test was a 2.7 m X 4.0 m space that included a desk and storage cabinets (Fig. 2). This setup gave the moths plenty of hiding spaces other than the traps, if they preferred to not go to a trap at all. Moth colony jars, active with adult *T. bisselliella*, were opened on a platform 0.74 meters above the floor and at a distance of 2.06 meters from the wall where the traps were placed. The traps that incorporated a textile square on the inside for this study were prepared by taking a textile from the freezer and placing it into the center of a sticky trap. These traps were made of milk-carton stock, wax-coated cardboard with the interior base of the trap coated with a 1 – 2 mm layer of sticky adhesive (Flat Trap adhesive trap, Insects Limited, Westfield, IN, USA). The dimensions of the traps were 20.32 cm X 10.16 cm X 3.81 cm. All four traps were set on the floor and spaced at a distance of 53.34 cm apart from each other. These locations are marked 1 through 4 in Fig. 2.

Webbing clothes moth colonies were reared in 2-Quart (1.89 liter) screw-top canisters with 5 pin holes on the upper side of the canister. The pin holes were made to allow air exchange in and out of the canister. The diet consisted of a mixture of chicken feather meal with 1% brewer's yeast by weight. The colony jars were opened and placed on their side to aid in the release the adult moths.

After one week of allowing the moths to move out of the colony jar and enter the test arena, the individual trap captures were counted and recorded and the traps were rotated to a new trap location in the arena. Also, after one week, the existing colony jar of live moths was removed and a new colony jar with freshly emerged adult moths was opened in its place. A total of 4 repetitions, totaling 4 weeks of release for each textile, were performed. Each trap in the study would spend one week's time at each location of the four without duplication of location during that 4-week period. A total of six individual 4-week trials were run in this study. Five of those studies involved each of the different textiles exposed at the short 0.3 mm distance to the lure. The sixth trial was a single trial of the Modern Synthetic Carpet exposed to pheromone from the greater distance of 152 mm.

3. Results

Totals of captured moths after 4 weeks varied from 107 to 323 based on the number of adult moths in the colony jars at the time of release and the attraction of the traps. Throughout the six trials, a total of 913 *T. bisselliella* were captured in the different traps. It is estimated that a total of > 2000

moths were released through these studies. The results of the capture numbers for each individual textile, as well as the controls and pheromone lures can be seen in Fig. 3 – 7.

A statistical analysis was prepared using the Kruskal-Wallis *H* test (Microsoft Excel 2013). The Kruskal-Wallis test is a rank-based non-parametric method for one-way analysis of variance test that compares the samples even though they may have different sample sizes. The results of the Kruskal-Wallis test can be found in Table 1.

Fig. 1 Image of pheromone exposure to textiles with anemometer
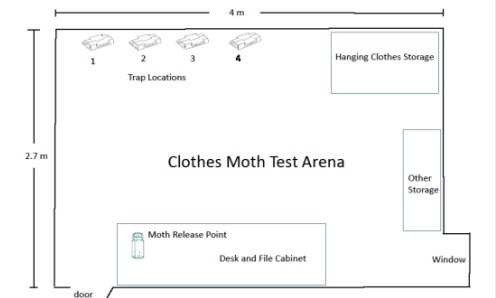
Fig. 2 Diagram of clothes moth test arena
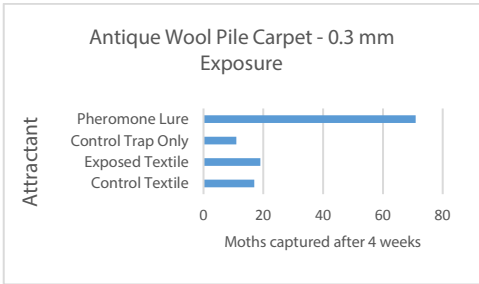
Fig. 3 - Choice test trap capture results for antique wool pile carpet exposed to pheromone at a distance of 0.3 mm.
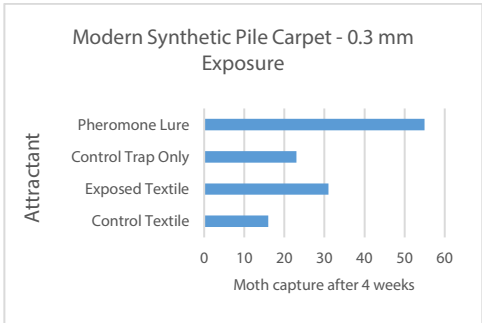
Fig. 4 Choice test trap capture results for modern synthetic pile carpet exposed to pheromone at a distance of 0.3 mm.
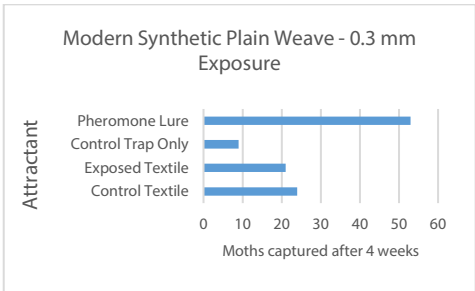
Fig. 5 Choice test trap capture results for modern synthetic plain weave exposed to pheromone at a distance of 0.3 mm.
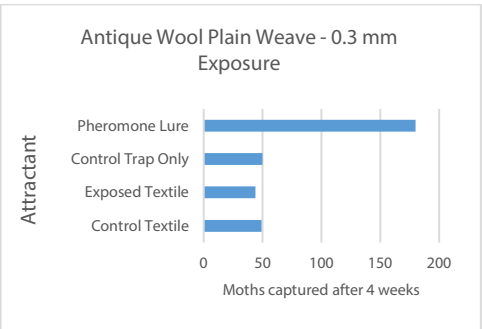
Fig. 6 Choice test trap capture results for antique wool plain weave exposed to pheromone at a distance of 0.3 mm.
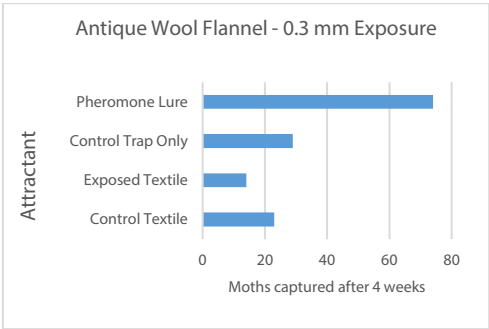
Fig. 7 Choice test trap capture results for antique wool flannel exposed to pheromone at a distance of 0.3 mm.

Tab. 1 Results of non-parametric Kruskal-Wallis tests comparing trap capture rates of combined textile types.

Pheromone Lure and Control Trap		Exposed Textile and Control Textile	
adjusted H	6.8182	adjusted H	0.0109
d.f.	1	d.f.	1
P value	0.0090	P value	0.9168
Control Textile and Control Trap		Exposed Textile and Control Trap	
adjusted H	0.0439	adjusted H	0.0982
d.f.	1	d.f.	1
P value	0.8340	P value	0.7540

4. Discussion

The procedures used to expose the textiles to the pheromones in this study represent what could be considered a worst-case-scenario for pheromone exposure in a real-world setting.

A lure with a relatively high dose of pheromone was used in this study. This is not always the case in a museum storage environment as pheromones with low dosages are commonly used. Also, pheromone traps are typically not placed in direct contact with museum objects as this could be detrimental to the object if it were to get stuck in the glue. Pheromone traps for pest insects and particularly traps for *T. bisselliella* are usually placed in open areas along the wall or on the floor so they can be inspected easily (Trematerra and Fontana, 1996). Even a pheromone trap that is in direct contact with a museum object is going to have the pheromone lure at a minimum of 1 cm away from the object due to the distance from the paper outside of the trap to the adhesive pad within where the lure rests. It may even be as high as 15 mm away depending on where the lure sits in the trap. These distances are considerably higher than the 0.3 mm that we used in this study.

Air currents are the mechanisms that translocate quantities of the sex pheromone from the lure into the air or onto a textile. In this study, a constant air flow was blown across the lure at a rate of approximately 40 meters/min ± 1.5 meters/min for a full 2-week period. This type of constant air flow across a lure and onto a textile is usually not seen in a museum storage setting unless the textile is placed directly between a return air vent and the pheromone lure or if the pheromone lure is placed directly in front of an air supply vent and the textile is positioned directly in the air path of that vent.

Although this study does represent a worst-case scenario for textile exposure to pheromone, this type of exposure theoretically could occur in a museum setting. Because of this potential, the questions of concern for this type of exposure need to be considered valid. Correlations between this study and other similar studies could not be done since other studies regarding the pheromone transfer from monitoring lures to textiles were not found in the available research.

There was a wide range of materials incorporated into the textiles that were studied here. Antique natural fibers were used in three of the five samples; wool pile carpet, flannel and antique wool plain weave. Also incorporated in some of these samples were synthetic materials that contained no

natural fibers at all. These were the new pile carpeting and modern synthetic plain weave. *Tineola bisselliella* larvae feed on a wide variety of dried material of animal origin (Griswold, 1944). This fact should theoretically make the woolen textiles more attractive than the synthetic textiles, at least to the female moths looking to lay eggs. When we look at the results from this study however, we find that only the antique wool carpet and the modern synthetic flat weave had apparently higher moth attraction than the control trap. No clear affinity for natural fibers over synthetic fibers could be found. It is possible that many of the females in the study were left unmated due to a large capture of the male moths in the traps. If this were the case, the unmated females were not looking for potential food sources to lay their eggs, so we did not see an affinity for the natural fibers. The addition of human sweat, urine or food stains to natural fibers can make these materials more attractive to *T. bisselliella* (Klass, 2010). A possible explanation for the low attraction is that the samples we used containing natural fibers did not contain any of these additional attractants.

5. Conclusions

The textiles in this study, whether exposed to pheromone or not, did not have greater captures than control traps (Table 1).

Given these results, it is unnecessary for museum staff to be overly concerned that they are making their textile collection objects more attractive to *T. bisselliella* if they are using pheromone monitoring traps within their collections storage. This study suggests that *T. bisselliella* monitoring traps are an effective, non-detrimental tool. The informational value gathered through use of the pheromone traps used to mitigate damage to collection textiles and objects, far outweighs any negative possibilities that the textiles themselves will attract pests into storage areas.

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Khapra beetle diagnostics

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Abstract

The khapra beetle, *Trogoderma granarium* Everts, is a serious pest of grains and stored dry food stuffs and is the subject of strict quarantine measures in many countries including Australia. Morphologically the khapra beetle can only be reliably identified by dissection by a limited number of skilled taxonomists. Suspect specimens found in grain products are usually the larvae or larval skins which are difficult to diagnose morphologically. Adult specimens are usually scarce and damaged. Due to their similarity, warehouse beetle (*Trogoderma variabile*) and other native *Trogoderma* spp. could be mistakenly identified as *T. granarium* with market access implicatons or could mask an incursion. Molecular diagnostic protocols have been developed for khapra beetle, but remain largely untested against other species of *Trogoderma*, some also capable of being pests. Western Australia has a broad large, poorly studied native *Trogoderma* fauna, many of which are still undescribed; their estimated number is possibly over 100 species. Occasionally native Australian species can occur in stored commodities. Their identification and at least separation from the pestiferous exotic *Trogoderma* presents a serious problem. The work in this paper has been undertaken in an attempt to distinguish *T. granarium* from Australian native *Trogoderma* and related Dermestid species by both morphological and molecular methods. Dermestid specimens were sourced mainly from a targeted survey around grain silos throughout Australia, using two trap types, inside and outside facilities. Khapra beetle specimens were sourced from different geographical locations around the world.

Keywords: *T. granarium*, PCR, native Australian *Trogoderma*, targeted survey, taxonomy.

Introduction

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is recognised as one of the world's most destructive pests of grain products and is the subject of strict quarantine measures in many countries. Khapra beetle is listed in the 100 "World's Worst Invasive Alien Species" by the Global Invasive Species Programme (Lowe et al 2000). Plant Health Australia has identified khapra beetle as one of the top 5 biosecurity threats to the Australian Grains Industry. By definition, khapra beetle does not occur in Australia, but there are occasional records of intercepts (Emery et al 2008; Day and White 2016). An incursion could lead to costly control and eradication efforts. Non-khapra beetle countries enforce quarantine restrictions on imported commodities from khapra beetle countries.

There are over 120 described *Trogoderma* spp. worldwide of which four are recognised as stored product pests, including *T. granarium*, *T. glabrum*, *T. inclusum* and *T. variabile* (Banks, 1994). In Australia there are over 50 described native *Trogoderma* species, and many more remain undescribed. None of these are pests but could accidentally get into grain stores and be misidentified. Due to their similarity, *T. variabile*, already in Australia, or native *Trogoderma* spp. could be mistakenly identified as *T. granarium* or could mask an early incursion of *T. granarium*.

Suspected *Trogoderma* specimens found in grain products are usually the larvae or larval skins which are difficult to diagnose morphologically (Banks 1994; Emery et al 1997). Adult specimens are usually scarce and damaged and need expert dissection for identification (EPPO, 2002; IPPC, 2012). Diagnostically the khapra beetle can only be reliably identified by a limited number of skilled taxonomists. Misidentification of *Trogoderma* and related Dermestids has the potential to seriously compromise Australian grain exports (Szito 1997).

The aim of this work was to develop a molecular diagnostic tool that could quickly discriminate between khapra beetle and native Australian *Trogoderma* fauna based on whole specimens or insect fragments.

The approach included a review and modification of a published diagnostic DNA method for khapra beetle (Olson *et al* 2014) as well as review and optimization of in-house protocols.

Thousands of native *Trogoderma* and related Dermestid specimens were used to verify the diagnostic components of this work. Native Australian *Trogoderma* specimens and related Dermestids were sourced from a national *Trogoderma* trapping program conducted throughout Australia between 2009-2011 at targeted sites around grain silos and ports. Khapra beetle material was sourced from overseas collections, of different geographical origin. The molecular approach included conventional PCR, real-time PCR and DNA sequencing methods. DNA was extracted from morphologically verified khapra beetle populations, field collected native Australian *Trogoderma*, warehouse beetle and other related pest Dermestids. Taxonomically verified target specimens were used to data mine for unique DNA sequence profiles.

1. Materials and Methods

Morphology – taxonomic verification of target species

Dermestid material from a national *Trogoderma* trapping program (2009 -2011) was a major resource for the project in terms of diversity of native *Trogoderma*, number of geographic sites and number of specimens (~17,000) in providing a broad range of *Trogoderma* species and *Trogoderma*-like species for DNA-validation work and generation of unique sequence profiles. A targeted trapping approach was used based on previous studies by Wright (1993) and Rees *et al* (2003) and data collected using hand held devices (PDAs) synchronised to desktop server database (Emery et al 2010). The survey involved setting two trap types at >70 seleted sites – both inside and outside grain silos around Australia (Botha et al 2012). The insect traps used in this study were commercially available products – Trece Storgard khapra beetle trap (Barak 2004), and a modified Lepidopteran wet trap (UniTrap) using *Trogoderma*-specific lures (Barak 1989). The survey was conducted between 2009 and 2011, with trap catch material collected on a monthly bassis and identified at the Department of Primary Industries and Regional Development (DPIRD), Western Australia.

Additional Dermestid material was provided by University of Western Australia collaborators (collected from Gnangara area of Western Australia). Ad-hoc specimens, specimens from smaller trapping projects, colony material and curated specimens were also used to build a diverse Dermestid collection for the project.

Khapra beetle specimens from different geographical locations were sourced through international contacts in Spain (colony, established 1956; origin: unknown), Canada (origin: Pakistan), Greece (origin: unknown, possibly Turkey), Germany (origin: Iran) and UK (Centre for Agriculture and Biosciences International (CABI); origin: unkown).

Morphological methodology included specialist insect handling, identification with chain-of-custody labelling for trace-back to collection site, date of collection, trap type etc. Thousands of specimens were pinned, labelled and data-based. Western Australian Department of Primary Industries and Regional Deveopment (DPIRD) taxonomists verified the specimens for the molecular development and verification in this project. Diagnostic image capture (photomontage) of the unique native *Trogoderma* identified was outside the scope of the project, nonetheless, some unique specimens were photomontaged and cross-referenced with specimen ID and DNA sequence codes.

Molecular diagnostics

The methodology included assessment of molecular (real-time PCR) khapra beetle protocols developed in previous Plant Biosecurity Cooperative Research Centre (PBCRC) projects (PBCRC20137,PBCRC60046), as well as testing a published DNA protocol (Olson et al 2014) on an extensive cohort of Australian native *Trogoderma* and khapra specimens from different geographical origin. Optimisation and development of new PCR primers for khapra beetle and

warehouse beetle (*T. variabile*) was also undertaken as part of this study. For DNA extractions and molecular procedures in the DPIRD Diagnostic Laboratory Service (DDLS), insect legs were removed from pinned, labelled adult specimens, or provided as larvae in etOH from multiple, labelled specimens, cross-referenced with DDLS codes for chain-of-custody.

The molecular protocols were tested for accuracy, specificity and reproducibility as outlined by the Australian Subcommittee on Plant Health Diagnostic Standards (SPHDS) instructions for National Diagnostics Protocols. The proposed research was designed to address the "International importance of accredited diagnostic laboratories using accepted diagnostic procedures" as written in the International Standards for Phytosanitary Measures (ISPM 27).

A 'blind-test' challenge using 30 insect specimens, including khapra beetle, warehouse beetle and a selection of related Dermestids (and non-Dermestids) was used to test the rigour of the protocol in a 'real-world scenario'.

Destructive and non-destructive methods for DNA extraction from larvae, adults and skin casts were tested. Below is a summary of molecular methods:

> Modified Olson qPCR (Olson et al. 2014) for the detection of *T. granarium* specific mitochondrial 16S ribosomal RNA (16S rRNA) gene.
> Conventional Folmer and Simon PCRs (Folmer et al. 1994, Simon et al. 1994) for the universal amplification and sequencing of the mitochondrial COI gene.
> Conventional – 16SAr PCR (Simon 1994, Cognato & Volger 2001, Olson et al. 2014) for the amplification and sequencing of arthropod mitochondrial 16S rRNA gene
> Universal Arthropod - 16SAr qPCR (Simon 1994, Cognato & Volger 2001, Olson et al. 2014) for the confirmation of successful DNA extraction from arthropod specimens.

Extraction options included:
A. Whole insects – remove 1–2 legs and transfer to a microcentrifuge tube containing 180 µL ATL buffer and 20 µL Proteinase K. Grind the sample using a sterile micropestle.
B. Larvae – a 'core biopsy' taken from the larvae using a fine gauged syringe and transferred to a microcentrifuge tube containing 180 µL ATL buffer and 20 µL Proteinase K.
C. Destructive – if the specimens are not required for further taxonomic work the entire larvae, adult or skin cast (or part thereof) may be homogenised in a microcentrifuge tube containing 180 µL ATL buffer and 20 µL Proteinase K using a sterile micropestle.
D. Non-destructive – place the entire larvae, adult or skin cast in a microcentrifuge tube containing 180 µL ATL buffer and 20 µL Proteinase K (larvae may be 'punctured' with a fine gauge syringe to aid extraction) and incubate at 56°C with gentle agitation for at least 1 hr (can be left overnight).

2. Results

Morphology – taxonomic verification of target species

The trapping program generated more than 17,000 Dermestid specimens, including at least 20 native *Trogoderma* species, which are yet to be formally described. In the project time-frame, 11 different native *Trogoderma* species have been identified, along with thousands of related Dermestid genera. Table 1 provides a summary of the Dermestid species collected and numbers that have been curated. Table 2 provides a summary of the non-dermestid species in the bi-catch trapped.

Tab. 1 Dermestid taxa recorded at grain storages in an Australian Dermestid trapping survey.

Dermestidae	Total numbers
Anthrenocerus	69
Anthrenus	24
Anthrenus verbasci	15
Attagenus	18

Dermestes	9
Dermestes maculatus	2
Orphinus	775
Phradonoma nobile	11
Thaumoglossa	81
Trogoderma (native)	3,793
Trogoderma variabile	12,111
Trogoderma granarium	0

Tab. 2 Non-dermestid Coleopteran taxa recorded at grain silos in an Australian Dermestid trapping survey.

Non Dermestidae
Anobiidae
Bostrichidae
Buprestidae
Carabidae
Chrysomelidae
Coccinellidae
Other Coleoptera
Cucujoidea
Curculionidae
Elateridae
Haliplidae
Hydraeinidae
Hydrophilidae
Laemophloeidae
Melyridae
Mycetophagidae
Nititulidae
Ptinidae
Tenebrionidae
Scarabeaidae
Silvanidae
Staphylindae

Molecular

The qPCR 'road-test'

A total of 1,618 *Trogoderma* and related Dermestid specimens underwent qPCR screening. The majority of the specimens consisted of 2-3 dissected insect legs, with the remaining insect pinned and labelled for reference. The khapra-specific 16S qPCR assay proved successful with a sensitivity of 100% and specificity of 97.20% when tested against the 1,618 specimens, including 61 known khapra isolates and 1,557 endemic beetles (Table 3). The performance of the 16S qPCR assay compared to the gold standard taxonomic identification is presented in Table 4. The performance of the modified Olson qPCR was within the recommended parameters of a validated diagnostic test.

Tab. 3 Total number of specimens tested by the Olson qPCR and the diagnostic sensitivity and specificity of the assay.

Total number of specimens	1,618
Total number of Khapra	61
Sensitivity	100%
Specificity	97.20%

Tab. 4 Confusion matrix detailing the performance of the Olson qPCR assay compared to the gold standard taxonomic identification. TP = true positive, TN = true negative, FP = false positive and FN = false negative.

	Taxonomic ID	
	Khapra	Non-khapra

| PCR ID | Khapra | 61 (TP) | 43 (FP) |
| | Non-khapra | 0 (FN) | 1514 (TN) |

Confirmatory sequencing

Sequencing of the DNA barcoding COI gene (mitochondrial gene cytochrome oxidase I) revealed >99% sequence homology with *T. granarium* specimens in GenBank. This result means that the qPCR test will rapidly identify positive khapra specimens, which can then be sent off for confirmatory sequencing at a third party laboratory, which is standard practice for NATA accredited Diagnostic Protocols, and current practice in the event of a 'real' incursion.

Molecular blind testing

The khapra beetle qPCR test correctly identified and discriminated khapra beetle specimens in the blind sample set (30 specimens), with no false positives or false negatives, with a results turn-around time of 2 days (non-urgent) (Table 5).

Follow-up sequencing to confirm the preliminary PCR diagnosis was undertaken by a third party facility (AGRF QEII Medical Centre) to simulate the diagnostic process that would occur in the event of a real incursion.

Sequencing results confirmed the positive khapra PCR test results, returning *Trogoderma granarium* partial 16S rRNA gene for all 4 khapra specimens. The four khapra samples were haplotyped as HT1 (Spanish 1956 colony); HT1 (Iran - German colony); HT2 (Pakistan – via Canada); HT2 (Pakistan via Canada).

A neighbour-joining tree for partial mitochondrial 16S rRNA gene sequences based on Olson-defined *Trogoderma* haplotypes was constructed (Fig. 1).

Tab. 5 Multiplex real-time PCR for the detection of *Trogoderma granarium* and *Trogoderma variabile* (in-house assay*)*

Species No.	Species ID	Species Description	*T. granarium*	*T. variabile*
0001	A1	*Trogoderma variabile*	-	+
0002	A2	Coccinellidae (native)	-	-
0003	A3	*Anthrenus* sp.	-	-
0004	A4	*Sitophilus oryzae*	-	-
0005	A5	*Trogoderma variabile*	-	+
0006	A6	*Anthrenus* sp.	-	-
0007	A7	*Anthrenus verbasci*	-	-
0008	A8	*Tribolium castaneum*	-	-
0009	A9	*Trogoderma* sp. (native)	-	-
0010	A10	*Trogoderma variabile*	-	+
0011	A11	*Anthrenus verbasci*	-	-
0012	A12	*Trogoderma granarium*	+	-
0013	A13	*Rhyzopertha dominica*	-	-
0014	A14	*Trogoderma variabile*	-	-
0015	A15	*Trogoderma variabile*	-	+
0016	A16	*Anthrenus* sp.	-	-
0017	A17	*Oryzaephilus surinamensis*	-	-
0018	A18	*Trogoderma granarium*	+	-
0019	A19	*Trogoderma* sp. (native)	-	-
0020	A20	*Trogoderma variabile*	-	+
0021	A21	*Anthrenus* sp.	-	-
0022	A22	*Trogoderma granarium*	+	-
0023	A23	*Cryptolestes pusillus*	-	-
0024	A24	*Trogoderma* sp. (native)	-	-
0025	A25	*Thaumoglossa* sp.	-	-
0026	A26	*Trogoderma granarium*	+	-
0027	A27	*Anthrenus verbasci*	-	-
0028	A28	*Anthrenus verbasci*	-	-
0029	A29	Coccinellidae (native*)*	-	-
0030	A30	*Trogoderma variabile*	-	+
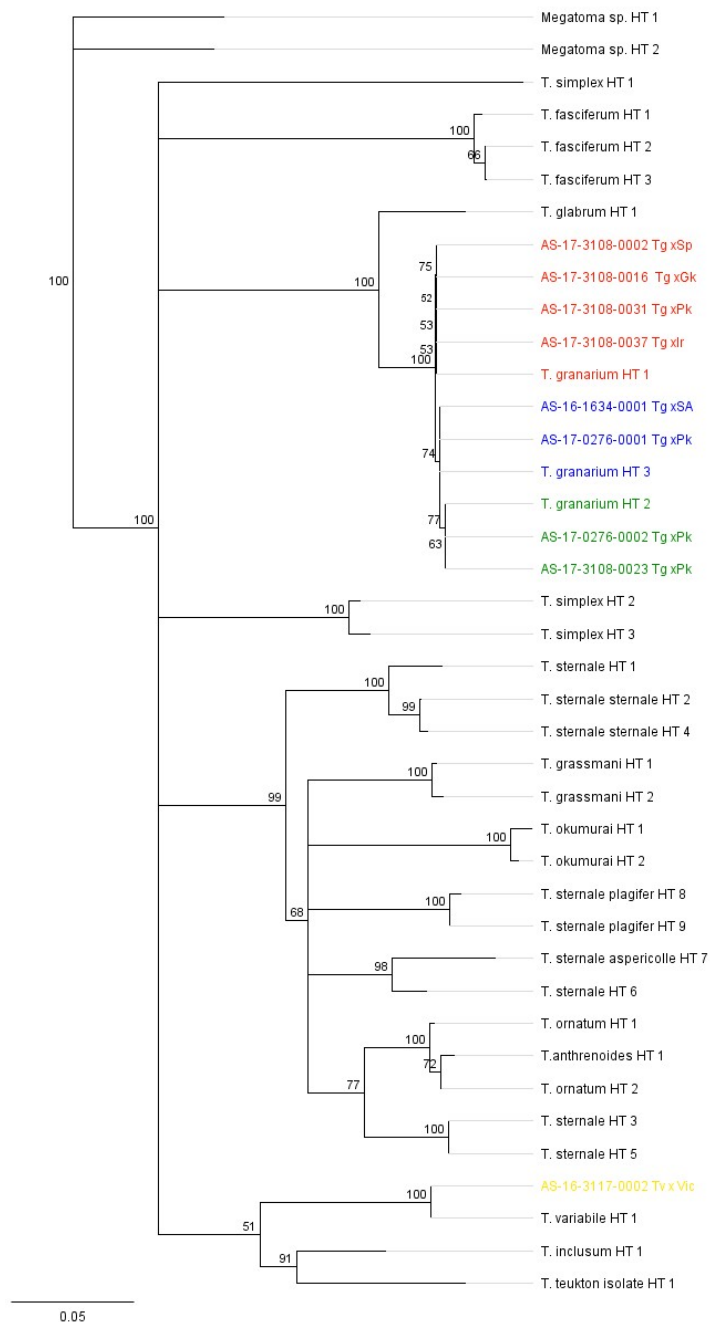
Fig. 1 Neighbour-joining tree for partial mitochondrial 16S rRNA gene sequences based on Olson-defined *Trogoderma* haplotypes (accessed via Genbank, NCBI). DPIRD sequencing results are denoted by laboratory accession numbers in colour (*T. granarium* HT 1 = red, HT 2 = green & HT 3 = blue and *T. variabile* in italics). Genetic distance was computed using the Tamura-Nei method. The tree is rooted to the outgroup species Megatoma sp. HT 1. The number on each node represents bootstrap probability based on 1000 replications.

Discussion

The project work has led to the development of a high-throughput qPCR diagnostic protocol for the species-specific detection of *Trogoderma granarium*. A second qPCR test for *Trogoderma variabile* was also developed. Additional 'universal' conventional (end-point) PCR assays form part of the diagnostic protocol which allow for the confirmation of identification via Sanger sequencing (if required). The protocol includes an optional qPCR method to quality control the DNA extraction process. This protocol is in routine use in our diagnostic DDLS facility, as a high-throughput Dermestid screening test.

As a result of this work, DPIRD has the capacity to undertake high-throughput PCR screening for the exotic khapra beetle which is absent from Australia. The PCR test offers a sensitive and specific quick 'first pass' screen for suspect khapra specimens adding to preparedness and planning options in the event of a pest incursion into Australia.

One of the advantages of the qPCR test is the ability to test insect fragments, damaged specimens and larvae that are almost impossible to identify morphologically. The project work has produced a Dermestid reference collection of more than 17,000 Dermestid specimens. This reference collection includes many previously unknown Australian native *Trogoderma* species and forms a unique and valuable legacy resource. Suspect Dermestid specimens can be tested in-house and their genetic sequences compared with in-house reference material, and against genetic reference profiles in publicly available databases (e.g. BOLD and GenBank). The diagnostic protocol developed for khapra beetle will be submitted shortly to the Subcommittee on Plant Health Diagnostics (SPHDS) in Australia for review as an accredited National Diagnostic Protocol for use throughout Australia (and Internationally). The Australian National Plant Biosecurity Diagnostic Network (NPBDN) publishes diagnostic protocols for priority pests online at:

http://plantbiosecuritydiagnostics.net.au/resource-hub/priority-pest-diagnostic-resources/.

It is anticipated that once approved by SPHDS, the khapra beetle protocol developed in this study will be published on the NPBDN website. The DNA sequences of Australian native *Trogoderma* and related Dermestids will be submitted to an internationally recognised genetic resource website at the conclusion of this study.

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Assessing drivers of maize storage losses in south west Benin using a Fractional Response Model

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Abstract

An assessment of drivers of maize storage losses was undertaken in south west Benin applying the Fractional Response Model on information collected from 400 smallholder maize farmers. Overall, respondents lose on average 10.3% of their harvest during the storage period. The average marginal effect obtained from the fractional response model of storage losses revealed that storage technologies, farmers' post-harvest attitudes, insects damage, the weather conditions and infrastructures played a significant role in the level of storage losses surveyed farmers have experienced. Farmers using bags and plastic containers have respectively reduced their storage losses by 6.7 and 7.8% compared to farmers using cribs. Considering the use of storage protectant, the results indicated that using ash, neem leaves, pepper or lemon lead to an increase of 4.1% of losses relative to

storing without any protectant. Drying after harvesting decreased by 1.9% the share of the quantity stored lost during storage. The percentage of maize lost increased by 5.1% for respondents who have reported insects as predators of their stored maize. Rain at harvest time increased the percentage of losses by 2.1%. A one-degree increase in temperature increased the percentage of maize loss by 4.4% and farmers who live at less than 26.5 km to the market have reduced by 0.17% of maize losses. Effective policies for a sustainable reduction of storage losses among maize farmers in the area should consider the need to discourage the use of cribs, ash, leaves, pepper and lemon as storage technologies. Farmers should avoid harvesting during times of rain, and should properly dry their produce after harvesting. Sustainable hermetic equipment should be promoted and farmers' access to markets facilitated.

Keywords: Maize; Storage equipment; Storage protectant; Storage losses; Fractional Response Model

1. Introduction

Each year, significant volumes of food are lost after harvest in Sub-Saharan Africa (SSA), the value of which is estimated at USD 4 billion for grains alone (World Bank, 2011). World Bank (2011) emphasizes that the high level of grain lost in developing countries after harvest, in addition to aggravating hunger, also leads to a waste of expensive inputs such as irrigation water, fertilizer and human labour. Storage is a critical stage in the food supply chain. In developing countries with hot climates, most smallholder farmers rely on sun drying to ensure that crops are well dried before storage. If unfavourable weather conditions prevent crops from drying sufficiently, such crops are subject to high losses during storage (Hodges *et al.*, 2014). The need to deal with post-harvest losses and to undertake innovative and impact oriented PHL research is critical for achieving food security and reducing poverty in the sub region (Affognon *et al.*, 2015).

A major obstacle in the efforts to mitigate storage losses in developing countries is the lack of accurate knowledge on the magnitude of losses as well as the linkage between drivers of such losses. Outdated contextual estimates of these losses could lead to the implementation of bad policies (Affognon *et al.*, 2015).

This paper offers a good understanding of the scope and nature of the problem of storage losses among maize farmers in south western Benin where maize is considered as an important food crop; mainly produced under rain fed agriculture by smallholder farmers and subject to important storage losses. The study is the first in Benin to assess drivers of storage losses in a multivariate setting. Planners and policy makers can rely on the results of the study to as early as possible in their decision cycle design appropriate and effective measures for storage loss reduction.

2. Materials and Methods

Data were randomly collected from over 400 farmers from September to October, 2016. Secondary information on temperature and rainfall pattern during 2015 were obtained from the local climate agency, known as ASECNA Benin/ Lokossa Station.

The dependent variable of interest in this study is the percentage of maize storage losses in south west Benin. The Fractional Response Model (FRM) has been defined for the first time by Papke and Wooldridge (1996) to deal with situations where the dependent variable is a proportion and its values are allowed to be zero or one. Authors have shown that the use of the Ordinary Least Squares (OLS), the censored regression (Tobit), or the transformed logistic normal model (the log-odds ratio of the dependent variable) in such cases are inefficient, as their error distributions will be heteroskedastic (Papke and Wooldridge, 1996; Kieschnick and McCullough, 2003). The Fractional Response Model is a non-linear model estimated using the Quasi-Maximum Likelihood Estimation (QMLE) method. The QMLE is asymptotically efficient and consistent compared to either OLS or Tobit. In the FRM model, a functional form for the dependent variable is chosen such that it imposes constraints on the response variable to ensure that predicted values will always lie within the closed interval [0,1].

The empirical FRM specification of storage losses retained in this study is:

$$E(Y_i/X_i) = G(X_i) = \mathbf{b_0} + \sum_{k=1}^{24} b_k X_{ik} + \varepsilon_i \quad E(Y_i/X_i) = G(X_i\beta) = b_0 + \sum_{k=1}^{n} b_k X_{ik} + \varepsilon_i$$

$$\tag{2.1}$$

Where $0 \leq Y \leq 1$ correspond to the percentage of storage losses; X_i represent the explanatory variables for each observation i and $\varepsilon^{\varepsilon}$ represents the error term. $G(.)$ is a distribution function similar to the logistic function.

Following Papke and Wooldridge (1996) and Wooldridge (2011), the generalised linear modelling (glm) was retained to fit the fractional response model for the percentage of storage losses in south west Benin.

3. Results

The volume of reported storage losses by maize farmers from the south western of Benin is on average 10.3% of the quantity harvested.

Storage equipment

The marginal effect computed from the fitted model showed that farmers using bags and plastic containers respectively have reduced their storage losses by 6.7 and 7.8% compared to farmers using cribs. There is however no difference between the predicted storage losses of users of rooms and cribs.

Storage protectant

Considering the use of storage protectants, the results indicated that using ash, neem leave, pepper or lemon leads to an increase of 4.1% of losses relative to storing without any protectant.

Drying

The results revealed that drying after harvesting decreased by 1.9% the share of the quantity lost during the storage period. Drying the harvest for a second time at home lowered the moisture content of maize and this significantly contributes to a loss reduction.

Insect attacks

The amount of maize lost during storage has increased by 5.1% for respondents who have reported insects as predators of their produce kept in stores.

Rains at harvest

The effect of rain at harvest time was significant and increased the percentage of losses by 2.1%. This result was expected, since rain at harvest time raises the issue of moisture content in harvested crops. The higher the wetness/moisture/dampness of the grain before storage, the higher is the likelihood of losing maize while being kept in stores.

Temperature

The temperature within the first three months of storage had a significant effect on the percentage of maize loss during the storage period. A one-degree increase in temperature increased the percentage of maize loss by 4.4%. The significant effect of temperature on losses is in line with the literature, where the climate conditions have been suggested as a factor in storage losses by Costa (2014). However, the study revealed a turning point of 26.8 over which the temperature contributes to losses reduction.

Market conditions

Market conditions have been tested through price and the distance to market. Prices do not significantly affect the percentage losses. However, the distance to market revealed a non-linear effect on the percentage losses. A one kilometre increase in the distance to market reduced by 0.2% of maize loss and this is true only when the distance to market is less than 26.5 km, the computed extremum. Beyond that, it contributes to storage losses. This result shows that distance to market remains an important issue when it comes to commercializing agricultural products.

4. Discussion

Cribs that are widely used are subject to storage losses. It suggests that awareness should be raised about the storage losses issue, as this is strongly related to the use of cribs in the region. The results show some limit within farmers' attitudes when it comes to preserving their maize product using storage protectant. The study revealed the irrelevance of using ash, neem leaves, pepper and lemon to store maize in south west Benin. The inefficiencies may be explained - without a proper investigation on the issue - by the fact that ash, pepper, lemon and neem leaves are commonly poured on the maize (especially in layers for neem leaves) with husk kept in stores. The fact that insects are damaging the grain itself and are even living inside the maize, the presence of husk between the used protectant and the stored product could prevent the effectiveness of the given protectant.

In the region, maize drying is commonly done in the field before harvest. However, some farmers reported drying their produce a second time before storage. This has contributed to storage losses reduction. Accordingly, dryer technologies with low fixed and operationalisation cost could be implemented in the region. This may help farmers reducing their losses by firstly harvesting after maturity of the crops and then drying adequately. Solar maize dryers could therefore be a better alternative.

Insect attacks remain a challenge for maize farmers. Insect infestation starts from the field when crops are not well treated and / or during the storage period. The effect of insects in damaging or destroying the edible part of the grain put in storage is well documented in the post-harvest literature (Hodges *et al.,* 2014), and that issue is not new. Unfortunately, insects continue to be a threat to maize farmers whose products are kept in stores. Recently, modern hermetic storage equipment have been suggested as a sustainable way to overcome the insect problem (Costa, 2014). Finally, farmers have to avoid harvesting during times of rain and their access to markets should be facilitated to effectively reduce losses that are likely to occur during storage.

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Insects and fungi in stored maize in Angola

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Abstract

In underdeveloped countries in Asia and Africa, non-effective post-harvest technologies and sometimes ideal environmental conditions for development of pests like insects, fungi, rodents and birds, can lead to damage of both raw or processed foods. Losses can achieve considerable proportions in dried vegetables used as food products, particularly in underdeveloped countries where food security problems are a daily basis routine. The major goal of the present study was the identification of insects and fungi associated with maize under local storage conditions in the Angola provinces of de Benguela, Bié, Cuando Cubango, Cuanza sul, Huambo, Huíla, Luanda, Malange and Namibe. A wide range of storage methods for cereals were sampled, from small containers of peasants and small farmers up to the large metal containers used by large agricultural companies and Estates. The achieved results will contribute for food security improvement in Angola and for the maintenance and preservation of good and healthy seeds at the traditional farmers' community level. The insect pests registered from the studied samples were *Cryptolestes ferrugineus, Gnatocerus maxillosus, Liposcelis bostrychophila, Oryzaephilus surinamensis, Rhyzopertha dominica, Sitophilus zeamais, Sitotroga cerealella* and *Tribolium castaneum*. The species *Prostephanus truncatus* was not found in the studied samples. Fungi in the genus *Aspergillus, Fusarium* and *Penicillium* were presented at a high incidence in all samples studied, although the relative abundance of different fungi species varied with the sample location.

Keywords: maize, insects, fungi, Angola, storage.

Introduction

In Angola, maize is the cereal with the highest production and one of the most consumed. An average maize yield of 640 kg/ha was reported for the period 2000-2010 (FAOSTAT, 2012). Although grain production in the country has increased, Angola still has a deficit of 3 million tons, achieving only 40% of consumption needs (INCER, 2014). Factors such as severe technical knowledge gaps, lack of incentives to producers, low fertility of soils, use of low-yielding varieties, non-application of technologies or lack of access to them, lack of access to production factors, lack of infrastructure for water management, lack of reliable storage structures, and low availability of credit resources greatly reduce the expected yields (Pacheco et al., 2011). There are a number of warehouse systems and warehouse types in Angola at the smallholder level. These warehouses are built with clay, sticks and covered with grasses or wood and grass. The poor condition of the warehouse structure, its hygiene and moisture control issues at the level of the small producer does not guarantee good phytosanitary status for the stored products.

Cereals storage is a specific agro-ecosystem, conditioned by several factors which are difficult to control, like temperature, relative humidity, water content, and oxygen availability (Barros, 1993). This is especially true in underdeveloped countries where technological innovations such as refrigeration and controlled atmospheres represent huge investments. Storage under deficient conditions can lead to insect or fungi attack, inducing organoleptic changes (taste, flavour and appearance), nutritional losses or even mycotoxin contamination. These cause significant economic losses and can represent serious health problems.

The insects of stored grains have certain preferences regarding temperature, relative humidity, water content and food. The interaction of these factors affect, directly or indirectly, the insects' proliferation rate and thus the possibility of causing damage and/or loss during storage of those products (Barros, 1996).

The objective of this work was to identify species of insect and fungi responsible for the deterioration of stored cereals present in samples of maize from Angola, and as a result contribute in some way to the improvement of the storage conditions of these products. Aim was not only to confirm the storage pests already identified in Angola in previous studies, but also to check for the presence of *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae). This species is a pest of stored maize and cassava, which became a major concern after being accidentally introduced in Africa.

This is pioneering and very important work for Angola. It covers approximately 50 % of the national territory evaluating the phytosanitary situation of stored cereals in the Benguela, Bié, Cuando Cubango, Cuanza Sul, Huambo, Huíla, Luanda, Malange and Namibe provinces, identifying all the insects and fungi present in the studied cereal samples.

Materials and Methods

Maize samples were collected randomly in Angola in different quantities and packed in plastic or paper bags, and transported to the laboratory in Lisbon. The samples were kept cool (0-4°C) after collection, during shipping and at the laboratry until being observed. These procedures took 7-8 days.

Samples originated from different sources, from small producers in the provinces of Benguela, Bié, Cuando Cubango, Huambo and Huíla, from the business sector, from the Pungo Andongo farm in the province of Malange and from the local markets of the provinces of Cuanza Sul, Luanda and Namibe. At the arrival of the samples in the laboratory they were cleaned and sieved for removal of stones, dust, crop pieces, excrement and insects. Then, using the Boerner divider the samples were subdivided for later entomological and mycological analyzes.

Insects analysis

The insects present in the maize samples, on arrival, were identified and recorded. The maize samples were then placed in glass bottles, identified with origin, arrival date and incubated at 27±1°C and 75-80 % relative humidity. The purpose of this procedure was to observe and identify the emergence of hidden adult insects inside the maize kernels.

Mycoflora analysis

Maize samples from six provinces of Angola were collected in sterilized containers and taken into the ISA laboratory. The maize samples were sub-divided into 110 kernel samples. These sub-samples were surface disinfected with 1 % sodium hypochlorite for two minutes, as describe by Pitt and Hocking (1997) and Magro et al. (2006).

Ten dried grains were placed in Petri dishes with 20 mL of Potato Dextrose Agar (PDA) medium with chloranphenicol (1 %). For each sample, ten replicates were made. Petri dishes with grains were incubated at 25°C for 7 days and then examined under a light stereomicroscope for fungal growth. Isolation of the colonies was made to obtain pure cultures. Slides of fungal growth were prepared and observed under a compound microscope for fungal morphology study. Identification was carried out using identification keys (Carmichael et al., 1980; Domsch et al., 1980; Onions et al., 1981; International Mycological Institute, 1991; Hanlin, 1997; Malloch, 1997; Pitt & Hocking, 1997; Barnett & Hunter, 1998; Samson et al., 2004).

Results

Insects

Table 1 shows a list of the insects identified in the maize samples from eight provinces of Angola. It was found that *C. ferrugineus*, *S. zeamais* and *S. cerealella* were present in all samples.

Tab. 1 Identified insects in stored maize samples from eight provinces of Angola.

Insect	Province							
	Benguela	Bié	Cuanza Sul	Huambo	Huíla	Luanda	Malange	Namibe
COLEOPTERA								
Cryptolestes ferrugineus	+	+	+	+	+	+	+	+
Gnatocerus maxillosus	+	+	-	-	-	-	-	-
Oryzaephillus surinamensis	-	-	+	-	-	+	+	-
Rhyzopertha dominica	+	+	-	+	+	-	+	+
Sitophilus zeamais	+	+	+	+	+	+	+	+
Tribolium castaneum	+	+	+	+	+	+	-	+
LEPIDOPTERA								
Sitotroga cerealella	+	+	+	+	+	+	+	+
PSOCOPTERA								
Liposcelis bostrychophila	-	-	+	-	-	+	+	+

Note: (-) without insects and (+) with insects.

Fungi

In this study, field and storage fungi were detected and identified in all samples. The field species isolated were *Diplodia maydis*, *Nigrospora* sp., *Rhizopus* sp., *Trichoderma* sp. and *Trichothecium roseum*.

The storage species isolated were *Aspergillus candidus*, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. parasiticus*, *A. wentii*, *Fusarium moniliforme*, *F. oxysporium*, *Penicillium citrinum*, *P. funiculosum*, *P. furcatum*, *P. islandicum*, *P. purpurogenum*, *P. variabile* and *P. pinophilum*. The presence of different taxa in samples from the six provinces is presented in the Table 2.

Tab. 2. Frequency of identified fungi in stored maize samples from six provinces of Angola.

	Province					
	Benguela	Bié	Huambo	Huíla	Malange	Namíbe
High frequency	*Fusarium moniliforme* *P. citrinum*	*A. clavatus* *A. flavus* *A. ochraceus*		*F. moniliforme*	*A. flavus*	*Trichothecium roseum*
Frequent	*Rhizopus sp.*	*P. funiculosum*	*A. flavus* *A. fumigatus* *A. ochraceus* *F. moniliforme* *P. variabile* *P. pinophilum*	*A. flavus* *A. parasiticus* *P. purpurogenum*		*A. flavus* *P. citrinum* *P. variabile*
Low frequency	*A. clavatus* *A. flavus* *F. oxysporum* *P. islandicum*	*A. niger* *A. parasiticus* *F. moniliforme* *Nigrospora* sp. *P. pinophilum*	*A. niger* *A. parasiticus* *P. citrinum* *Trichoderma* sp.	*A. candidus* *A. niger* *A. wentii* *Diplodia maydis* *P. funiculosum* *P. furcatum*	*P. funiculosum* *P. furcatum*	*A. candidus* *A. niger* *F. moniliforme* *P. funiculosum* *Rhizopus* sp.

P. pinophilum
Rhizopus sp.

Discussion

The results show that most of the insects present in the studied samples belong to the Coleoptera order, confirming the results obtained by Amaro & Gouveia (1957), Carvalho (1984) and Matos (2004). Data support the conclusion of no differences in species in relation to the sample collection site; i.e., insect species found in samples collected in the silos are the same as those obtained in the local market.

The presence of the same insect species that have been identified in the other provinces is highlighted in Malange, which is somewhat worrisome given that the maize sample from this province belongs to a major agricultural company, which theoretically has good technical advice and practices, while the other samples are from small local producers and markets.

In all of the provinces where maize samples were collected the presence of *P. truncatus* was not detected. It is of paramount importance to continue this work by collecting a larger number of samples, for each province, for each type of storage, in maize, from the small farmer to the large storage companies, to detect the arrival of *P. truncatus*, a devastating pest already present in many African countries. Ensuring continuous training and implementation of pesticide regulation are also a priority for Angola.

Field fungi colonize maize grains only when the water activity (aw), temperature and relative humidity are high. However, as a result of an adaptation to low aw, fungi belonging to *Aspergillus* spp., and *Penicillium* spp., also designated as storage fungi, are able to invade the maize grains stored at aw levels considered as safe. They are frequently responsible for causing serious losses, even before they were visually detected. They affect negatively the product's appearance, flavour, odour and nutritional content. They also may produce mycotoxins with large impact on public health (Magro, 2001). It is important to emphasize that *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp., are potential mycotoxins producers. It is fundamental to improve and control the maize storage conditions as well as the cleaning process before any further grain processing.

Acknowledgements

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Automated detection and monitoring of grain beetles using a "smart" pitfall trap

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Abstract

A smart, electronic, modified pitfall trap, for automatic detection of adult beetle pests inside the grain mass is presented. The whole system is equipped with optoelectronic sensors to guard the entrance of the trap in order to detect, time-stamp, and GPS tag the incoming insect. Insect counts as well as environmental parameters that correlate with insect's population development are wirelessly transmitted to a central monitoring agency in real time, are visualized and streamed to statistical methods to assist effective control of grain pests. The prototype trap was put in a large plastic barrel (120lt) with 80kg maize. Adult beetles of various species were collected from laboratory rearings and transferred to the experimental barrel. Caught beetle adults were checked and counted after 24h and were compared with the counts from the electronic system. Results from the evaluation procedure showed that our system is very accurate, reaching 98-99% accuracy on automatic counts compared with real detected numbers of adult beetles inside the trap. In this work we emphasize on how the traps can be self-organized in networks that collectively report data at local, regional, country, continental, and global scales using the emerging technology of the Internet of Things (IoT). We argue that smart traps communicating through IoT to report in real-time the level of the pest population from the grain mass straight to a human controlled agency can, in the very near future, have a profound impact on the decision making process in stored grain protection.

Keywords: pitfall trap, sensors, Internet of Things, stored grain, beetle pests.

Introduction

Low tolerance of the presence of insect pests in stored grain requires the development and implementation of detection and monitoring methods that are sensitive enough to detect early pest infestation to prevent quality and economic losses (Trematerra, 2013). Today, the innovative uses of sensors and networks targeting animals are starting to be translated into new ecological knowledge (Portet et al., 2009). Traps equipped with a detection sensor and wireless communication abilities have some distinct advantages against manual monitoring. They can monitor insect populations 24 h a day, upon their entrance to the trap, every day of the year, in dispersed nodes across a variety of fields, simultaneously, and all counts and recordings can be permanently stored in a cloud service. Another distinct advantage is the determination of the precise onset of an infestation. Real-time reporting, opens new grounds in stored product research and mainly in crop protection as – besides a timely control action in response to a pest infestation – it can help in the evaluation of the impact of a control treatment and therefore reschedule future actions if necessary.

Pitfall traps are typically used for monitoring several species of stored-grain beetles (Coleoptera) in silos, warehouses and processing plants (Reed et al., 1991). They are placed inside the bulk grain near the external surface. The cone-shaped device is made of clear plastic and has a removable perforated lid, which allows insects to enter, but not escape. Various pheromone lures targeting different species may be used. Many destructive beetle pests of stored grain may be monitored by this type of trap: the flour beetles *Tribolium* spp. (Tenebrionidae), the grain weevils *Sitophilus* spp. (Curculionidae), the lesser grain borer *Rhyzopertha dominica* (F.) (Bostrichidae), the cigarette beetle *Lasioderma serricorne* (F.) (Anobiidae) and the khapra beetle *Trogoderma granarium* Everts (Dermestidae) (White et al., 1990; Neethirajan et al., 2007).

Our approach aims at reducing the necessity of human-in-the loop in any intermediate processing stage of the workflow and reserve the need of expert entomologists only for the highest abstraction layer: the interpretation of the data received (trap catches) normally presented in the form of georeferenced maps and the corresponding decision making and action planning based on pest Economic Injury Levels (EIL) population thresholds that are applied in the frames of Integrated Pest Management (IPM). Our work focuses on leveraging the quality of service of remote surveillance of pest populations to a better and cost-effective status than sparsely applied human inspection.

Materials and Methods

We have embedded our electronics into the Pitfall trap (EDIALUX, Bornem, Belgium) for monitoring populations of beetle pests of stored grain. There is always an emitter of light opposite to a receiver of light and the path of the incoming insect passes in between. The interruption of the path of light effects a voltage drop that exceeds a threshold and constitutes a count. Both receiving and emitting elements are deployed as 1D linear arrays that are long enough to cover the entrance to the trap. In the pitfall trap, an insect can enter from any hole of the lid. In order to avoid blind spots in the field of view we need to have a uniform field sensing insect sizes ≤ 0.5 mm. We used 16 LEDs and the same number of photodiodes and both emitter and receiver have a light diffuser. All sensors are operated in pulse mode i.e. there is no constant flow of light from emitter to receiver but a pulse train is emitted.

For the purposes of our study, a prototype (Fig. 1) equipped with a linear array of five Light Emitting Diodes (LED) opposite to 5 receiving photodiodes was evaluated. The prototype trap was put in a large plastic barrel (120lt) with 80 kg maize. Adult beetles of various species were collected from laboratory rearings and transferred to the experimental barrel. In order to ensure trap catches a large number of adult beetles was used resulting in an infestation level of more than 15 adults per kg maize. Caught beetle adults were checked and counted after 24h and were compared with the counts from the electronic system.
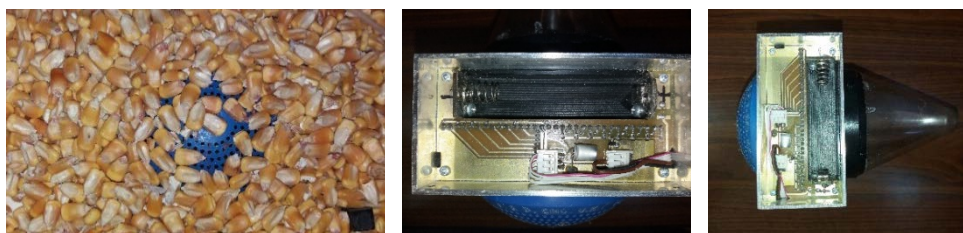
Fig. 1 The "smart" Pitfall trap. A sheet of light covers the lid entrance. Photo interruption due to a falling insect produces a voltage variation that is turned to a count. Counts as well as environmental parameters and a time stamp are transmitted wirelessly and uploaded to server.

Results & Discussion

Results from the evaluation of the prototype traps are presented in Table 1 and Fig 2. As it is clearly concluded from our data, our system is very accurate, reaching 98-99% accuracy on automatic counts compared with real detected numbers of adult beetles in each trap. The accuracy of our system in detecting adult beetle catches is also shown by the very high (r > 0.99 in all cases) correlation between the generated signals and actual numbers of insects caught in the trap.

Tab. 1 Number of actually detected (manual inspection) and automatically counted (electronic sensors) adult beetles in "smart" pitfall trap

Species	Actually Detected	Automatically Counted	Correlation coefficient (r)
	59	62	
C. ferrugineus.	45	49	0.9912
	67	74	
	31	34	
O. surinamensis	11	12	0.9978
	24	25	
	15	15	
R. dominica	23	24	0.9976
	24	26	
	21	21	
S. oryzae	32	36	0.9900
	29	30	
	13	13	
T. confusum	26	30	0.9912
	34	36	
	14	14	
R. ferrugineus.	45	49	0.9999
	67	74	

Single trap inside grain mass, insect density >15 adults / kg grain
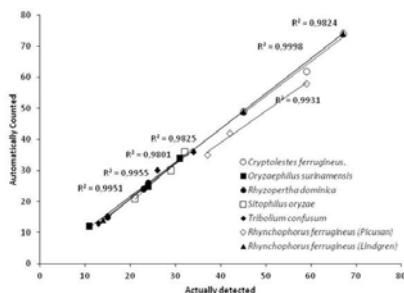
Fig. 2 Accuracy of the automatic counting in comparison with actual detection. The values of the linear regression coefficient R^2 prove that our system is 98-99% accurate (when detected and counted values are the same then R^2 equals to 1)

Only a few remote pest monitoring systems, based on wireless communication technology, have been evaluated in the past, with varying accuracy. The oriental leafworm moth *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) was effectively monitored by an ecological monitoring system combining GSM transmission technologies with mechatronics with accuracy ranging from 71 to 100 (Shieh et al., 2011). Average accuracies of 96.3% (Liao et al., 2012) and 94.9% (Deqin et al., 2016) were demonstrated by automatic monitoring systems counting the catches of the oriental fruit fly *B. dorsalis*. Other automated systems with image analysis technology also proved to be reliable in detecting mainly whiteflies and moths, with accuracies ranging from 70 to 100% (Xia et al., 2012; Ding and Taylor, 2016; Boissard et al., 2008; Lopez et al., 2012; Guarnieri et al., 2011). The accuracy of our system is higher than almost all of the abovementioned monitoring systems.

In this work we establish a connection between sensors' readings, pest population level and predictive models to ensure timely and effective control treatments. Acceptance of automated monitoring practices will raise doubts about the reliability of data collected without expert's intervention. The optoelectronics need to reduce their errors in order to reach comparable analysis to that done by experts. A long-term field operation is needed in order to identify the cause of possible false alarms and detection misses and sensor failures in sometimes harsh conditions before applying the output of such data-collection schemes to modeling and policy. We believe current results are sufficient to warrant further exploration on insect surveillance. Insect surveillance can provide insight into the effects of insecticide efficiency, reduce its use and shape our understanding of pest problems in agriculture. Provided we continue improving the reliability of devices and services and perform real-field, long-term trials we will upgrade automated practices to the level of being indispensable to farmers, policy makers and stakeholders.

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Detection and estimation of population density of bean weevils (Coleoptera: Bruchidae) in stored pulses via bioacoustic analysis

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Abstract

Stored product insects, produce acoustic emissions by moving, feeding or ovipositing inside the grain mass. These sounds can be used not only for detection purposes, but also for population density estimation. Acoustic emissions of adults of *Acanthoscelides obtectus* (Coleoptera: Bruchidae) and *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) were recorded infesting various pulses in varying population densities from 1 to 500 adults/kg product. The acoustic analysis system is being described. Population density, type of grain and pest species had significant influence on the number of sounds. The system was 100% precise in negative predictions and considerably successful in positive predictions. The system was very accurate (80-100%) in detecting insect presence even in the "critical" density of 1 adult/kg product, the most common threshold for classifying a stored mass as "infested" or "not infested". Our study suggests that automatic monitoring of the infestation state in bulk grain is feasible in small containers. This kind of service can assist reliable decision making if it can be transferred to larger storage establishments (eg. silos). Our results are discussed on the basis of enhancing the use of acoustic sensors as a decision support system in stored product IPM.

Keywords: Stored Pulses, Bioacoustic, Detection, bean beetles, Density Estimation

Introduction

More than 500 species of beetles have been reported to be associated with various stored grain products (cereals and pulses) and almost 100 of them may cause significant quantitative or qualitative losses. It has been estimated that between one quarter and one third of the world grain crop is lost each year during storage. The key for successful management of stored grain pests is not only early detection, but also an accurate population density estimation of the pest (Rajendran and Steve, 2005).

Acoustic detection is a very promising method for early detection of insects inside the grain mass (Eliopoulos et al., 2015; Hagstrum et al., 1996; Mankin et al., 2011; Potamitis et al., 2009 and others). Insects of stored grain generate sound by eating, flying, egg laying, or locomotion. Reliability and

efficacy of acoustic sensors has been greatly increased in the last few years as a result of the development of improved acoustic devices and signal processing methods (Mankin et al., 2011). Apart from detection, very few studies have evaluated the potential of the acoustic method in estimating the population density of the pest inside the grain mass (Hagstrum et al., 1988, 1990).

The aim of our study is to propose and evaluate an automated monitoring procedure for IPM implementation in grain handling and storing facilities. The main unit is composed of a piezoelectric sensor and a portable acoustic emission amplifier connected to a computer. The software analyses the vibration recordings of the piezoelectric sensor, performs signal parameterization and eventually classification of the infestation severity of adult beetles inside the grain mass in four classes, namely: Class A (densities ≤1 adult/kg), Class B (densities 1-2 adults/kg), Class C (densities 2-10 adults/kg) and Class D (densities >10 adults/kg). Our results are discussed on the basis of enhancing the use of acoustic sensors as a decision support system in stored product IPM.

Materials and Methods

For the purposes of our study, we used adults from two important beetle pests of stored pulses. We used the grain that each species is most commonly associated with in natural conditions. Specifically, we recorded acoustic emissions of the bean weevil *Acanthoscelides obtectus* (Say) (Bruchidae) on kidney and butter (giant) beans and the cowpea weevil *Callosobruchus maculatus* (F.) (Bruchidae) on broad (fava) beans.

Our system was adopted by Eliopoulos et al. (2015) and consisted of a 14cm long piezoelectric sensor mounted on the end of a probe that was pushed into the grain (hard wheat) and a portable acoustic emission amplifier (AED-2010L, Acoustic Emission Consulting, Inc., Fair Oaks, CA) connected with a computer. The experimental procedure (grain preparation, recording methodology etc) is described in detail by Eliopoulos et al. (2015). Each treatment (recording of the desired species and number of adults in the desired grain mass) was replicated 5 times. Recordings from uninfested pulses was used as control.

Various infestation densities were tested during the present study (1, 2, 10, 20, 50, 100, 200 & 500 beetle adults/kg grain). We proceeded into inserting the piezoelectric probe and taking 5 recordings per jar. We have grouped insects' density in 4 distinct classes: Class A (densities ≤1 adult/kg), Class B (densities 1-2 adults/kg), Class C (densities 2-10 adults/kg) and Class D (densities >10 adults/kg). We apply supervised learning techniques to our dataset as we know the class labels (i.e. we set the infestation densities). The task is given the counts/min of the unknown test jar the classification algorithm must predict the Class (i.e. severity) of the infestation.

In operational mode, the computer receives a vibration recording from the sensor which turns into counts of enumerated pulses (counts/min). From these counts/min it infers the distribution of probabilities over infestation severity classes A-D. By peaking the probability distribution the algorithm can output a single decision as well.

Our data (number of recorded sounds expressed as counts/min) were subjected to analysis of variance in order to evaluate the main effects. ANOVA was performed by using SPSS v.18.0. (SPSS Inc, 2009).

Results and Discussion

The mean numbers of counts/minute that were recorded during the present study are presented in Fig 1. The increase on population density (number of adults inside the grain mass) was always followed by an increase in recorded sounds. The differences were not always significant. The linear model was very effective in describing the relationship between population density and number of sounds given that values of R were high (>0.80) (Fig. 2).

The type of grain had notable influence on the number of sounds. This was observed in the case of *A. obtectus* in small (kidney) and large-sized (butter) beans. The number of sounds was significantly

higher when bean weevils were inside the kidney bean mass irrespective of population density (F1,88 = 12.61; P = 0.0007) (Fig. 1).

It has been well documented that acoustic sensors may be very effective in detecting insect presence in the grain mass (Eliopoulos et al., 2015; Hagstrum et al., 1991). However, there are only a few studies focusing on the estimation of pest density using bioacoustic technology. The first attempt was made by Hagstrum et al. (1988) using various densities of *R. dominica* larvae in wheat, and counting the produced sounds, with a piezoelectric microphone and earphones. They concluded that insect sounds increase with pest density and that the accuracy of estimation of insect densities with the acoustical method was comparable to that obtained with a standard grain trier. Following studies by the same research team revealed that acoustic sensors can be used for density estimation not only in experimental bins (Hagstrum et al., 1990, 1991) but also in real silos (Hagstrum et al., 1994, 1996) with very satisfactory results. Our results cannot be compared with those of the above mentioned studies because of the completely different methodology they used. For example, Hagstrum et al. (1996) correlated the number of sounds with pest density using 140 sensors on 7 cables in grain bins, checking each sensor for 10 sec 27 times per day.
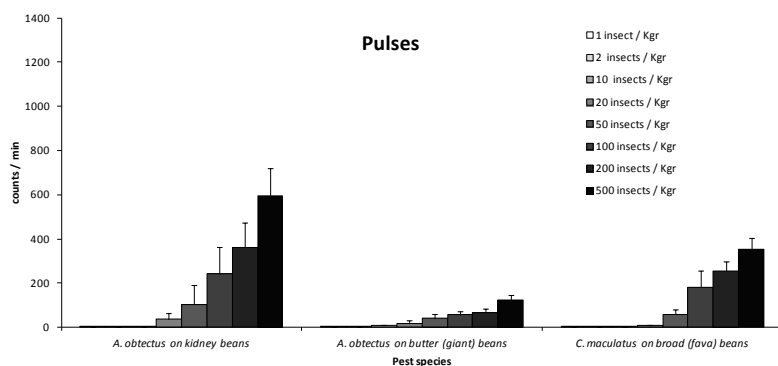
Fig. 1 Mean number of sounds recorded inside the pulses mass during the present study.
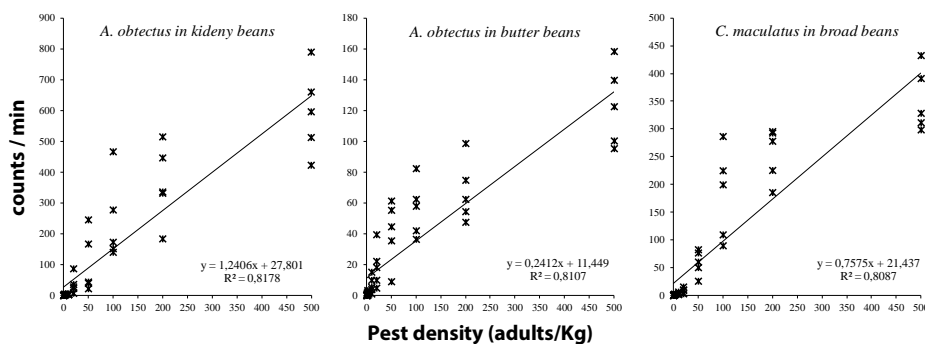
Fig. 2 Linear relationship of pest population density with number of sounds recorded in stored pulses

The type of pulse was an important factor that influenced the number of sounds. Although, sound is transmitted over longer distances in grains with a larger inter-kernel spacing, such as maize and butter beans (Hickling et al., 1997), we found that more sounds were generated when adult beetles were in small-sized grain like kidney beans. The reason for this should be that insects have smaller free space to move and they "interact" with kernels more often in small sized grains. Vick et al. (1988) also demonstrated that number of sounds produced by *S. oryzae, R. dominica* and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) varied significantly when they were in a different type of grain (rice, corn or wheat).

Our study suggests that automatic monitoring of the infestation state in bulk grain is feasible in small containers. This kind of service can assist reliable decision making if it can be transferred to larger storage establishments. Very soon and with further technological development (e.g. piezo electric sensors embedded in cables submerged in the grain) the acoustic methodology can provide a quick and easy way, not only of detecting, but also of estimating pest population density in larger establishments of stored grain facilities.

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PHID-Coleo - a database identification tool for wood-boring beetles in plant health interceptions

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Abstract

Recent examples for the introduction of wood-breeding beetles in Europe include the asian longhorn beetles *Anoplophora* spp. and *Aromia bungii* (red-necked longhorn beetle). These and other woodboring beetle species pose a high risk of economic damage to trees and wood products. Smaller beetles like the powderpost beetles from the families Bostrichidae and Lyctidae also have the potential for causing considerable damage. These are often not identified adequately during inspections of wood packaging materials, making it impossible to assess their risk for becoming invasive. This project will aim at closing that gap. Our project PHID-Coleo (= Plant Health Identification of Coleoptera) has the objective to develop new diagnostic tools for the identification of potentially invasive and economically important beetles that can be found in wood packaging materials. The identification methods include classical identification keys based on morphological characters as well as molecular methods based on DNA analysis by PCR (barcoding). The methods for species identification will be supplemented by molecular analyses of introduced populations to clarify within species variations. Such methods will make it possible to determine the taxonomic relationship of samples from different areas and to draw conclusions about the introduction pathways, resulting in more efficient monitoring of the invasive species and preventing their spread. PHID-Coleo will build a freely accessible database of relevant species which are potentially invasive.

Keywords: Cerambycidae, Bostrichidae, morphological diagnostics, molecular diagnostics, population analysis.

1. Global trade: introduction pathways for potentially invasive insect species

An increasing number of imported goods from all over the world are reaching European ports every day. These are often accompanied by untreated wood in the form of wood-packing material (WPM). The international transportation of WPM (e.g. as wooden dunnage, pallets or crates) is recognized as an important pathway for the introduction of non-native harmful insects. For this reason, specific international phytosanitary measures (International Standard for Phytosanitary Measures No. 15 or ISPM15) have been developed by the Food and Agriculture Organization of the United Nations (FAO) within the framework of the International Plant Protection Convention (IPPC) (IPPC, FAO 2013).

To prevent the introduction of harmful species this standard specifies that wooden materials (> 6 mm) used for shipment have to be free from living organisms, when being exported into a country following the ISPM15. In practice, this usually requires WPM to be debarked and then heat treated or fumigated. If such treatments have not been done adequately, wood boring insects may survive in WPM and then be introduced into the importing country. Therefore, inspections still need to be conducted at European ports. These inspections are aimed at an accurate identification of any intercepted species as well as preventing them from entering the country. But infested WPM are not always recognized properly and intercepted during the inspections at the import control and of course only in random samples. In such cases, non-native species can enter the country, may survive under suitable conditions (e.g. the availability of suitable hosts) and become established. This can result in serious damage to crops or forest trees. Recent examples of such introductions into Germany are the Asian long-horned beetle (*Anoplohora glabripennis*) (Mühleisen & Zimmermann 2016) and the rednecked long-horned beetle (*Aromia bungii*) (LfL Bayern 2018).

In case a new species is repeatedly found in the field, several possibilities need to be considered and distinguished, including whether a) the species is already established, b) there have been repeated introductions or c) the samples are related to each other. Identifying which of these possibilities apply with new molecular tools can help to improve the monitoring of invasive species and preventing their spread.

2. The problem of species identification and the necessity of pest risk analysis

The species identification is necessary, because it is the basis for pest risk analysis (PRA) conducted by the regulatory national plant health organization (e.g., the Julius Kühn-Institute for Germany). The PRA analyses the risks and possible consequences of the introduction of a new pest, under inclusion of biological (e.g. climatic tolerance, available hosts) and other scientific information. It also identifies the phytosanitary measures required to protect plant resources against a new

potential pest. In case of an introduction event, the PRA helps decision makers to react quickly and in the most suitable way (JKI - Julius Kühn-Institut 2018).

The morphological identification of non-native species is difficult, since identification keys are often unsuitable for the inspection teams, are unavailable, or are only available in a foreign language (Ohbayashi & Nisato 2007). This is especially the case for the immature stages of frequently imported groups of insects like Cerambycidae and Bostrichidae (Wang 2017, Geis 2002).

Another problem arises, when the sample is in a bad physical condition (e.g., a crushed larva) and identification traits have been lost. A molecular identification based on DNA barcoding analysis can help in such cases (Wu et al. 2017). For this, a genetic marker (a short sequence) of the specimen's DNA is compared with DNA references from an online database. If both DNA sequences match, the examined sample is most likely the species that has been deposited as reference in the database. Unfortunately, in databases such as NCBI there are error rates of up to 20%, because some species had been misidentified before they were barcoded (NCBI 2018). Such erroneous references must be recreated to meet quality standards and accreditation requirements in diagnosis.

At the same time other databases like the Barcode of Life Database, BOLD, with higher quality standards are yet often incomplete (Ratnasingham & Hebert 2007). Also, the Q-BOL Project which covered a broad range of organism groups of pests and diseases and resulted in the database Q-Bank, still does not include e.g. important Cerambycid beetles such as *Batocera lineolata* or *Saperda candida* (Q-Bank 2017). In Europe the important PM7 diagnostic protocols for identification are usually limited to some more relevant species that are already regulated (EPPO 2018). Available molecular references for species associated with WPM are currently very limited. Therefore, relevant species that have been found in WPM so far and are likely to be mistaken with similar species have to be barcoded to fill that gap and to build up reliable datasets for their use by diagnostic laboratories.

3. The project activities of PHID-Coleo

The project PHID-Coleo (**P**lant **H**ealth **ID**entification of **Coleo**ptera) has been designed in Germany as a cooperative project between the plant protection service of Baden-Württemberg in Karlsruhe (LTZ) and the University of Hohenheim (UHOH). The project was launched in 2017 and runs for three years until June 2020 (Bauer & Zimmermann 2018).

PHID-Coleo aims at developing new diagnostic tools for the identification of potentially invasive and economically important beetles which are associated with WMP. In addition, it aims to develop new molecular methods for the analyses of already established exotic species. Introduction pathways and relationships between existing populations of invasive species need to be investigated as fast as possible to predict the invasive potential and to prevent economical damages.

The activities of the project are divided into three sections, with sections one and three being implemented by the Centre for Agricultural Technology Augustenberg (LTZ), Department 33, Zoological Diagnostics in Karlsruhe, Germany and section two by the Departments of Live Stock Population Genomics and Applied Entomology at the University of Hohenheim, Germany.

Project activies - Section 1: Morphological and molecular key

The activities under section one aim at new identification tools for potentially invasive and economically important species of false powderpost beetles and long-horned beetles (Coleoptera: Bostrichidae and Cerambycidae, respectively). According to the European phytosanitary alert system EUROPHYT these are the most important groups of insects that can be found in WPM (EUROPHYT 2018). For this, classical identification keys based on morphological characters are being developed for the relevant species of these groups. The keys will not only consider the adult beetles but also the immature stages, because usually only the larvae are found in WPM.

The classical keys will be supplemented by molecular methods for species identification. This will make it possible to deal with the smallest tissue samples and physically damaged specimens. Thus, species can be identified quickly and easily in the laboratory.

In collaboration with entomologists and according to recently published species lists (Geis 2014, Eyre and Haack 2017), the project partners have identified more than 100 species, which were confirmed to be associated with WPM. These are currently being documented photographically and molecular references are being developed. The keys will also be available in a printed version and molecular data will be published as well in established online barcoding databases.

Project activies - Section 2: molecular analysis of insect populations

The activities under section two aims at developing a detailed understanding of the population dynamics of invasive beetles and their dispersal. *Anoplophora glabripennis*, the Asian long-horned beetle (ALB) is serving as a model example. Investigations of intraspecific nucleotide variations should help to understand the relationship between populations found at different infestation sites, e.g. in Germany.

Genetic markers for the molecular comparison of individual populations of invasive species will be selected and provided for diagnosis (Hasselmann et al. 2015). They should help plant protection services to trace and identify new infestation events and to understand introduction pathways. A higher resolution of the ALB population structure will be achieved by using a larger amount of genetic markers for mitochondrial and genomic DNA. A set of molecular tools such as sequencing, conventional microsatellite analysis and state-of-the-art single-nucleotide polymorphism screening (Nolte et al. 2005, Gruber et al. 2013) will provide a broader spectrum of genetic markers than available now. For the genotype analysis specific software for measuring genetical differences will be used (Pritchard et al. 2000).

The project partners are collecting genetical material of the ALB from different infestation sites in Germany, as well as from other countries for comparisons, including Europe and the native habitat of this species in Asia. Research results about the intraspecific genetic differences of ALB populations indicate that there are variations in the D-Loop region and the cytochrome oxidase subunits 1 and 2 of the mitochondrial genome, as well as in the microsatellite regions of the genomic DNA. A preliminary analysis with microsatellites in the PHID-Coloe project showed promising results. The genome of the ALB was ´screened´ and approximately 700 regions with tandem repeats have been found, of which 25 microsatellites had been tested, that have not been used in ALB-studies so far. After sequencing, eight of them showed considerable differences in length and in the number of so-called repeats in comparison to the provisional reference genome (McKenna et al. 2016).

Project activies - Section 3: open identification keys and an expert network for identifying beetles intercepted during plant health inspections

Under section three, the results obtained under the previous two sections will be published as booklets, printed identification keys, and as well online in the form of a freely accessible database for interested scientists, plant health services and zoological diagnostic laboratories. Diagnostic workshops will also include training for companies that provide barcoding services. Workshops and single training will be offered during the project, but also beyond the term of the project. Interested parties may contact the PHID-Coleo partners.

A further aim of the project is to establish a long term collaborative network in the field of molecular pest diagnosis which will include plant protection professionals, entomologists, research scientists (e.g. from state institutes and museums) as well as commercial companies. This collaborative network should continue its activities after the end of the project for a quick and safe identification of future interceptions of unknown insect species.

Acknowledgement

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Visible Near Infrared Hyperspectral (VNIH) technique to differentiate Trogoderma variabile reared on different commodities

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Abstract

Under Trogoderma sp, some comes major stored grain pest, which are of economic concern and most of the times accurate identification becomes very difficult. Under this study a new diagnostic system using visible near-infrared hyperspectral (VNIH) imaging methods is developed to address identification gaps for T. variabile. This technique is useful because different materials have unique reflectance spectra, and this difference in reflectance spectra can be used to identify various constituents in an image. For this study both larvae and adult were studied for T. variabile fed on wheat, maize, canola, barley, oats and rice for more than 4 generations. Each individual insects killed by ethanol were scanned using a hyperspectral imaging system from 400 to 1000nm. Matlab 2016b was used to develop predictive model for hyperspectral image classification. Deep neural network approach gave more than 90% accuracy for both larvae and adult stages fed on different commodities. From this result we can say that T. variabile on the different host can lead to difference in VNIH reflectance spectra. This is one of most fundamental factors for development of robust VNIH technique as diagnostic tool.

In search of a new attractant for monitoring *Stegobium paniceum* L. (Coleoptera: Anobiidae)

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Abstract

Stegobium paniceum (L.) is a major pest for several stored products worldwide. Monitoring methods for this species, based on pheromone traps, are affected by the complexity and expensiveness of the chemical synthesis of the pheromone isomer [(2S,3R,1'R)-Stegobinone] and/or by its lost of efficacy after two weeks at room temperature. So other semiochemicals that can be exploited for monitoring this species are highly desirable. In this study was tested the behavioral response in two-choice olfactometer of *S. paniceum* adults to Volatile Organic Compounds (VOCs) collected from colonized substrate. The elution of the headspace collection from *S. paniceum* colony elicited attraction of both sexes. The GC-MS chemical analysis of the extract indicated the presence of several alkanes, alcohols, aldehydes and fatty acids, some of them already reported to attract other stored product coleopteran pests and promising candidates for further studies to test their attractiveness on *S. paniceum*.

keywords: drugstore beetle, monitoring, attractant, volatile organic compounds, headspace

1. Introduction

The drugstore beetle *Stegobium paniceum* (L.) (Coleoptera: Anobiidae), , is among the major pests for a wide variety of dry and durable stored agricultural products (Edde et al., 2012). Drugstore beetle females produce a sex pheromone that induces attraction behavior of males, with the highest responses 5–12 days after adult emergence (Kuwahara et al., 1975; Kodama et al., 1987).

However, the response to this synthetic pheromone in trapping experiments often had unsatisfactory results (Mahroof and Phillips, 2007). This can be due to the complexity and expensiveness of the chemical synthesis of the pheromone isomer [(2*S*,3*R*,1'*R*)-Stegobinone] at high purity grade and/or by its lost of efficacy after two weeks at room temperature (Kodama et al., 1987). For these reasons, it is important to investigate alternative attractants or pheromone synergists for monitoring and/or mass trapping *S. paniceum* adults. The *Volatile Organic Compounds* (VOCs) produced from the insect colonies of *S. paniceum* could be useful in order to develop an efficient and economically sustainable attractant for the drugstore beetle as positively exploited for *Lasioderma serricorne*, a related anobiid species (Buchelos and Trematerra, 1998; Mahroof and Phillips, 2007). To date, the olfactory responses of *S. paniceum* to VOCs of its colonies and their volatile chemical composition have never been investigated. In this study, the behavioral responses of *S. paniceum* adults to VOCs from colonized substrate were evaluated in a two-choice olfactometer and analyzed by gas chromatography-mass spectrometry (GC-MS).

2. Materials and Methods

Colony VOCs collection

VOCs from a *S. paniceum* colony (insects plus rearing substrate) were collected by a dynamic headspace method (pull system). To collect volatiles, 90 grams of the rearing substrate were placed into a cylindrical plastic container. About 200 unsexed adults of *S. paniceum* were added to the rearing media in the plastic cylinder. Volatiles were collected for 24 hours on adsorbent tubes filled with 40 mg of Tenax-TA and 40 mg of Carbotrap B, both Supelco (Bellefonte, PA, USA). The adsorbent materials were fixed in the adsorbent tubes using glass wool. To generate a flow rate of charcoal-filtered air (200 ml/min) a vacuum pump was used. Headspace samples from empty cylindrical plastic boxes and oven bags were used as controls. Volatiles trapped in the tubes were eluted with 500 µl of cyclohexane. All samples were stored in screw cap vials at -20 °C until chemical and behavioral studies.

Chemical analysis

GC-MS analysis of the colony headspace samples were performed on an Agilent 5890 GC system, equipped with a DB-5ms column, interfaced with a 5973 quadruple mass spectrometer. The GC oven temperature was set at 40°C for 5 min, then, increased by 10°C/min to 250°C. Identification of compounds was carried out by comparison of mass spectra and retention times with standard compounds purchased from Sigma Aldrich (Milan, Italy). When synthetic standard was missing, the identification was made by using the NIST 98 library and by Kovats retention indices (Adams, 2007).

Two-choice olfactometer bioassays

To validate the two-choice olfactometer used in our behavioural experiments, the sex pheromone [(2*S*,3*R*,1'*R*)-Stegobinone] was tested in preliminary experiments on *S. paniceum* adults. Subsequently, 200 µl of headspace elution was tested versus a blank headspace collection. The olfactometer consisted of a glass chamber (cm: 26 long × 17 wide × 13 high) covered by a glass lid and illuminated by a lamp positioned 1 m above the instrument. Each side of the chamber was covered outside with white printer paper to eliminate potential distractions to beetles and to diffuse light. Two pairs of white plastic cups (0.2 l and 0.3 l) were used as olfactometer arms. For each bioassay, one beetle was released inside the chamber through the entry-hole on the long side of the chamber. The presence of the beetle inside the olfactometer arm was verified after 24 hours. The position of the stimuli in the arms was switched after each replication to avoid the influence of the olfactometer position on beetle choices. Before each replication, to prevent the accumulation of odors, the collection cups and the ramps were changed while the glass chamber was cleaned with acetone and dried by paper towel and a hair-dryer.

3. Results

Chemical analysis

Overall, twenty-four VOCs were found in the GC-MS analysis of the elutions from the colony of *S. paniceum*. Among them, nineteen compounds were identified whereas five were unknown. The most abundant compounds were hexanoic acid (12.4%), heptanoic acid (9.5%), decane (9.1%), 4-methyldecane (8.5%), and nonanal (8%), contributing to 47.5% of the total composition. Other compounds detected were heptanal, 1-octen-3-ol, octanal, limonene, 3,6-methyl decane, 2-phenylethanol, tridecane, tetradecane and hexadecane. Stegobinone comprised 0.3 % of the total volatile profile.

Olfactometer bioassays

The results of the behavioral experiments are summarized in Fig. 1. The olfactometer was validated by the response elicited by the sex pheromone that attracted males ($x^2 = 6.3$, df = 1, $P = 0.01$, N= 30) but not females ($x^2 = 2.4$, df = 1, $P = 0.1$). The elution of the headspace collection from *S. paniceum* colony elicited attraction of both sexes (males: $x^2 = 11.6$, df = 1, $P = 0.0007$, N = 30; females: $x^2 = 5.1$, df = 1, $P = 0.02$, N = 35).
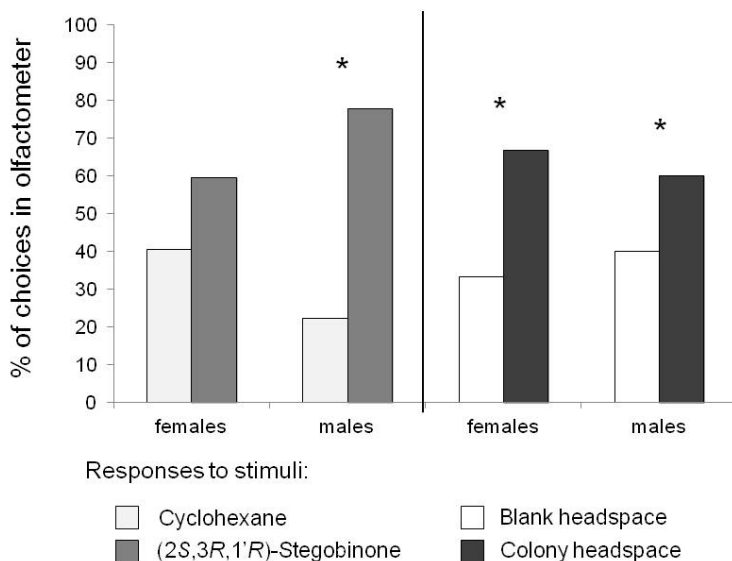
Fig 1. Percentage of choices of *S. paniceum* females and males to sex pheromone and to VOCs of the colony. * $P \leq 0.01$

Discussion

Our behavioural results showed that the elution from the dynamic headspace collection of the colony of *S. paniceum* elicited attraction for both adult sexes (Figure 1). Among the compounds identified in the chemical analyses of the colony VOC, several alkanes, alcohols, aldehydes and fatty acids have been already reported to attract other stored product coleopteran pests as *Oryzaephilus* spp., *Trogoderma* spp. and *Cryptolestes ferrugineus* (Levinson, 1978; Pierce et al., 1990, 1991). Interestingly, some of the chemicals detected in our analysis also co-occur in volatiles emitted from Chinese medicinal plant materials that elicit attraction toward *S. paniceum* adults (Cao et al. 2018).

Since *S. paniceum* sex pheromone attracts only males and loses its attractive capacity after two weeks at room temperature (Kodama et al., 1987), it is desirable to exploit alternative attractants for monitoring this pest. In anobiid beetles that feed on stored products, the use of food volatiles that, acting as kairomones, synergize the pheromone lures have been successful tested on *L. serricorne* (i.e. VOCs from *Capsicum* spp.) (Mahroof and Phillips, 2007). Similarly, the results of our study showed that the headspace elution, containing the VOCs from *S. paniceum* colony, is an attractant for both sexes of this pest species. In this context, our study gave the basis for the development of a new alternative and sustainable attractant for trapping the drugstore beetle. Further investigations are in progress aimed to identify which are the behavioral-active VOCs of the entire chemical composition involved in the attraction of *S. paniceum* adults.

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Field trials on attractiveness of the synthetic sex pheromone of the four-spotted bean weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae).

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Abstract

Quarantine pests of legumes pose a threat to many countries of the world including Russia. Pests that can enter the country as a result of the transportation of regulated articles (by sea, air, road, rail, etc.) pose a particular danger (Shutova, 1970; Dankvert *et al*., 2009). Monitoring and identification of legume pests is complicated by the fact that small beetles have a hidden mode of life. One of the most dangerous quarantine pest is the four-spotted bean weevil *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), which is widespread throughout the world and can cause serious economic losses in agriculture of Russia. Research work on the identification, synthesis and laboratory evaluation of the synthetic sex pheromone of *Callosobruchus maculatus* was carried out at the All-Russia Plant Quarantine Center (Bykovo, Moscow region). Tests have shown that synthesized sex pheromone of *C. maculatus* has a high attractiveness for males. An effective dose of pheromone that attracts males of the four-spotted bean weevil has been found at the laboratory and is equal to 0.5 µg per

dispenser. Thereafter tests have shown that the concentration of pheromone above 2 µg does not cause behavioral response in beetles and doesn't result in contact with the stimulus. Dispensers with doses of pheromone from 4 to 8 mg have been used with a Delta trap in storage. The use of pheromone traps can help in pest identification, decreasing or complete avoidance of repeated treatments with chemicals at low pest population. The results of this study will be presented and discussed on the basis of laboratory and literature data.

Keywords: *Callosobruchus maculatus*, synthetic sex pheromone, pheromone trap, monitoring, plant quarantine.

Introduction

Polyphagous bruchids of genus *Callosobruchus* Pic (Coleoptera: Bruchidae) are the most dangerous pests of legumes. The most severely damaged crops are mung bean (*Phaseolus aureus*), chickpea (*Cicer*), pigeon pea (*Cajanus*), green pea (*Pisum sativum*), field bean (*Vicia faba*), cow pea (*Vigna*), soybean (*Glycine max*), lentil (*Lens*) and bean (*Phaseolus*) – in these crops seed damage can reach up to 100%. Beetles not only reduce the yield in fields, but also are imported to storages with harvested seeds and continue developing as storage pests without diapause. Therefore, pests drastically reduce the food value and crop quality in many legumes. Totally the genus *Callosobruchus* includes 15 species. In practice, 4 species of this genus are most often imported to Russia, namely: *Callosobruchus phaseoli* Gyll. – cosmopolitan seed beetle, *C. analis* L. – Graham bean weevil, *C. chinensis* L. – Chinese bean weevil and *C. maculatus* F. – four-spotted bean weevil (Sadomov and Mordkovich, 2004). All these species are similar in biology, harmfulness and habitats. Morphologically they clearly differ in adult form, but larvae are indistinguishable. Species of genus *Callosobruchus* are designated in the unified list of quarantine objects of the Eurasian Economic Union (approved by the Council Decision of the Eurasian Economic Commission dated November 30, 2016 № 158, entered into force from 1st of July 2017).

Fig. 1 Male imago of the four-spotted bean weevil *Callosobruchus maculatus* F.

Fig. 2 Male imago of the four-spotted bean weevil *Callosobruchus maculatus* F. feeds on a pea seed.

In practice, the Russia's federal service for veterinary and phytosanitary surveillance uses a complex visual method to identify the four-spotted bean weevil. But one may significantly reduce labor costs and improve efficiency of quarantine monitoring of beetles by using pheromone traps in the field and storages for monitoring and identification (Smetnik *et al*., 1986; Burov and Sazonov, 1987; Phillips *et al*., 1996). Synthetic pheromone was synthesized at the Department of Synthesis and Application of Pheromones in All-Russia Plant Quarantine Center in 2012. In laboratory tests with olfactometer synthesized pheromone has attracted males of *C. maculatus* effectively at dose of 0.5 µg. The experiments as well have shown that the concentration of pheromone above 2 µg does not cause behavioral responses by beetles and doesn't result in their contact with the stimulus (dispenser).

Field trials on attractiveness of synthetic sex pheromone of the four-spotted bean weevil were conducted on the basis of the "Nikola Pushkarov" Institute of Soil Science and Agroecology in Bulgaria as part of bilateral R&D work. Trials were carried out in order to determine the biological activity of pheromone of the four-spotted bean weevil *C. maculatus* F. for early detection in the area of its distribution.

Materials and Methods

Field trials

Field trials were conducted in 2014 from 15th of August till 14th of September. The experimental field is located in the village of Skryt (*41°23'53"N, 23°12'27"E*), which is suburb of Petrich, Blagoevgrad region, close to the border with Macedonia. The trial has been set at the field of *Vigna sinensis* L. in the time of the ripening of beans when the beetles cause the highest danger.

Delta traps with sticky inserts were used for trials, at the center of which the dispensers with correspondent doses of pheromone of *C. maculatus* were placed. Insect monitoring was carried out within a month from the date of installation, in total 6 records were carried out. Pheromone doses applied to the dispenser are the following: variant I (control)– 0 mg, II – 1 mg, variants III and IV – 2 and 3 mg, respectively. A rubber cork has been chosen as a material for the dispenser, which has the property of prolonged and gradual release of the chemicals for a long period of time. The number of replications over all variants was 3.

Storage trials

In 2015 and 2017, trials on the biological activity of the pheromone during storage of legumes were conducted in the storage at the Institute of Soil Science and Agroecology in Kostinbrod (Bulgaria). Trials were carried out in a facility with a total area of 25 m^2. In a storage, damaged leguminous crops infested by *C. maculatus* in the field were stored. *Vigna radiata* L. and *Vigna sinensis* (L.) Walpers were stored crops in 2015 and 2017 respectively, that had been grown in the southwest of Bulgaria in the territory of ecological agriculture and were not treated by pesticides during periods of cultivation and storage. In the course of trial, there were no other insect pests and diseases on beans. The average temperature in the storage ranged from 23°C to 25°C, and relative air humidity was 65-70%. The experimental traps were placed in a randomized way throughout the area of the storage at a height of 1.5 m at available places (Lebedev *et al.*, 1984; Dospekhov, 1985). The distance between traps was not less than 2 meters.

In 2015, the doses of pheromone applied to the dispenser were the following: variant I – 2 mg, II – 10 mg, variants III and IV – 6 and 20 mg, respectively. Rubber cork was used as dispenser for variants I and III, and spongy material wicks for variants II and IV. A Delta trap with sticky glue insert was used for catching imago of *C. maculatus*. The research was carried out on *Vigna radiata* L. over 26 days (from 8th of August to early September). There were 5 replications and 6 surveys were done during the season. In 2017, an identical trial was set up during the storage period of beans of *Vigna sinensis* L. Walpers. The following doses of pheromone (by variants) were used: I – 2 mg, II – 4 mg, III – 8 mg and IV – 16 mg. There were 6 replications and 5 surveys were done during the season. Two types of traps were used: Delta and "Book" (storage trap type); dispenser with the appropriate dose of synthetic sex pheromone of the four-spotted bean weevil was placed to the center of the traps. Insect monitoring was carried out for 41 days (from 2nd of August to 11th of September). The recording and sampling of insects from traps was made every 10 days from the period of adult emergence and the beginning of insect flight.

Obtained data was processing by statistical methods and differences determined with least significant difference test (LSD).

Results

Field trials

Results of 2014 trial showed that the dynamics of imago flight to traps was extremely low, that was probably due to the low number of beetles in the field. Statistical differences (*LSD test, F_{05}, t_{05}*) between the tested variants were not revealed (Table 1). However, traps with the dispenser of variant II (1 mg of synthetic pheromone per dispenser) showed the highest attractiveness, and can be recommended for quarantine monitoring. Traps with dispensers III and IV (2 mg and 3 mg pheromone on the dispenser, respectively), and control traps (variant I without pheromone application) showed similar results (Table. 1). Variant II with 1 mg of pheromone had the highest results among the selected doses and control (*F*=4,76).

Tab. 1 Number of male insects caught during the period of flight.

variants	pheromone doses	average number of male beetles caught per one trap	significance
I (control)	0 mg	*1.4*	n.s.
II	1 mg	*2.4**	s.
III	2 mg	*1.1*	n.s.
IV	3 mg	*1.1*	n.s.

LSD₀₅ = 0.94

Storage trials

Results of trials in 2015 showed that the largest number of caught male insects recorded in traps were with dispenser IV, with 27.3 individuals per trap during the flight period. Average number of male beetles per one trap in variants I, II and III were 12.7, 18.7 and 10.3 individuals, respectively (Table 2). At the same time, the average number for all variants (x_{avg}) was =17.25 male individuals per trap. The ratio of females (f) and males (m) in different variants was: I – 19 f:m 38, II - 5 f:m 56, III - 10 f:m 31 and IV - 17 f:m 82. The ratio of the total number of caught insects - 207 males and 51 females, i.e. 80.2% and 19.8%, respectively.

A long period of monitoring allowed us to estimate the dynamics of flight and the number of beetles of *C. maculatus*, it was stable and quite high.

Tab. 2 Trials in 2015: Number of male and female insects caught during the period of flight.

variants	pheromone doses	average catching of male beetles per one trap	significance	total number of caught males per one trap	total number of caught females per one trap
I	2 mg	*12.7*	n.s.	38	19
II	10 mg	*18.7*	n.s.	56	5
III	6 mg	*10.3*	n.s.	31	10
IV	20 mg	*27.3**	s.	82	17

LSD₀₅ = 2.58

Statistical data processing has shown that there was a significant difference in insects caught among the tested variants. At the same time, variants I and II had the lowest attractiveness and number of caught insects was at the same level. Thus, for quarantine monitoring of the four-spotted bean weevil we can recommend the dispenser in the form of a spongy material wick with a dose of 20 mg of pheromone per dispenser.

Results of the trial conducted in 2017 did not reveal significant differences in the attractiveness among pheromone traps with tested types of dispensers: nearly the same number of insects was caught in variants with doses of 2, 4 and 8 mg (from 1.6 to 1.9 beetles per trap). The total number of captured insects was 67, of which 64 males and 3 females, representing 95.5% males and 4.5% females. The ratio of female (f) and male (m): I – 1:15, II - 2:12, and III and IV variants caught only

males, 16 and 10, respectively. At the same time average number for all variants was 2.3 individuals per trap.

Tab. 3 Trials in 2017: Number of male and female insects caught during the period of flight.

variants	pheromone doses	average catching of male beetles per one trap	significance	total number of caught males per one trap	total number of caught females per one trap
I	2 mg	2,7	n.s.	15	1
II	4 mg	2,3	n.s.	12	2
III	8 mg	2,7	n.s.	16	0
IV	16 mg	1,7	n.s.	10	0

$LSD_{05} = 8.47$

Most attractive type of trap was Delta with variants IV (8 mg) and I (2 mg). The "Book" type trap caught significantly fewer insects than Delta. Average number of caught insects in "Book" type trap was extremely low (0.3 males per trap for the entire period of flight), while the trap Delta consistently showed high results compared to the latter and the number of caught males on average for all variants was 2.3 individuals, that is 2 times more than for the "Book" trap type.

Traps with variants IV and I caught only males of *C. maculatus*, 16 and 15 individuals respectively. Pheromone dispensers with doses of 8 mg and 2 mg were the best attractive substance for catching insect males. In variants II (4 mg) and V (16 mg), the number of captured males was 12 and 10, respectively. It is possible to make the assumption that under conditions of closed spaces (storages) these dispensers may cause an effect of insect disorientation.

The attractiveness of all pheromone traps used in the trials was high enough throughout the research period; therefore, dispensers were not replaced.

Discussion

Trials were carried out according to the original method developed at All-Russia Plant Quarantine Center. It allowed us to draw a conclusion that the synthetic sex pheromone of the four-spotted bean weevil *C. maculatus* possesses biologically active properties and is attractive for males of this pest species. Based on results of the field trials in 2014, it can be concluded that variant II (1 mg of pheromone per dispenser) showed the highest results among the selected doses ($F=4,76$). It should be assumed that variant II is most effective for attracting individuals of the four-spotted bean weevil into traps in early flight of insects in the field during the ripening of beans.

Evaluation of attractiveness of various doses of pheromone showed the possibility of disorientation of the four-spotted bean weevil in storage during the trial in 2015. At the same time, in a closed room with a constant temperature and humidity the dose of applied pheromone may vary in dependence on dispenser type used in traps. For example, capture efficiency was the optimum when using Delta type traps with a wick and applied pheromone in dose of 20 mg rather than when using an insulin cork as dispenser.

Storage trial in 2017, taking into account the dynamics of male numbers of *C. maculatus* attracted by pheromone, Delta traps with variants 4 (8 mg) and 1 (2 mg) were the best options in terms of attractiveness. In almost all cases, the "Book" trap type attracted significantly fewer insects than the Delta. In all variants, average number of caught insects in the "Book" trap type was 2 times lower than for Delta trap.

A long period of monitoring allowed us to estimate the dynamics of flight and the number of beetles of *C. maculatus*, during the trial it was stable and quite high.

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A Multi-parameter Grain Detection System Based on Industry 4.0

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Abstract

A multi-parameter grain detection system based on industry 4.0 was used to map all kinds of sensors and devices into multiple network addresses through the integration of equipment, to realize the local visual perception and the network transmission of various grain data, using software plug-in architecture technology to build online extension of the software to achieve the corresponding grain multi-parameter monitoring plug-ins; setting sensor and device communication protocol standards to achieve remote monitoring of various grain situation data on the scene equipment Remote debugging and maintenance work to form a remote data center and equipment maintenance center. The system is compatible with a wide range of heterogeneous sensors and devices online and with a high degree of online scalability.

Keywords: grain detection system; Industry 4.0; the integration of equipment

Introduction

The grain detection system is a system that uses modern electronic technology to detect, store and analyze ecological parameters of stored grain. The current system has major problems such as single function, poor compatibility, poor extension capability, and low level of intelligence. The design architecture lacks systemic considerations. It is difficult for different manufacturers and different kinds of data to be integrated in one system. The system integration of different sensors and equipment is difficult and incompatible, and it is unable to achieve integrated collection, control and data transmission, and it is no longer adaptable to new demands.

The stored grain detection system implemented in this paper is an information system under the Industry 4.0 architecture. It relies on the heterogeneous sensor universal access hardware platform and integrated equipment deployed in the field to achieve real-time perception of multiple stored grain condition data and cooperative control field equipment, intelligent system based on this platform can achieve continuous evolution and upgrade of the system, use of big data technology to analyze the correlation relationship between sensor data, accurately extract characteristics of stored grain, and form an online extension and maintenance of the stored grain detection application system, the core of which is compatible with a variety of heterogeneous sensors and devices online, has a high degree of online scalability.

System implementation

Overall design structure of system

The system design adopts the idea of automatic evolution and divides the system into four subsystems: (1) universal access hardware platform and integrated equipment for the front-end granary; (2) software system for the grain depot monitoring center; (3) background data and maintenance center software system; (4) stored grain condition big data analysis application platform. as shown in Fig.1.
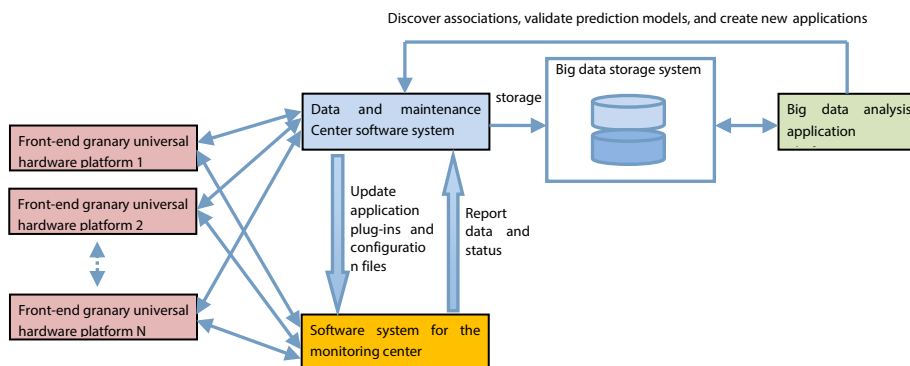
Fig.1. System Design

Each front-end general hardware platform and integrated equipment is installed in one granary, and is responsible for connecting different sensors and control devices according to user needs. Its design adopts the concept of Industry 4.0 and maps various on-site sensors and control devices into independent IP addresses. All sensors and devices can communicate over Ethernet, thus shielding the heterogeneity of various sensor and device communication physical layers. In addition, the heterogeneity of the communication protocols of different devices is packaged and transparently transmitted using the communication protocol packaging technology. This platform can also push data to different data terminals at the same time, and allows legitimate login clients to perform remote device debugging.

The software system for the grain depot monitoring center is a core software platform installed in the local control room of the grain depot. It adopts a full plug-in framework and can be upgraded to adapt to different sensors and devices through automatic plug-in upgrade. The service can be upgraded by authorizing download of the latest application.

The background data and maintenance center software is responsible for receiving and analyzing data from different sensors and electromechanical devices. It has a monitoring function and stores the data in a large data storage system. This software system is developed using a full plug-in framework and the system can adds and updates newly developed plug-ins and pushes the plug-in to the user software system for the grain depot monitoring center that purchased the application.

The stored grain condition big data analysis application platform is used to find out the relationship between sensor data in data analysis, develop prediction models, promote the development of new applications and plug-ins, and provide users with new services.

Design and Implementation of universal hardware platform and integrated equipment

"Universal hardware platform and integrated equipment" includes a general hardware interface platform and a field integrated equipment, as shown in Fig.2.
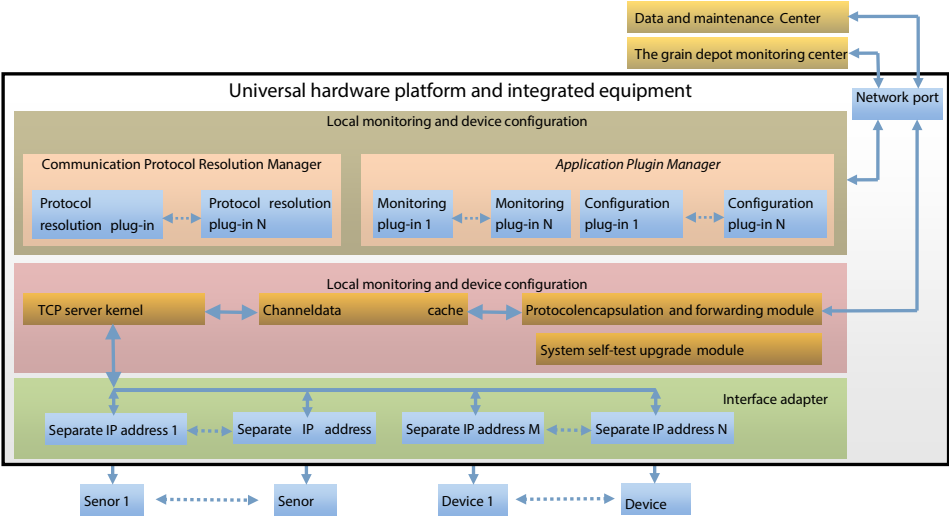
Fig.2. Universal hardware platform and integrated equipment Design

The universal hardware interface platform connects different sensors and control devices according to the user's needs. Its design uses the idea of the Internet of Things to map various on-site sensors and devices into separate IP addresses. All devices communicate through the network. It shields the heterogeneity of communication and physical layers of various sensors and devices, adopts the secondary packaging technology of communication protocols to transparently transmit data of different devices, and facilitates the rapid deployment of new sensors or devices in the future; while taking into account the local monitoring of stored grain conditions The data can also be pushed to different data centers at the same time to form a source node of big data information that can adapt to the future development.

The integrated equipment adopts an integrated industrial control computer running Windows operating system. The application program adopts a framework structure and plug-in mode. The developed application program has strong reliability, flexibility, and compatibility. It realizes the on-site visual display of stored grain condition data and system configuration and high-speed and flexible network data transmission. The software has an online upgrade function.

Software system for the grain depot monitoring center

"Software system for the grain depot monitoring center" is a software platform used by users. It adopts a full plug-in framework to develop and adapt to different sensors and equipments through automatic plug-in upgrade. At the same time, it can remotely apply for authorization to the data center and download the latest ones. The application of stored grain condition is used for online upgrades, as shown in Fig.3.
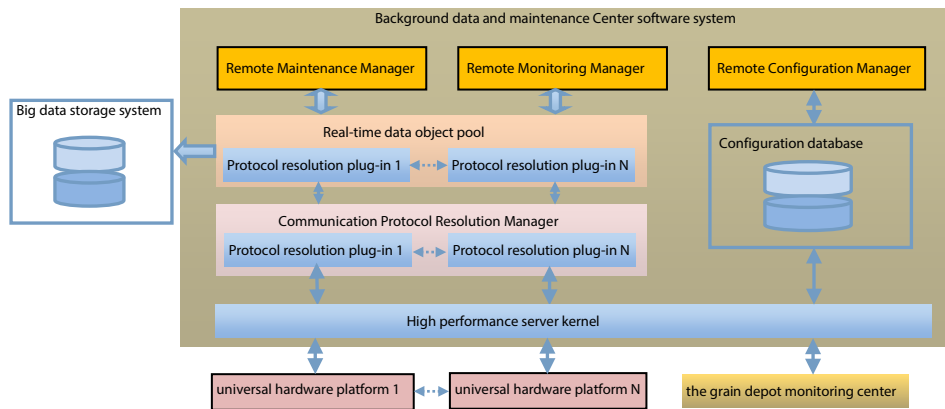
Fig.3. Software system for the grain depot monitoring center
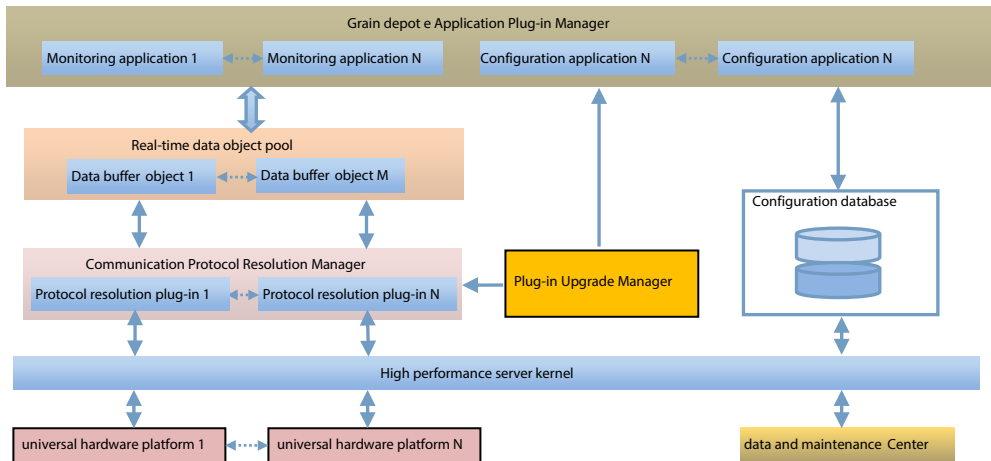
Fig.4. Background data and maintenance Center software system

Background data and maintenance center software system

"Background data and maintenance center software system" is a software platform installed in the data center. It is responsible for receiving and analyzing the transparent transmission data sent from different granaries of each grain depot. The system is developed with a full plug-in framework. Each plug-in can correspond to different field devices. In the data center, all the data information in the grain depot can be remotely monitored, and the equipment can be remotely commissioned and maintained in a transparent transmission mode to form a maintenance center. A variety of new application softwares are developed for application release on this platform; data from grain depot are stored in the big storage system to form an internal data center, as shown in figure 4.

Stored grain condition big data analysis application platform

"Stored grain condition big data analysis application platform" is used to find out the correlations among sensor data in the stored grain condition big data storage system based on hadoop, to develop prediction models, and to promote the development of new applications and plug-ins to form new high-additions to users. The value service is delivered to users through the " Background data and maintenance center software system ".

Conclusion

The system adopts a universal network platform design. Upgrading the front-end sensors and equipment will not affect the system. It only requires the development of relevant interface plug-in dynamic links. The system software of the data and maintenance center is developed by the plug-in architecture, and the system function expansion only needs to replace or add different dynamic connection blocks, which has good scalability. With the universalized front-end design, the integration process for installing or upgrading different sensors or devices will be standardized. With remote on-line device debugging capabilities, and the system is very maintainable. The system provides data mining tools based on historical data, finds the relationships among data, develops prediction models, and optimizes configuration information. A sustainable and intelligent evolutionary system is finally formed, which can generate value for users for a long time.

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Global establishment risk of stored products beetles

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Abstract

Stored-product beetles were regarded as some of the most important stored-product pests in the world. Predicting which one in hundreds of potential invasive stored-product beetles is the most likely to invade a region presents a significant challenge. A global presence/absence dataset, including 201 economically significant stored beetles in 143 countries/regions, was analysed using a Self-Organizing Map (SOM) to categorize regions based on similarities in species assemblages. This method is able to rank these stored-product beetles based on risk of establishment indices (values between 0 and 1). From the six countries/regions selected from each continent, we can have an overview of the global invasive risk of this group of beetles. We also found that those countries geographically close were clustered together by the SOM analysis because they have similar beetle assemblages and therefore represent greater threats to each other as sources of invasive stored-product beetles.

Keywords: stored-product beetles, Coleoptera, self-organizing map, establishment risk

The stored-product beetles (Coleoptera), include more than 300 species in 40 families and cause about 85% of stored pest damage (Zhang et al., 2015). These taxa are of quarantine significance since the beetles are usually small in size, have a broad host range, and have a high reproductive ability and dispersal capacity, and, in addition, the grain depot can offer a stable environment and abundant food for the establishment of alien species. For example, *Trogoderma granarium* originated from Asia, was first reported in California in 1953, where it caused 220 million dollars in loses amounting to 10% of income from agricultural products (Chu et al., 2008). Predicting which one in hundreds of potential invasive stored-product beetles is the most likely to invade a region is of significant importance for global trade policies such as China's 'Belt and Road' program.

A Self-Organising Map (SOM) (Kohonen, 1982), which is a type of Artificial Neural Network (ANN), has been used in the past to simultaneously rank and prioritize a large number of invasive species by their likelihood to establish in a region (Cereghino et al., 2003; Worner and Gevrey, 2006; Paini et al., 2010; Paini et al., 2011; Morin et al., 2013; Singh et al., 2013; Qin et al., 2015). We initially extracted the distribution data from the Crop Protection Compendium (CABI, 2018), Pest China dataset and monograph. Subsequently results of the presence (1) or the absence (0) of each stored beetles in each geographical area in the database comprised a 201×143 matrix (201 species in 143 countries). The analysis was performed by using Matlab and SOM Toolbox (version 2.0) (Laboratory of Information and Computer Science, Helsinki University of Technology, http://www.cis.hut.fi/projects/somtoolbox/). SOM indices were then extracted for all stored beetles for each country/region of the world.

Establishment likelihood lists of all the 201 beetles were generated for all 143 countries included in the analysis. The top 10 ranked species, which are currently absent in each country were extracted from the full lists of SOM indices and we present the top ten ranked species for six countries (China, USA, Nigeria, Chile, Italy, and Australia) (**Tab. 1**). We also examined how the SOM clustered the countries identifying which countries have the most similar stored beetle assemblages (**Fig. 1**). All 143 countries/regions were clustered into 66 neurons. We noted that many of the countries clustered together by the SOM analysis were also geographically close to each other, which suggests a species present in a country will be of greater threat to a neighboring country that is in the same cluster.

A SOM analysis could be used as an initial screening process to reduce the large numbers of potential invasive species to a more manageable number (Paini et al., 2011), the SOM indices for stored beetles currently absent from a country could be used to guide debate on which species should be listed for national surveillance needs to achieve early warning. More importantly, the SOM indices could provide a first screen of the beetles prior to going through more systematic risk analysis (Morin et al., 2013).
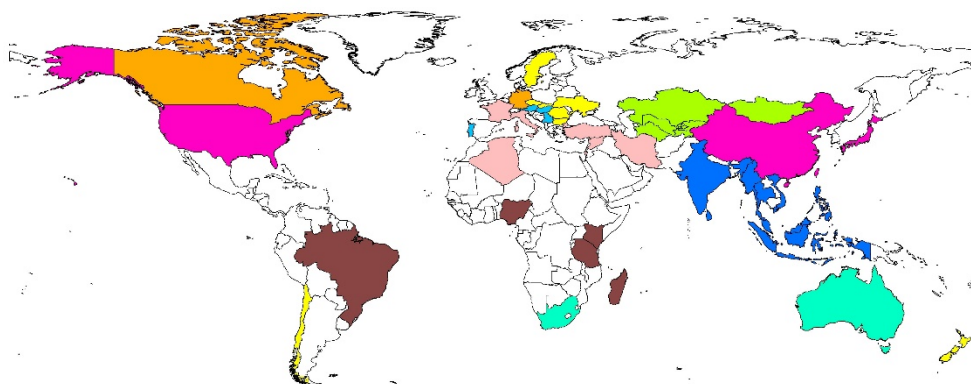
Fig. 1 Map of world showing country clustering (same color) based on stored-product beetle assemblages that were allocated to the same neuron in a SOM analysis and hence those countries that have the most similar stored beetle assemblages.

Tab. 1 Top 10 stored-product beetles species for each of six countries, representative of each continent (except for the Antarctic) that are currently absent but have the highest likelihood of establishment if introduced.

China	SOM Index	Nigeria	SOM Index	The United States	SOM Index
Trogoderma inclusum	0.57	*Callosobruchus analis*	0.77	*Gibbium aequinociale*	0.49
Dinoderus bifoveolatus	0.42	*Cylas formicarius*	0.76	*Dermestes coarctatus*	0.41
Callosobruchus ademptus	0.41	*Sinoxylon conigerum*	0.71	*Dermestes tessellatocollis*	0.41
Carpophilus mutilatus	0.37	*Urophorus humeralis*	0.65	*Dermestes vorax*	0.41
Reesa vespulae	0.35	*Gibbium aequinociale*	0.49	*Dermestes vorax var.albofasciatus*	0.41
Trogoderma angustum	0.35	*Dinoderus minutus*	0.40	*Dermestes freudei*	0.41
Bruchus brachialis	0.33	*Hylotrupes bajulus*	0.38	*Attagenus unicolor japonicus*	0.41
Trogoderma anthrenoides	0.31	*Cryptamorpha desjardinsii*	0.37	*Anthrenus nipponensis*	0.41
Bruchus signaticornis	0.31	*Xylopsocus capucinus*	0.37	*Orphinus japoonicus*	0.41
Prostephanus truncatus	0.29	*Minthea rugicollis*	0.37	*Atholus depistor*	0.41

Chile	SOM Index	Italy	SOM Index	Australia	SOM Index
Tenebroides mauritanicus	0.12	*Bruchus signaticornis*	0.48	*Attagenus unicolor simulans*	0.72
Hylotrupes bajulus	0.90	*Bruchus affinis*	0.47	*Orphinus japoonicus*	0.68
Tribolium castaneum	0.46	*Gibbium aequinociale*	0.44	*Bruchus rufipes*	0.65
Sitophilus oryzae	0.39	*Cryptolestes pusillus*	0.40	*Pseudeurostus hilleri*	0.64
Trichoferus campestris	0.32	*Bruchidius incarnatus*	0.33	*Mesomorphus villiger*	0.59
Trogoderma granarium	0.29	*Bruchidius trifolii*	0.33	*Thorictodes erraticus*	0.54
Cryptolestes pusillus	0.29	*Sapronus subnitescens.*	0.30	*Carpophilus delkeskampi*	0.53
Anthrenus polonicus	0.28	*Thorictodes heydeni*	0.28	*Cryptolestes ugandae*	0.42
Reesa vespulae	0.26	*Phradonoma nobile*	0.26	*Bruchidius terrenus*	0.36
Oryzaephilus mercator	0.19	*Ptinus clavipes*	0.23	*Holoparamecus signatus*	0.35

Acknowledgement

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Session 4

Engineering for Stored Product Protection and Pest Prevention

Bin coring: a simple practice for improving aeration performance and saving energy

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Abstract

The coring operation consists of removing the center portion of the grain mass, or core of the silosilo, to improve airflow distribution. Additional benefit of this practice is the elimination of a significant portion of the fine material, which is a source of fungal inoculum and feed for insects. The effect of coring on airflow distribution through a grain mass has been previously addressed, but the effect on energy savings was not fully quantified. Thus, the goals of this reseach were: 1) to quantify the airflow increase due to the coring operation of a silosilo full of wheat; and 2) to quantify the reduction on fan runtime and energy consumtion due to improvement in airflow distribution and airflow increase after coring. The effect of coring on airflow was quantified using the AireAr software, and the effect on aeration efficiency was studied through simulation using a specialized software (PHAST-FDM). For levels of coring (0%, 3%, 5% and 8% of total grain mass) and four levels of non-uniformity of airflow (center side difference) (30, 20, 10 and 0) were considered. Results indicated that the coring operation reduced the total time to achieve cooling, number of fan run hours, and fan power consumption. The main effect of the coring operation was the increase in specific airflow (up to 45% increase). Energy savings increased with coring, obtaining savings of 11%, 28% and 30% for 3%, 5% and 8% of coring, respectively. It was concluded that coring the silosilo by unloading from 3 to 8% of the stored grain mass is a recommendable practice, because it increases the specific airflow rate and airflow uniformity, reduces fan run hours and generates energy (and cost) savings.

Keywords: airflow resistance, airflow uniformity, simulation, fine material.

Introduction

One of the most frequent problems in storage facilities is the accumulation of fines in the center (core) of the silosilo. Fine material is defined as pieces of broken grains, foreign matter and weed seeds. Fine material occupies the void spaces in the grain mass, reducing the porosity of grain and increasing airflow resistance (Grama et al., 1984; Haque et al., 1981). When loading a silosilo through the center of the silosilo, fine material tends to concentrate in the center of the grain mass and increasing the airflow resistance in the core. Consequently, air velocity and specific airflow are lower in the core than in the periphery of the grain mass.

The coring operation is one of the most simple and recommended practices for improving the storability of the grain mass. The coring operation consists in removing the center portion of the grain mass, or core of the silosilo. When unloading a silosilo from the center opening in the floor, the first grain to come out is the grain of the core of the silo, which also contains most of the fines concentrated in that location of the grain mass (Bartosik and Maier, 2006). Removing most of the fines from the silo not only improves the airflow distribution, but also reduces the risk of developing insects and molds in that area or the silo.

Bartosik and Maier (2006) measured the concentration of fine material and air velocity at the center and periphery of the grain mass for 15 on-farm natural air/low temperature (NA/LT) in-silo corn drying and conditioning experiments. It was observed that the accumulation of fine material in the core was up to 232% higher than at the periphery. This accumulation of fines at the core of the silo resulted in non-uniform airflow distribution. It was observed that, on average, there was 74% more

airflow at the side (close to the silo wall) than at the center of the silo (ranging from 24 to 222%). Simulation was used to study the effect of non-uniform airflow caused by fine material accumulation at the center of the silo and the grain peak produced after loading the silo. They concluded that operators of NA/LT in-silo drying systems could reduce drying costs from 25 to 33% by leveling the grain peak after loading the silo. Additional reductions in drying costs from 18 to 22% could be achieved by installing effective grain spreaders or by coring the grain mass. Later, Lawrence and Maier (2011) developed a non-uniform airflow model using the finite volume method to predict air velocity for cored, peaked and leveled grain mass configurations.

Coring silos for long term storage, even though a known practice among elevator managers, it is not consistently implemented. Typically, during coring from 3 to 8% of the grain mass is unloaded. Cardoso et al. (2008) evaluated the fine material distribution in wheat silos and the effect on airflow. They found that unloading about 3% of the grain mass was required to remove most of the fines. Additionally, they concluded that the coring operation can increase not only the airflow uniformity but also the total airflow in the silo.

Simulation was used in the past to quantify the effect of fine material and non-uniform airflow on the performance of natural air/low temperature in-silo drying systems (Bartosik and Maier, 2006) with the PHAST-FDM model. However, not sufficient information was generated about the improvement in airflow distribution due to the coring operation, amount of grain to be unloaded during coring, reduction of the cooling time during aeration, and potential energy consumption reduction derived from this best management practice.

Thus, the goals of this reseach were: 1) to quantify the airflow increase due to the coring operation of a silo full of wheat; and 2) to quantify the reduction on fan runtime and energy consumtion due to improvement in the airflow distribution and airflow increase after coring.

Materials and Methods

Airflow estimation

The metal silo considered for this study had a cone botton with 30° inclination, 8.5 m diameter and 10 m heigh to the eave, with a centrifugal aeration fan of 2 HP (1.49 KW) (Chicago Blower, SQDA Agro-200 – 1410 RPM). The grain considered for the study was wheat at 14% moisture content (m.c.) and test weight of 76 kg/hl (0.76 t/m^3).

The effect of the coring operation on total airflow was evaluated with the AireAr software (http://online.inta.gov.ar:8080/aireAr/mainMenu). This software compares the performance curve provided by the fan manufacturer with the system airflow resistance curve computed with the Shedd equation and the set of parameters provided in the ASABE standard for wheat (ASAE, 1999) (a= 8,410, b= 2.72, Multiplier = 1.2) (Fig. 1), and estimates the resistance and the total and specific airflows in the silo (Bartosik et al., 2009).
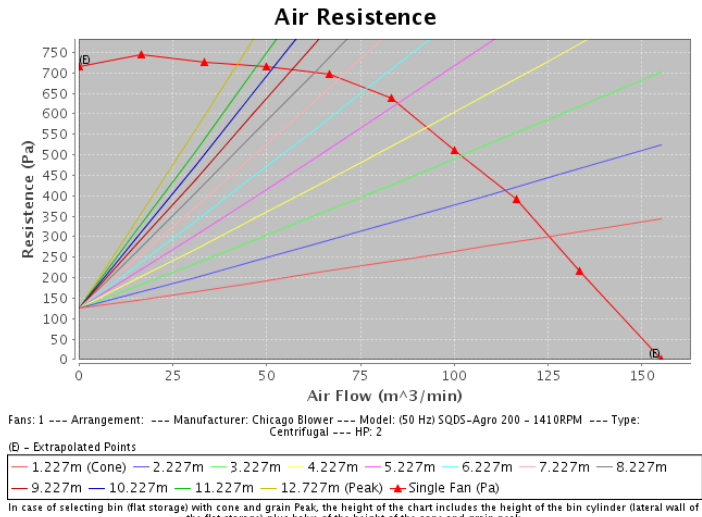
Fig. 1. Screen capture of the AireAr software showing the fan performance curve and the airflow resistance curves for the base condition.

The base silo configuration, before coring, had a grain peak of 3.0 m at the center with a total capacity of 509.6 t, and a multiplier of 1.2 was considered in the Shedd equation to account for the packing effect of the grain on airflow resistance. Three coring alternatives were evaluated (3%, 5%, 8%), which resulted with different differential height in the grain peak and different amounts of stored wheat. Additionally, the Shedd equation multiplier was proportionally reduced according to the coring percentage to account for the "loosening" effect in the grain mass, which reduces airflow resistance (Table 1).

Table 1. Height differential of the grain peak at the center of the silo, amount of unloaded grain and amount of remaning grain in the silo before and after coring.

Parameter	Base condition (Before coring)	After coring		
		3%	5%	8%
Shedd multiplier	1.2	1.1	1.05	1.0
Peak heigh differential (m)	3	1.95	1.2	0
Amount of grain (t)	509.6	494	484	468
Amount of unloaded grain (t)	-	15.6	25.6	41.6

Aeration simulation

The Purdue Post-Harvest Aeration and Storage Simulation Tool – Finite Difference Method (PHAST-FDM) is a numerical model that solves the heat and mass transfer during in-silo drying and conditioning in two dimensions (x, y) (Bartosik, 2005). To solve the problem of non-uniform airflow rate through the grain mass, PHAST- FDM simulates two grain columns: one for the core, and the other for the periphery. The heat and mass transfer equations are solved independently for each column, and the model assumes no interaction between them. PHAST-FDM accepts different non-uniformity factors (NUF) for airflow rates that can be entered by the user. The NUF was defined as the center-periphery difference with respect to the average airflow rate: (airflow periphery – airflow center / [(airflow periphery + airflow center) / 2] × 100). For instance, an NUF of 30% for an average airflow rate of 1 m/s indicates that the airflow rate is 0.85 m³/(min t) at the center of the silo and 1.15 m³/(min t) at the periphery. The PHAST-FDM model with the center-periphery differential airflow rates was validated for predicting MC changes in different grain layers for several on-farm NA/LT in-silo drying tests (Bartosik, 2005; Bartosik and Maier, 2006).

The initial average conditions for the cooling aeration simulation were 14% m.c. and 30°C temperature. It was assumed that the wheat was harvested on January first at Balcarce, South-East Buenos Aires province, Argentina. The final condition for the simulation was achieved when wheat reached 18°C average temperature and 19°C maximum temperature. The PHAST-FDM model was supplied with hourly temperature and relative humidity data of 13 different years. The silo considered had the same configuration as decribed in the previous section. The simulated aeration strategy turned on the fan whenever the ambient temperature was below 18°C. The airflow was obtained with the AireAr software as described in the previos section for four different coring percentages (0 (before coring); 3; 5 and 8%). Four NUF levels were considered (30 (before coring); 20; 10 and 0). A NUF of 30 represents the situation before coring, in which the difference in airflow between the center and periphery of the gran mass was the greatest, while a NUF of zero means that the airflow distribution was completely uniform (perfect coring).

Table 2. Combinations of specific airflow rates obtained for different coring percentages and non-uniformity factors considered for the aeration simulations with the PHAST-FDM model.

Non-Uniformity Factor	Coring percentage			
	0	3	5	8
30	X *	-	-	-
20	-	X	X	X
10	-	X	X	X
0	-	X	X	X

* Base line corresponding to the silo condition before coring

Energy savings

Energy savings due to the coring operation was computed taking into account the fan electrical power consumption (kWH) and substracting the electrical power needed to unload the silo for the coring operation. Fan power consumption was obtained by multiplying the fan runtime hours obtained for each simulation condition by the fan power (kW). For computing the electrical power consumption related to coring (unloading the silo) it was assumed a silo unloading auger of 5.5 kW and a bucket elevator of 11 kW were used with a conveying capacity of 60 t/h. The coring operation time was estimated as 0.0, 0.26, 0.49 and 0.63 hours for coring conditions of 0, 3, 5 and 8%, respectively, and the corresponding power consumption values were 0, 4.29, 7.04 and 11.44 kWH.

Results

Airflow estimation

Before coring, the silo full of wheat had a total capacity of 509.6 t, with a grain peak of 3 m at the center. Under that condition, airflow resistance against the fan was 720 Pa, which resulted in a total airflow rate of 42.1 m^3/min. Thus, before coring, the specific airflow was 0.083 m^3/(min t). The coring operation increased the specific airflow in three ways. First, after coring there is a lower grain depth, which reduces the airflow resistance and, hence, the total airflow provided by the fan increases. Second, the "loosen" effect of the coring further reduces the airflow resistance, which also increases the total airflow. Third, as the total amount of grain in the silo decreased, the specific airflow increased. Table 3 shows the total airflow, speficic airflow and airflow resistance for the different configurations considered in the study.

Aeration performance

The speficic airflows obtained in Table 3 were used as input in the aeration simulation runs carried out with the PHAST-FDM program for the four coring levels. Table 4 shows the average results of 13 years of simulation for each evaluated condition (coring % from 0 to 8%, and NUF from 30 to 0). In the base situation (before coring and NUF of 30), the total time to complete cooling from 30°C to less than 18°C was 1055 h (44 days). The accumulated fan runtime was 307 hours (fan was "on" 29%

of the time), and the aeration power consumption was 457.4 kWH. The final grain condition was 13.7% m.c. and 17.6°C.

Table 3. Total airflow provided by the fan, specific airflow and static pressure in the aeration system before coring and after different coring percentages.

Parameter	Base condition (Before coring)	After coring		
		3%	5%	8%
Total airflow (m³/min)	42.1	47.6	51.4	55.9
Specific airflow (m³/(min t))	0.083	0.095	0.108	0.12
Airflow resistance (Pa)	720	716	713	708

As the percentage of coring increased, the total time to complete cooling and the fan runtime hours decreased, while the energy savings regarding the base situation (no coring) increased.

Coring 3% of the grain mass resulted in a reduction of the total time to complete cooling to 909 hours and fan runtime hours to 269 hours. The aeration power consumption was 400.8 kWH, while the electrical power consumed by the unloading auger and the bucket elevator for the coring operation was 4.29 kWH. This resulted in an average energy saving of 52.3 kWH or 11% of the base condition.

For a coring percentage of 5%, the total time to complete cooling was reduced to 761 hours and fan runtime hours to 216 hours. The resulting aeration power consumption was 321.3 kWH, while the electrical power consumed by the unloading auger and the bucket elevator for the coring operation was 7.04 kWH. This resulted in an average energy saving of 129.0 kWH or 28% of the base condition.

For a coring percentage of 8%, the total time to complete cooling was further reduced to 727 hours and fan runtime hours to 207 hours. The resulting aeration power consumption was 308 kWH, while the electrical power consumed by the unloading auger and the bucket elevator for the coring operation was 11.44 kWH. This resulted in an average energy saving of 138.1 kWH or 30% of the base condition.

As the resulting airflow after coring became more uniform (NUF decreased from 30 to 0), the total time to complete cooling and the fan runtime hours decreased, while the energy saving regarding the base situation (no coring) increased. Across all percentages of coring, fan runtime hours and energy savings regarding the base condition (no coring and NUF of 30) for a NUF of 20 were 234 hours and 22.1%, respectively, while for a NUF of 0 (no airflow difference between center and side) the fan runtime decreased to 228 hours and the energy saving increased to 24.2%.

Table 4. Results of the PHAST-FDM simulation runs showing the time to complete cooling, fan runtime, aeration power consumption, and average final moisture content and temperature, and the computed coring electrical consumption and total energy saving due to coring for the four coring levels evaluated.

Coring	NUF	Time to complete cooling (hs)	Fan runtime (hs)	Aeration power consumption (KWH)	Coring power consumption (kWH)	Energy saving (kWH)	Final average m.c. (%)	Final average temp. (°C)
Before (0%)	30	1055	307	457.4	0	0	13.7	17.6
3%	20	916	272	405.2	4.29	47.8	13.7	17.5
	10	909	269	400.8	4.29	52.3	13.7	17.5
	0	902	266	396.3	4.29	56.8	13.7	17.6
	Avg	909	269	400.8	4.29	52.3	13.7	17.5
5%	20	770	217	323.3	7.04	127.0	13.7	17.6
	10	757	215	320.3	7.04	130.0	13.7	17.6
	0	757	215	320.3	7.04	130.0	13.7	17.6
	Avg	761	216	321.3	7.04	129.0	13.7	17.6
8%	20	752	213	317.4	11.44	128.6	13.7	17.5
	10	717	205	305.4	11.44	140.5	13.7	17.5
	0	713	202	301.0	11.44	145.0	13.7	17.6
	Avg	727	207	308	11.44	138.1	13.7	17.6

Discussion

The coring operation has a main effect of increasing the specific airflow for aeration. As a portion of the grain is unloaded, the total depth of grain is reduced, the path of the air through the grain mass is shortened, and the airflow resistance is reduced. An additional reduction in airflow resistance is obtained by the "loosening" effect of the grain mass. As a result of the reduction of airflow resistance, the fan total airflow increased (Table 3). The airflow increase depends on the characteristics of the fan (fan performance curve shape) and the operational condition of the fan. For instance, if the aeration fan has a performance curve that does not change much with static pressure (e.g., a high speed centrifugal fan), then the reduction in airflow resistance due to coring will have little effect on total airflow, and vice versa for an axial fan (with a fan performance curve that changes with static pressure). In addition to the increase in the total airflow, the specific airflow also increased due to the reduction in the amount of grain after coring. In this study, the increase on specific airflow was estimated up to 45%. Cardoso et al. (2008) reported an increase of 63% in the measured airflow after 3% of coring a silo with 700 tonnes of wheat.

The simulation of the effect of coring on airflow performance showed that the coring operation reduced the total time to achieve cooling, the fan runtime hours, and the fan power consumption. The reduction in fan power consumption was achieved through the reduction in the fan runtime hours. The electrical power consumption of the unloading auger and bucket elevator for coring the silo was always lower than the savings achieved, implying that coring always has an economical benefit (Table 4). The energy saving increased with coring, obtaining an energy saving of 11%, 28% and 30% for 3%, 5% and 8% of coring, respectively. Based on these results, 5% of coring was the most convenient, because this amount of coring had the larger marginal benefit in energy savings.

The improvement of airflow uniformity after coring also reduced the fan energy consumption, although to a lesser extent. For a NUF of 20 the total energy savings was 22.1% (across all coring percentage levels) while for a NUF of 0 (no airflow difference between center and side) the energy savings only increased to 24.2%. This implies that the main benefit of coring was through the increase in the specific airflow.

Additional benefts of coring, besides energy savings, also must be considered. A reduction in the time for achieving cooling objectives has consequences reflected in the final quality of the grain. For instance, total cooling time for the base condition was 1055 hours, while 5% of coring with a NUF of 0 shortened the total cooling time to 757 hours. Shortening cooling time by 12 days may provide important benefits preventing insect development (Navarro and Donahaye, 2005). Additionally, coring removes a significant proportion of the fine material from the silo (Cardoso et al., 2008), and fine material was reported to have higher mycotoxin concentration than whole grain (Abbas et al., 1985).

Thus, coring the silo by unloading from 3 to 8% of the stored grain is a recommendable practice, because it increases the specific airflow and airflow uniformity, reduces fan runtime hours and generates energy (and cost) savings. Additionally, by reducing the cooling time and eliminating the fine material reduces the risk of insect development and mycotoxins formation.

Acknowledgement

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Application of transverse ventilation in grain storage in China

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Extended abstract

In China, mechanical ventilation technology has been researched and applied since the 1950s. Beginning in 1998, large-scale grain warehouses started to be built with national government support. The mechanical ventilation technology, namely the "four-in-one" technology, was promoted enormously during this period. In the "four-in-one" system, the aeration technology was based on the vertical aeration system with ventilation ducts temporarily fixed on the floor of the warehouse. The airflow passed vertically through the grain bulk from the bottom to the surface or vice versa with air being pushed by fans, and the heat and moisture from the grain exchanged with the air during vertical aeration. This vertical ventilation system has been widely used for the last twenty years, but it is complex and inconvenient, and also air distribution is uneven.

To fix these problems, Chinese researchers developed a new transverse ventilation technology as shown in Fig. 1. In this system, aeration ducts are mounted along the opposite interior walls of the warehouse and air travels horizontally through the grain mass. A large number of pilot scale tests and warehouse applications have been done from 2010 to 2014.
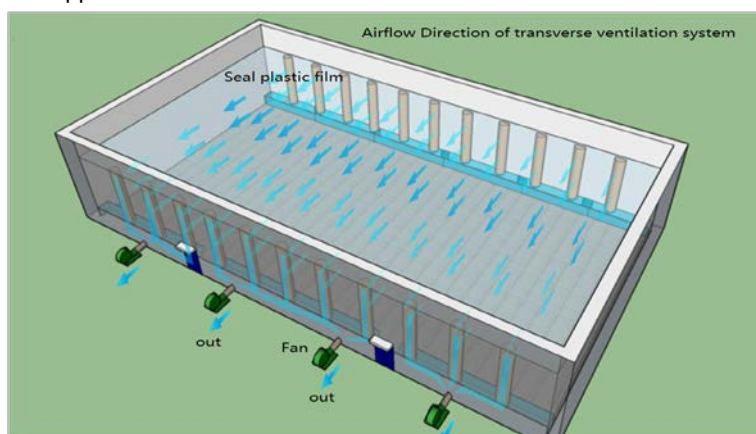
Fig. 1 The new transverse ventilation system.

The grain surface is sealed by plastic film during storage to prevent air from escaping through the surface layer during aeration and gas during fumigation. During aeration, the airflow is sucked from

one side of the aeration ducts and exhausted out the fans after horizontally passing through the grain bulk. With nearly five years of application, it has been demonstrated that the storage technologies in this new ventilation system, such as aeration, grain cooling, fumigation and controlled atmosphere treatment can be done effectively, ande grain moisture loss during ventilation can be reduced by 0.3-0.5 percentage points. Alos, the efficiency of loading and unloading grains can be increased by 100% as compared to the vertical ventilation system because on-floor ducts do not need to be removed during the unloading process.

Therefore, application of the granary transverse aeration system will obtain better economic and operational benefits as summarized in Tab. 1.

Tab. 1 Evaluation of vertical and transverse ventilation system.

No.	Evaluation index	Vertical ventilation	Transverse ventilation	Remark
1	Ventilation uniformity	80-85%	90-95%	Increase of 10%
2	Percent of moisture loss during ventilation	0.7-1.0%	0.2-0.3%	Reduced by 3-5 times
3	Capacity of grain load/unload/hour	50 t/hour	>100 t/hour	Increase of 100%
4	The load/unload cost of per ton	5.0 ¥/t	3.0 ¥/t	Reduced by 40%
5	Labor cost	high	low	Reduced by 50%
6	Depreciation expense	high	low	Reduced by 20%
7	Labor intensity	high	moderate	
8	Mechanization level	low	high	

Until now, the transverse ventilation system has been applied in more than twenty provinces throughout China, and the quantity of stored gain has reached 3 million tons of warehouses storage capacity that is equipped with the new transverse system.

Technical and Economic Evaluation of Ambient and Chilled Aeration Strategies to Maintain the Quality of Paddy Rice During Storage in a Tropical Climate

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Abstract

Warm and moist conditions of some tropical climate regions make it difficult to use ambient aeration to cool stored grain, which contributes to pest problems and increases dependence on chemical control as part of grain management strategies. Grain chilling is a non-chemical alternative to cool grain stored under high risk climatic conditions. The objective of this research was to use computer simulation to evaluate the technical and economic viability of using grain chilling compared to four ambient aeration strategies developed for paddy rice stored under the tropical climatic conditions of the North Pacific coast of Costa Rica. The minimum grain temperature achieved through ambient aeration at the end of the six-month simulated storage period was 30.8°C, using an aeration strategy based on a grain-ambient temperature differential greater than 10°C. Grain chilling lowered the average grain temperature from 35°C to below 15°C in 117 hours and the maximum average temperature it registered after six months of storage was 15.5°C. The economic evaluation of the ambient aeration and chilling strategies determined that the operational costs of grain chilling were 1.83 US $/t lower than ambient aeration plus chemical control of pests. However, the initial cost of the grain chiller made the net present cost (NPC) of the grain chilling strategy 0.22 US $/t higher than the cost of ambient aeration plus

chemical control over a 10-year analysis. Several potential financial options were analyzed to make the grain chiller economically feasible for a rice miller in Costa Rica.

Keywords: Paddy rice, ambient aeration, grain chilling, economic feasibility.

Introduction

The conditions of high temperature and relative humidity (RH) during most of the year in most tropical climatic regions limit the cooling capacity of ambient grain aeration. This is why in this climate ambient aeration is used mainly to maintain the grain temperature and moisture content (MC) in equilibrium with the average ambient conditions, which avoids the development of hot spots and prevents condensation on walls and roofs (Lawrence and Maier, 2011; Noyes and Navarro, 2002).

A limited number of research studies have come up with strategies that give viable options for aeration in tropical climates. One of these studies was presented by Sinicio and Muir (1998), in which they determined that using a difference of 6°C between the average grain and ambient temperature, at an airflow between 0.08 and 0.16 m³/min/t, provided the best storage conditions for wheat during eight month storage (only 0.1% shrink loss) under Brazilian conditions.

Aeration during night time or early morning hours has also been considered a technically viable option for tropical climates since lower temperatures during these hours have reasonable cooling effect on stored grain (Monroy and Valencia, 1978; Recio, 1999). However, the risk of rewetting grain is a restriction for using lower temperatures in this latitude, but according to Noyes and Navarro (2002), the RH is usually lower in the plenum due to the heat of compression produced by the aeration fans. According to Noyes and Maier (2002), for every ~248 Pa (1 in. of water column) of static pressure (SP) that is generated in the aeration system, the temperature of the air passing through the aeration fan can increase by ~0.5°C (1°F). According to Zeledon and Barboza (2000) (unpublished), the RH inside the plenum can be between 6 and 18 percentage points drier than the ambient air.

Grain chilling is an alternative to ambient aeration that allows cooling of grain under 20°C in weather conditions where otherwise it would not be possible. This helps limit or stop completely insect population growth (Fields, 1992). This technology has proven to be effective for cooling grain to below 17.5°C in relatively short periods of time (80-300 hours) in silos between 500 and 5000 metric tons (t), located in tropical regions of Argentina, Brazil and Israel (Calderon, 1972; Lazzari et al., 2010; Roskopf and Bartosik, 2009).

The high purchase price of grain chillers and the lack of economic studies that complement the technical studies has limited the implementation of this technology more widely in some tropical regions. One of the only studies that has made an effort to evaluate the true value of this kind of investment in the long term was developed by Rulon et al. (1999), in which they analized the economic feasibility of a grain chilling prototype developed by Purdue University using the Net Present Cost (NPC) methodology that analyzes the net cost of an investement through its life cycle. This study demonstrated that the grain chilling technology was highly competitive compared to the cost of using ambient aeration plus chemical control.

The obejctive of this resesearch study was to use computer simulation to evaluate the technical and economic feasibility of using grain chilling compared to four ambient aeration strategies developed for paddy rice stored under the tropical climatic conditions of the North Pacific coast of Costa Rica.

Materials and Methods

Ambient aeration and grain chilling computer simulation model

The ambient aeration and grain chilling strategies were analyzed using a finite element computer simulation model adapted from Lawrence and Maier (2011) and based on the storage conditions of paddy rice in the North Pacific region of Costa Rica, also called Guanacaste. For the development of

the computer model, five years of weather data (2010-2014) during the storage period of paddy rice in this region (November to May of next year) were collected. The initial conditions of the paddy rice were determined at 13% MC and 35°C, assuming it would go into storage directly from the dryer, and the physical properties such as bulk density, porosity, and thermal properties which were retrieved from ASABE standards D241.4 and D243.4.

The storage structure used in the computer model was a corrugated steel silo of 1500 t (diameter-to-height ratio of 1.0), which is commonly used for long term storage in this region. The aeration system of these silos consisted of one 20 HP centrifugal fan and a perforated false floor. Using these specifications, the airflow rate of the ambient aeration fan was determined to be 0.22 m³/min/t (~0.2 cfm/bu) and the static pressure (SP) produced by the aeration system was determined to be 2070 Pa (~8.3 inches of water column) (Dickinson and Morey, 2013). This SP would cause an increase of the aeration air of approximately 5°C according to Noyes and Maier (2002) which was accounted for in the ambient aeration simulations.

Based on the analysis of the climatic conditions of the region and the structural conditions of the storage structure, the following ambient aeration strategies were proposed:

Run ambient aeration fan when ambient temperature is less than or equal to 24°C and ERH in the plenum is less than 70%.
Run ambient aeration fan from 6:00 a.m. to 8:00 a.m. and from 5:00 p.m. to 7:00 p.m.
Run ambient aeration fan from 5:00 a.m. to 9:00 a.m. and from 5:00 p.m. to 9:00 p.m.
Run ambient aeration fan whenever ambient temperature is 10°C lower than grain temperature in the top section of the grain mass.
The grain chilling strategy was programmed to start the grain chiller as soon as the paddy rice entered the silo and continue the cycle until the top section of the grain mass reached 15°C. The input data for the development of this strategy was collected from field trials developed on wheat storage in Kansas, U.S.A., during the summer of 2015 and 2016 (Morales-Quiros, 2017). The grain chiller used for these trials has a rated capacity to cool 100 to 170 t of grain per 24 hours of continous operation on silos of up to 1800 t, according to the manufacturer (Coolseed, 2016).

Net Present Cost economic model

The cost of the ambient aeration strategy with the best results from the previous section, based on fan run hours, MC, and final grain temperature, was compared with the cost of the grain chilling strategy using the NPC methodology developed by Rulon et al. (1999). The NPC economic model calculated the net cost of the investment over its life cycle (10 years for the grain chilling equipment), using factors like annual interest rate, tax rate, rate of return on equity and percent of business financed by debt. This information was collected from financial entities in Costa Rica.

The NPC of the ambient aeration strategy was calculated based on the power requirement of the aeration fan, maintenance labor, sampling labor and shrink loss. Due to the fact that it is not possible to control pests only with ambient aeration in this region, the cost of this strategy included fumigation cost, insecticide application, personnel safety equipment cost, application labor, among others. This information was collected from agrochemical companies and local rice milling industries.

The NPC of the grain chilling strategy was calculated based on factors like purchase price of the grain chiller (US $74700, according to the manufacturer), power requirement, installation and maintenance labor, sampling labor and shrink loss. Additionally, financial options for making the grain chilling technology feasible for the Costa Rican rice miller were also analyzed. Some of these alternatives were a leasing option, improving the capacity of the grain chiller, and premium sale price of paddy rice treated with the grain chilling technology. This information was compiled from previous field experience, financial entities and local rice milling companies.

The NPC calculations were based on a hypothetical rice milling company with six silos of 1500 t of paddy rice each, which is the average for the region, stored for six months.

Results and Discussion

Ambient aeration and grain chilling strategies

The results of the computer model demonstrated that it is possible to use low temperature, high RH air to aerate paddy rice since the temperature increase of approximately 5°C in the plenum will reduce the RH by approximately 20percentage points. This means that it is possible to use ambient air of up to 90% RH because in the plenum the RH will decrease to 70%. Similar observations were made by Zeledon and Barboza (2000).

The first ambient aeration strategy (Ambient temp. ≤24°C, plenum RH <70%) only reduced the average of the 5-year average temperatures of the grain mass by two degrees (35°C to 33°C), without noticeable MC variation.

The second and third ambient aeration strategies (2 and 4 early morning and night time fan run hours, respectively) showed adverse results because the temperature of the grain mass essentially remained unchanged but the average of the 5-year average MC was reduced dramatically due to the lack of restrictions for the conditions of ambient air that could be used in these strategies. The number of fan run hours were also excessive in these strategies (729 and 1458 hours, respectively). For this reason, the period of aeration was limited to between November and January of the storage period, which are the months with the lowest minimum temperature of the year. This helped to reduce the average of the 5-year average of rice temperature to approximately 33°C, without noticeable MC variation. This modification also reduced the fan run hours to 312 and 624 hours, respectively.

The fourth strategy (grain-ambient temp. difference ≥10°C) was the one that reduced the average of the 5-year average paddy rice temperature the most by the end of the six-month storage period, down to 30.8°C. This strategy increased the average of the 5-year average MC by 0.1% and required the least amount of fan run hours (214 ±43 hours) among all ambient aeration strategies analyzed (Fig. 1).
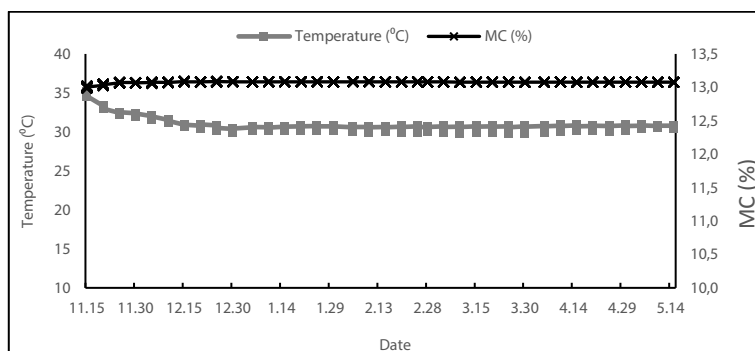
Fig. 4 Five-year average of the average grain temperature and moisture content (MC) profile of paddy rice stored from Nov. 15th to May 15th in Guanacaste, Costa Rica and aerated using Strategy 4.

The computer simulation of the grain chilling strategy showed that the average of the 5-year average grain temperature was reduced from 35°C to below 15°C in 117 hours of active chilling, and remained below 15.5°C for the six months of storage. Nevertheless, the average of teh 5-year average paddy rice MC increased by 0.2 percentage points with this strategy (Fig. 2).

Preserving paddy rice at low temperature demonstrated to be effective at controlling *R. dominica* and *Sitophilus* spp. for 60 days of storage in Brazil (Lazzari et al., 2006).
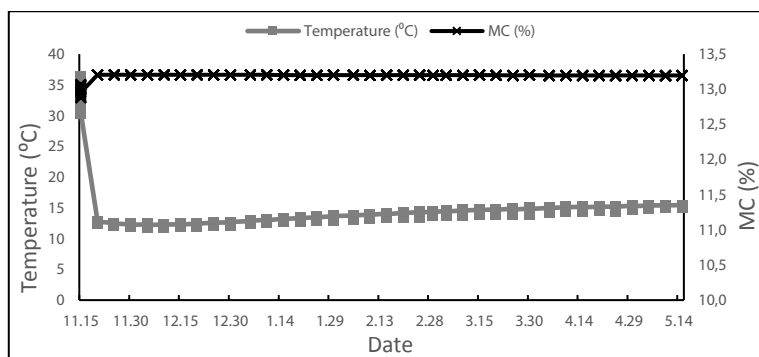
Fig. 5 Five-year average of average grain temperature and moisture content (MC) profile of paddy rice stored from Nov. 15th to May 15th in Guanacaste, Costa Rica and aerated using the grain chilling strategy.

NPC economic analysis

Since the fourth ambient aeration strategy was the one that required the least amount of fan run hours, and thus resulted in highest energy savings, and was also the one that maintained the lowest grain temperature throughout the six months of storage among all ambient aeration strategies, it was chosen for the NPC economic analysis. Its feasibility was compared against the feasibility of the grain chilling strategy.

The NPC economic analysis showed that, although the operational costs of running the ambient aeration fan in the fourth ambient aeration strategy were low, the added cost of the chemical control of pests increased the annual operational costs of this strategy up to 2.36 US $/t. On the other hand, the annual operational cost of running the grain chiller was only 0.53 US $/t, given that preserving the paddy rice at temperatures below 20°C in a climatic region, where otherwise it would not be possible, replaces the need for chemical control. Similar observations were made by Rulon et al. (1999).

Although the grain chilling strategy predicted to reduce the annual operational costs of the Costa Rican rice milling company, the high initial investment of the grain chilling equipment (US $74700) increased the total NPC of this strategy. It resulted in an annual amortized NPC of 1.51 US $/t, while the annual amortized NPC of the fourth ambient aeration strategy plus chemical control was 1.29 US $/t (Fig. 3), i.e., 14.5% lower.
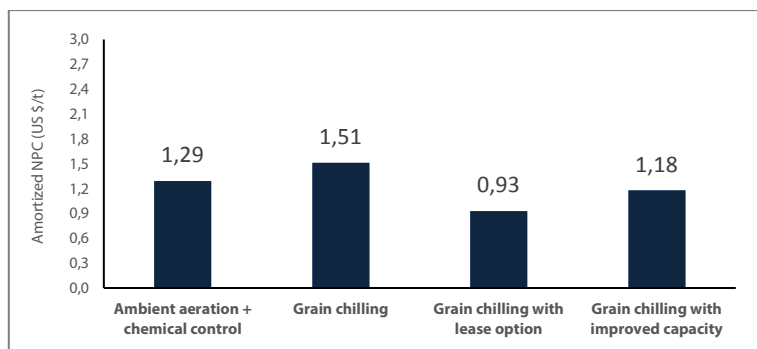
Fig. 6 Amortized Net Present Cost (NPC) of ambient aeration Strategy 4 and the grain chilling strategy with financial options for reducing the NPC.

In order to lower the NPC of the grain chilling strategy a leasing option was analyzed. It showed that leasing the grain chilling equipment for an annual rate lower than US $11,000 for a 10-year period

(assumed useful life time of the equipment), would reduce the NPC of chilling from 1.51 to 0.93 US $/t (Fig. 3), or -38.4%. Although this option would increase the annual opertional cost of the grain chilling strategy from 0.53 US $/t to 1.77 US $/t due to the addition of the annual leasing payment, this cost would still be lower than the operational cost of the fourth ambient aeration strategy plus chemical control, i.e., 2.36 US $/t.

Another feasible option for financing the grain chiller, according to the NPC economic analysis, was to increase the capacity or number of tons treated with the grain chilling technology, which would dilute the cost per ton of the grain chilling strategy. This analysis showed that increasing the number of silos of 1500 t treated with the grain chilling technology from six to eight would reduce the amortized NPC from 1.51 US $/t to 1.18 US $/t, i.e., -21.9% (Fig. 3). This seems like an achievable quantity for the rice milling industry of Costa Rica since there is usually more than one harvest per year. The rice companies are receiving paddy rice basically all year, which would justify the purchase of the grain chiller given that it would be utilized throughout the year, lowering the NPC further.

A third financial option for the grain chilling strategy, according to the NPC economic model, would be to sell the rice treated with the grain chilling technology as a value-added product because it would be free of residues from postharvest pesticides. If it were possible to incease the sale price of this product by US $0.50 to US $1.00 per ton, this would reduce the amortized NPC of the grain chilling strategy below 1.29 US $/t (amortized NPC of the ambient aeration plus chemical control strategy).

Conclusions

The ambient aeration strategy based on a grain-ambient temperature differential of 10°C or higher showed the best results on final grain temperature, moisture content and fan run hours; nevertheless, it was not possible to reduce the average temperature of the paddy rice below 30.8°C by the end of the six-month storage under the tropical conditions of Guanacaste, Costa Rica. On the other hand, the grain chilling strategy reduced the average grain temperature below 15.5°C in less than five days, and paddy remained below this temperature for the rest of the six-month storage period. This would potentially reduce insect populations and eliminate the requirement for chemical control.

The grain chilling strategy reduced the annual operational costs of the Costa Rican rice milling company, according to the NPC economic analysis, but the high initial cost of the grain chilling equipment made the amortized NPC of this strategy higher than the amortized NPC of the ambient aeration strategy plus chemical control. The leasing option of the grain chilling equipment at a reasonable price, increasing the capacity (number of tons treated) of the grain chiller, or charging a premium sale price of the paddy rice treated with the grain chilling technology, are all feasible options for reducing the amortized NPC of the grain chiller.

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CHILLING TEMPERATURE AND LOW MOISTURE CONTENT TO KEEP SOYBEAN GRAIN QUALITY DURING STORAGE

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Abstract

Soybeans are used as food, feed, oil and fuel. Losses may happen at harvesting, transportation, and mainly during storage. Moisture content (MC %) and temperature (T °C) of the soybean grain mass during storage are the main factors affecting quality, quantity and value of the product by favoring the development of microorganisms and insects. Large grain chillers have been used to maintain soybean quality and reduce insect infestation during storage. To evaluate the effect of MC and temperature on the quality parameters of soybean seeds, samples were stored at 58±2% RH, with five different MCs, at 15 °C (chilling temperature) and 30 °C (average temperature inside silos in Brazil) for 180 days. The following was observed: reduction in the MC at higher temperature; the weight of soybeans was maintained at either temperature when the MC was at about 12%; MC above 14% reduced the weight value independent of storage temperature; at 15°C the weight of 1,000 seeds was maintained during storage; low MC and temperature kept germination and vigor of the seeds at high rates; low MC and temperature reduced electrical conductivity; there was no noticeable influence of the storage temperature, regardless of the MC of the beans, on the free fatty acid content. In general, quality attributes tend to be reduced during storage, being more remarkable at higher temperature and MC of the seeds. In conclusion, the temperature of 15ºC, which simulates grain cooling conditions, favors the maintenance of quality, quantity and value of soybean for long-term storage.

1. Introduction

Soybean is one of the most important crops in Brazil and worldwide. It is used as food, feed, oil and fuel. Quality and quantitative losses in soybeans may happen during harvesting, transportation, and mainly in storage. Moisture content and temperature of the soybean mass during storage are the main factors that affect quality, quantity and value of the product.

The main cause of weight loss in stored soybeans is consumption of the dry matter (starch, proteins and fats) by storage fungi (Christensen and Meronuck, 1986). Lazzari (1997) stored soybeans for six months at 15 and 25 °C and water content varying from 13.9 to 22.1% wet basis (% w.b.). He concluded that the higher the temperature and the water content, the greater the fungi infection and consequently the dry matter loss which could range from 0.24 to 1.25% at 15 °C and from 0.39 to 36.6% at 25°C.

Teixeira (2001) mentions low temperature associated with drying of soybeans, allows a longer storage time without compromising quality during this critical period. Teixeira (2001) also mentions that grain with moisture content between 16 and 18.5% (% w.b.) can be stored safely for 3 to 18 months at cooling temperatures of 3 to 10°C, inhibiting the development of fungi, insects and the germination loss of seeds. It is important to consider that artificial cooling can be a cost effective alternative to aeration with ambient air. Considering the benefits, cooling soybean kernels during storage might be a valuable technology to reduce postharvest losses, although the effects of low temperatures and different water contents of soybean seeds need more complete evaluation.

In order to determine the benefit of cooling technology on the quality of soybean seeds, samples were stored at 58+2% RH, with five different water contents, at two temperature levels. The following parameters were evaluated: 1. variation in water content, 2. seed weight, 3. apparent specific mass, 4. weight of 1,000 kernels, 5. electric conductivity, 6. germination, 7. accelerated aging and 8. fat acidity.

2. Materials and Methods

The experiment was carried out in the Preprocessing and Storage of Vegetable Products laboratory, Department of Agricultural Engineering, University of Viçosa (UFV), Minas Gerais, Brazil. A 2 x 5 factorial experimental design was implementd in two climate-controlled chambers: one at 15°C simulating the cooling condition, and the other at 30± 2°C simulating storage temperature conditions prevalent in most areas of Brazil. The relative humidity was of 58+2% in both chambers. The subplots were five water content levels: 12, 14, 16, 18 and 20%, and five storage intervals (0, 45, 90, 135, and 180 days), for seven parameters evaluated, with three replicates.

Soybean seeds from the BIOAGRO/UFV experimental units were used, with initial water content of 22% (% w.b.). The seeds were dried in a fixed bed dryer, with air heated to 40°C using a LPG burner. The gravimetric process was used to obtain the water content of soybeans at 20, 18, 16, 14 and 12%. The variation of the mass of the evaporated water during drying from the initial water content was estimated by separating the fractions to obtain each of the desired levels. The final water content was measured after the seeds were in equilibrium at room temperature expressed as percentage wet basis (% w.b.). The soybean samples were packed in plastic bags measuring 0.40 x 0.45 m, with a capacity of 5 kg, and stored under the defined conditions, from April to October 2011.

Water content, weight of one thousand seeds, germination and accelerated aging were measured according to the methodology described in Brazil (2009). The electrical conductivity in the solution containing the soybean seeds was made using the "Glass System" (Vieira & Carvalho, 1994). The specific mass was determined using a weight scale with a capacity of 1 liter. The ethereal extract and total titratable acidity were performed according to the methodology described by Silva (2002).

3. Results

The temperature of the seeds remained practically uniform in most samples, with variations below 4°C at different points. The data indicate that the natural convection of the intergranular air was

minimal, reducing the mass transfer (water vapor) between the grains and the intergranular air and promoting stability in the water content of the grain (Fig. 1).

Water Content

At 15°C and 58+2 % RH, the values of the initial water contents were 12.6, 12.4, 15.9, 17.2 and 19.7% (Fig. 1A). After 180 days of storage, the water contents were 12.9, 12.8, 15.8, 17.0 and 19.7%, showing that seeds with water content lower than 13% suffered small increments in their humidity values, while those containing initial water contents of 15.9 and 19.7% did not present moisture content alteration (Table 1).

At 30°C, the initial water contents were 11.5, 13.6, 15.9, 17.8 and 20.0%. After 180 days of storage, the respective water contents were 11.2, 13.3 and 15.0%, without noticeable variation between the initial and final moisture contents. As expected, seed samples with the initial water contents of 17.8 and 20.0% were totally deteriorated by fungal activity at 90 days of storage (Fig. 1B and Table 1).
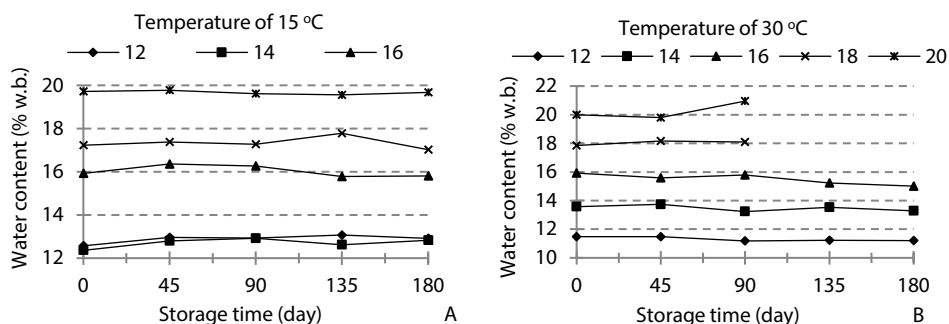
Fig. 1 Water content of soybean seeds stored at 15°C (A) and 30°C (B) for 180 days at 58+2% RH.

Seed Weight

For the seed weight variation (Table 1), it was found that at 15°C moisture gain was obtained for the drier seeds (12.6 and 12.4% initial water contents). Seeds with water contents equal to or greater than 15.9% lost water during the storage period.

Total soybean loss was found in the product stored at 30°C when its initial water contents were 17.8 and 20.0%. There was a slight gain in moisture when the product was stored with initial water contents of 12.6 and 12.4% at 15°C. Moisture losses of 24.4 kg t^{-1} and 21.4 kg t^{-1} occurred at 30°C with the initial water contents of 11.5 and 13.6%, respectively.

Apparent Specific Mass

When storing at 15°C, the highest value of apparent specific mass was 690.2 kg m^{-3} and the lowest was 636.6 kg m^{-3} for the seeds with initial water content of 12.9 and 19.7%, respectively. It was observed that soybeans with a lower water content had the specific mass unchanged after 90 days of storage (Fig. 2A). For soybeans with the other water contents, variation of the specific mass values was observed, which could be attributed to the tendency of adjustments related to hygroscopic equilibrium. However, soybeans with the initial water content of 19.7% had the specific mass reduced from 650.6 to 636.1 kg m^{-3}, indicating a mass loss for this qualitative attribute and that, even at the temperature of 15°C, this water content was too high for storing soybeans for 180 days. Storage fungi could grow in soybeans with moisture content above 16% and temperature of 15°C after 90 days in storage.

The results of the apparent specific mass reduction of the soybean seeds at 30°C with water contents ranging from 11.5 to 20.0% are shown in Fig. 2B. The seeds with water contents of 17.8 and 20.0% were badly degraded after 90 days in storage. The lowest observed value of the specific mass was 637.6 kg m^{-3} at 90 days of storage, when soybean seeds were infected by fungi of different species. The highest value was 691.7 kg m^{-3}, when soybeans had a water content of 11.2% at 135 days of storage.

Tab. 1 Initial and final water content, mass alteration and weight variation of soybean samples at 15°C and 30°C, at five levels of moisture content for each temperature, stored at 58+2% RH for 180 days.

Temperature (°C)	Water content (%w.b.)		Mass change (weight%)	Weight variation (kg t^{-1})
	Initial	Final		
15	12.6	12.9	(+) 2.70	(+) 27.0
	12.4	12.8	(+) 3.72	(+) 37.2
	15.9	15.8	(-) 0.81	81.6
	17.2	17.0	(-) 1.16	11.6
	19.7	19.7	(-) 0.25	2.5
30	11.5	11.2	(-) 2.43	24.4
	13.6	13.3	(-) 2.13	21.4
	15.9	15.0	(-) 5.71	57.2
	17.8	-	(-) 100.0	Total
	20.0	-	(-) 100.0	Total
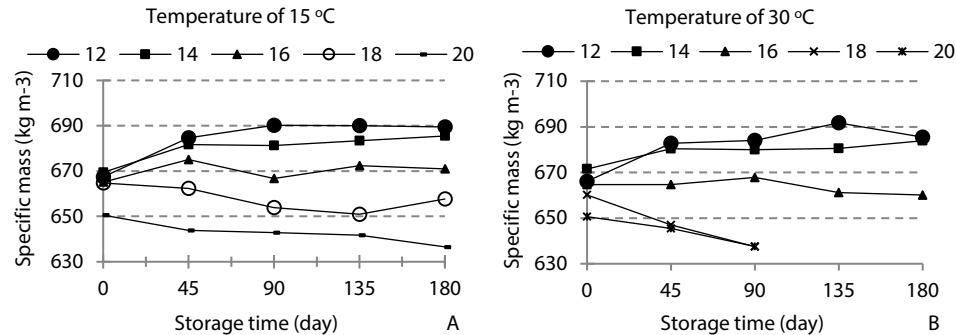
Fig. 2 Apparent specific mass (kg m^{-3}) of soybean with different water content stored at 15°C (A) and 30°C (B) for 180 days at 58+2% RH.

Weight of 1,000 Soybean Seeds

At 15°C, after 180 days the weight of 1,000 soybean seeds was between 188 and 181.8 g for the seeds with water contents between 16 and 20% (Fig. 3A). At 30°C, the weight was between 200 and 180 g for the samples between 16 and 20%, by the 90th day (Fig. 3B). In the beginning of storage, the weight of 1,000 seeds with water contents of 16 and 20% were of the order of 195 g and those with moisture of 12, 14 and 18% had a similar weight of 175 g. After 180 days of storage, only the seeds with a water content of 18% experienced weight reduction, resulting in a range of 180 to 185 g.

For the soybean samples stored at 30 °C, smaller dispersions were observed in the values of the weight of 1,000 seeds with the different water contents. However, these values were lower, ranging from 170.0 to 193 g, as compared to the samples stored at 15°C, after 180 days.
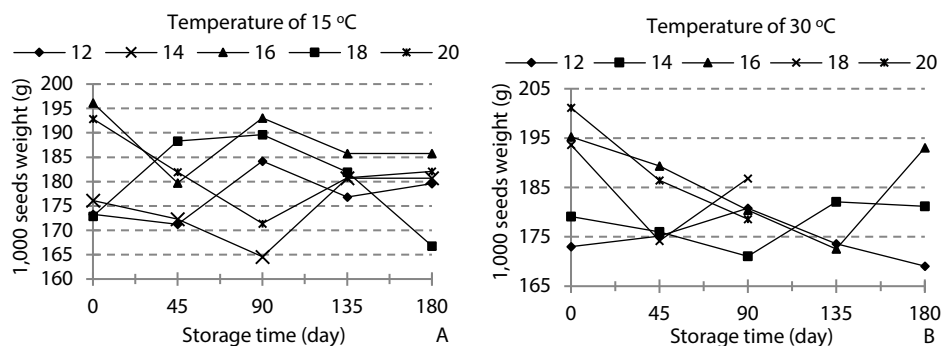
Fig. 3 Weight of 1,000 soybean seeds with five different water contents (% w.b.) stored at 15ºC (A) and 30ºC (B) for 180 days at 58+2% RH.

Electrical Conductivity

The average values of the electrical conductivity of soybeans stored at 58+2% RH, 15ºC for 180 days, with water contents between 12 and 20%, ranged from 72.6 and 80.0 μS cm^{-1} g^{-1}, respectively (Fig. 4A). At 30ºC, the average values ranged from 96.5 to 95.1 μS cm^{-1} g^{-1} (Fig. 4B).

It was observed during the storage period at 15°C that there was an increase in the electrical conductivity values of the order of 60 to 100 μS cm^{-1} g^{-1} (Figure 4A). For a water content of 20%, this value reached close to 140 μS cm^{-1} g^{-1}, indicating greater degradation of the product with higher water content after 180 days of storage. In soybean stored at 30°C, the damage was more intense at higher water contents. At a water content of 16%, the electrical conductivity ranged from 56.1 to 322 μS μS cm^{-1} g^{-1}. For a water content of 12%, the variation was from 68.8 to 141 μS cm^{-1} g^{-1}, and for a water content of 14%, it ranged from 62.0 to 191. μS cm^{-1} g^{-1}.

Germination

The average value of the germination index for soybean seeds stored at 15°C with a water content of 12% was above 98.7% from zero to 180 days of storage (Fig. 5A). With 14% water content, the variation was from 100 to 97.3%; at 16% it was from 99.3 to 91.3%; at 18% it varied between 99.3 and 88.7%, and at 20% between 100 and 55.3%. After 90 days of storage, there was a reduction in the germination index of the seeds with water content of 20%, from 100 to 86.7%.

In soybean seeds from the same samples stored at 30°C with the same water contents, higher deterioration rates were observed as compared to storage at 15°C. It was observed that after 45 days of storage the germination index of soybean seeds with water content of 18 and 20% reduced from 98.7 to 74% and from 100 to 24.7%, respectively (Fig. 5B). After this period, seeds with these water contents were totally degraded. The samples with 16% water content had the germination index reduced from 100 to 25.3% by the 90th day and to 0% by the 135th day. At 14%, the reduction in the germination index was from 99.3 to 10.7%, and at 12% from 100 to 92.7%,by the 180th day.
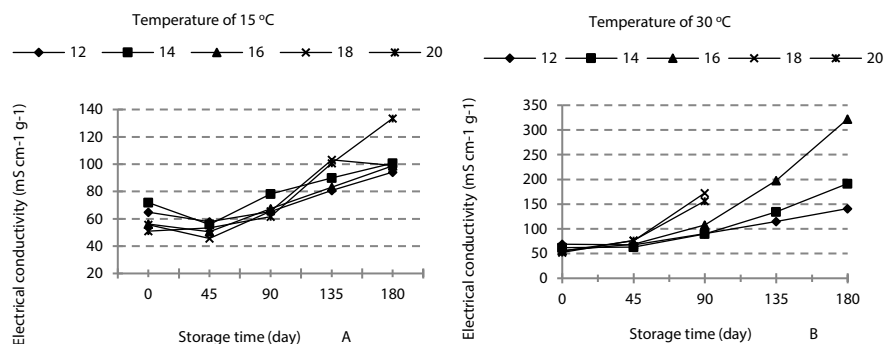
Fig. 4 Electrical conductivity of soybean seeds with different water contents (% w.b.) stored at 15°C (A) and 30°C (B) for 180 days at 58+2% RH.
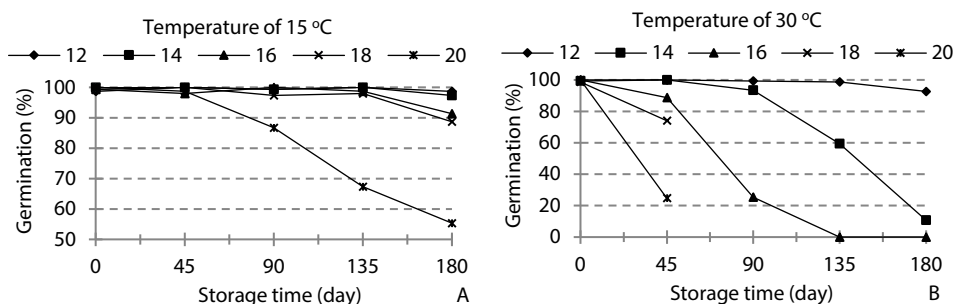
Fig. 5 Germination of soybean seeds with different water contents (% w.b.), stored at 15°C (A) and 30°C (B) for 180 days at 58+2% RH.

Accelerated aging

Figures 6A and 6B show the deterioration of soybean seeds with different water contents measured by the vigor index (accelerated aging) when stored at 15 and 30°C.

For storage at 15°C (Fig. 6A) the higher the water content, the higher the seed degradation index. At 12%, the accelerated aging index varied between 100 and 99.3%; at 14% it stabilized at 100%; at 16% it was reduced from 97.3 to 77.3%; at 18% it was reduced from 99.3 to 72.7%, and at 20% it was reduced from 99.3 to 44.7%.

At 30°C for the same variety and water content, the seed degradation rate increased as compared to storage at 15°C. Reduction in the vigor index from 99.3 to 39.3% and from 98.7 to 9.3% was observed for the 18 and 20% water contents, respectively. At 45 and 90 days of storage the vigor index decreased further to 0%. For water content of 16%, the reduction was from 100 to 5.3% at 90 days, and to 0% at 135 days of storage. At 14%, the vigor reduction was from 99.3 to 68.7% at 90 days of storage, and to 0% at 135 days. At 12%, the reduction was from 100 to 63.8% after 180 days of storage.
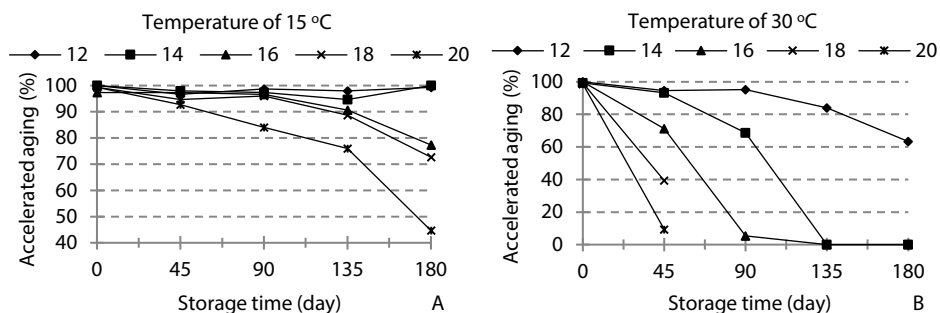
Fig. 6 Accelerated aging of soybean seeds with different water content (% w.b.) stored at 15ºC (A) and 30°C (B) for 180 days at 58+2% RH.

Fat acidity

There was little variation in the fatty acid content during the entire storage period at 15°C, ranging from 0.94 to 2.32% at the beginning of the storage period. These acid contents had a noticeable increase for both temperatures after 135 days of storage. There was an accentuated decrease from about 4.0 at 135 days to about 1% at 180 days. At 30°C, variation between 0.48 and 1.2% was observed for soybeans stored at water contents between 12 and 16%. The seeds stored at 18 and 20% water contents were spoiled after 90 days of storage. Influences of temperature and water content on the fatty acid content were not observed.

Figures 7A and 7B show the variations in the fatty acid index of soybeans stored at different water contents and temperatures.
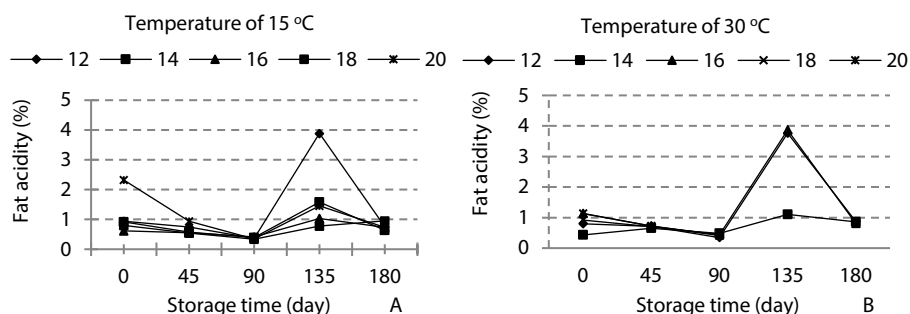
Fig. 7 Fat acidity of soybean seeds with different water content (% w.b.) stored at 15ºC (A) and 30°C (B) for 180 days at 58+2% RH.

4. Discussion

The results showed that 15°C for each water content was a more adequate storage condition than 30°C for maintaining the initial seed characteristics including the sample with the highest water content. The higher temperature resulted in a greater reduction in water content of soybeans during storage. According to Christensen and Meronuck (1986) and Lazzari (1997), the higher the temperature and the water content, the greater the fungi infection and the resulting increase of dry matter loss and grain deterioration, as observed here in our experiment.

Our results demonstrated that for the same equilibrium relative humidity conditions at 15°C, the mass losses due to drying were lower than those at 30 °C. The specific mass values of soybeans stored at 15 and 30°C can be maintained when their water content was low (12 to 14%). For water content above 14%, there was a reduction in the specific mass values, independent of the storage

temperature. The main cause of the weight loss in stored soybeans was consumption of dry matter (starch, proteins and fats) by storage fungi (Christensen and Meronuck, 1986; Lazzari, 1997).

The higher the apparent specific mass value of soybeans, the lower its water content. The apparent specific mass of 12% soybeans is considered to be on the order of 750 kg m^{-3}. In our tests, after 90 days in storage soybeans with water content of 17% and above were so badly degraded due to infection by microorganisms that it was impossible to carry out the laboratory tests. On the other hand, the samples with lower water contents and lower temperature had the expected variation in the values of the specific mass indicated in the literature. Our results show stability of this quality attribute during 90 days at 15°C. However, a longer storage time of about 135 days resulted in lower apparent specific mass caused by fungi growth, despite the lower temperature.

According to Brazil (2009), the weight of 1000 soybean seeds varies according to their water content. Storage temperature of 15°C maintained the mass of 1000 seeds with a weight value higher than at 30°C. The variations observed in our tests, considering the studied range, may indicate the influence of possible dispersion of the values of water contents of individual soybean seeds in relation to the average value observed, and even a certain independence of the weight of 1000 kernels in relation to the water content. Petter et al. (2014) found mean values of 146 ± 14.2 g while in the study of Morais et al. (2014) they were in the range of 159.8 to 178.1 g, which are lower than those observed in the present study. These differences can be attributed to the agronomic characteristics of the varieties studied and the moisture content variation of individual soybean seeds (Lazzari, 1997).

The electrical conductivity test can be considered an auxiliary resource to assess early aging and possible damage to cell walls, allowing ionic solutions to be formed as a function of cell leakage. At both temperatures, the electrical conductivity increased with increasing storage days, indicating loss of soybean quality. For healthy soybeans, the values may vary depending on the variety studied; however, in the same variety, an increase in temperature and water content of soybeans results in greater damage to the cell walls of the seed. Researchers observed values of 56 µS cm^{-1} g^{-1} for the "Embrapa 48" soybean and 46 µS cm^{-1} g^{-1} for the "Paradise" variety. Low values of electrical conductivity indicate low leakage and consequently high physiological quality. The higher the temperature and water content of soybeans, the greater the increase in electrical conductivity and the resulting physiological damage, as observed in our tests, agreeing with Woodstock, cited by Simoni (2007), who mentions that seeds stored at low temperatures have less tissue deterioration.

Due to their sensitivity, the results of the germination test showed the importance of reducing the temperature and water content of the seeds in order to carry out storage safely, aiming at the physical, biochemical, nutritional and sanitary aspects of these seeds. Germination can be influenced by temperature, water content and length of storage. Under the same storage condition and for the same variety, the increase in water content resulted in a reduction in germination index at the end of the storage period for both temperatures, but it was considerably more accentuated at 30°C. Thus the lower the storage temperature, the higher the rate of germination and vigor during storage of soybean seeds. However, according to Lazzari (1997), even cool soybean seeds can be spoiled if stored with high water content.

The vigor index (accelerated aging) is another quality attribute that can be used to verify the physiological degradation of seeds. It was observed, at both storage temperatures, that there was a qualitative loss during storage as a function of the higher water content of the product. The higher the water content, the higher the seed degradation index. Our results indicate that soybeans with 14% is a moist product for storage in the natural environment. It was observed that for the same variety, even with low storage temperature, physiological degradation of the seeds may occur due to the higher storage water content. Thus, at a given temperature, high water content tends to reduce germination and vigor of the seeds. Also, time of storage reduces those two important parameters for stored seeds.

Another attribute of great importance for evaluating the quality of soybeans during storage is the acidity index. Soybeans are the main oil source for human consumption in Brazil and there is a maximum acidity limit for the commercialization of the product. In our tests, regardless of the water content of the seeds there is no noticeable influence of the storage temperature on the acidity of the soybean fat. The behavior of this parameter did not follow an expected pattern. According to Christensen and Kaufmann (1969), the vigorous development of fungi and their lipases at a specific moment of the deterioration of the seed increases the free fatty acids value. This explains the drop observed in our graphs, which could be the result of the consumption of portions of the fatty acids by fungi.

Overall, quality, quantity and value attributes of stored soybeans tend to reduce with storage time, being more remarkable at higher temperature and higher moisture content. One can conclude that the temperature of 15ºC, which simulates grain cooling conditions, favors quality maintenance of soybean seeds within a range of water content considered safe for storage. This range should be below 14%, because at or above this level of water content the soybean seeds are considered a moist product and may deteriorate during the storage period, unless the seed mass is stored under cooled conditions.

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Assessment of a mobile solar biomass hybrid dryer for insect disinfestation in dried maize grains

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Abstract

Considerable losses of stored food grains occur through insect infestation in tropical countries because climatic conditions are conducive for insect activity throughout the year. Studies have shown that in order to kill stored grain insects of all life stages temperatures above 50℃ are required. However, grain simply laid in the sun or placed in a solar dryer does not reach such high temperatures. This study describes the use of a 1 tonne batch capacity mobile solar biomass hybrid dryer for disinfestation of infested maize and prevention of F1 progeny emergence in stored maize grains. To assess the effect of temperature and exposure period on mortality of maize weevils, infested maize in experimental cages were exposed for 3 and 6 hours of thermal disinfestation

treatment in the dryer. Comparing the heat generated in the dryer under hybrid mode operation where additional heat is generated by a biomass furnace in addition to solar, a mean temperature of 67°C was recorded compared to a mean ambient temperature of 36°C. Results showed that there was no significant difference (p < 0.05) in mortality of maize weevils during disinfestation treatment for 3 and 6-hour exposure periods. Mortality of 100% was obtained for samples disinfested in the highest tray (level 4) in the dryer. After 30 days of storage of disinfested maize grains, there was no emergence of F1 progeny from the maize grains exposed for 3 and 6 hours. Effect of ambient temperature and open sun exposure periods in the control set-up resulted in low mean percent mortality. Also, samples from the control set-up at both 3 and 6-hour exposure periods showed emergence of F1 progeny after storage. From this study, it can be concluded that an exposure period of 3 hours (or perhaps even less) in the solar biomass hybrid dryer could prevent damage by *Sitophilus zeamais* to stored maize grains after thermal disinfestation at a mean temperature of 67°C.

Keywords: Mobile solar biomass hybrid dryer; disinfestation; maize weevil, mortality,

Introduction

Maize is mostly destroyed by insects such as the maize weevil (MW), *Sitophilus zeamais* and the larger grain borer (LGB), *Prostephanus truncates*. Maize at harvest usually contains too much moisture (20-25%) which is a favourable environment for the growth of fungi and infestation of insects that normally cause grain damage (Folaranmi, 2008).

In Ghana, postharvest losses of maize occur in both the major and minor season which covers the period of April-August or Septmber and Septmber-December, respectively, especially in the middle belts of Ghana (Opit et al., 2014). Quantitatively, losses at harvest may be as high as 20% by weight of grains harvested by an average Ghanaian farmer (Ofosu, 1995 cited in Seidu et al., 2010). The major physiological, physical and environmental causes of postharvest losses are crop perishability; mechanical damage; excessive exposure to high ambient temperature, relative humidity and rain; contamination by spoilage fungi and bacteria; invasion by birds, rodents, insects and other pests; and inappropriate handling, storage and processing techniques (World Bank, 2011).

On the global scale, it is estimated that over two million tonnes of grains are destroyed annually by insects, moulds, rodents, birds and other pests (FAO, 2005). The MW is the most important insect pest of stored maize in tropical and sub-tropical countries (Ukeh, 2008). MW bores a hole through the grain kernel, consumes the endosperm, lays eggs in the holes and multiplies as their generation increases thereby causing vast damage to maize (Parker, 2008). In Ghana, out of an estimated total annual harvest of 250,000-300,000 tonnes of maize, about 20% is lost to MW (Obeng-Ofori and Amiteye, 2005). Therefore, it is important to mitigate these and other postharvest losses to ensure food security in Ghana (Opit et al., 2014). Infestation of maize by insects occurs mostly in the field due to delayed harvesting and also during maize storage. Postharvest activities such as timely harvest, shelling, drying and storage is a major concern because proper handling and storage generates more income to farmers. Grains can be stored for several purposes. Maize can be stored for short term (4-5 months), season-long (6-9 months), and long-term storage for more than 9 months (Mejia, 2008). Since storage is an important aspect of food security in developing countries (Kimenju et al., 2010), it is therefore important to store grains such as maize properly to prevent quality, physical, nutritional and biological losses which may occur.

Several techniques are employed in the storage of grains in developing economies. Some of these techniques include the use of traditional methods, botanical method, biological method, manipulation of drying and storage conditions, and use of synthetic chemicals. Synthetic chemicals are well known for insect pest control due to the important role these chemicals play in reducing storage losses. However, the disadvantages posed by the use of the chemicals such as risk to human health when inhaled and the toxic residues on food products, insect resistance due to its continuous use as well as high cost of these chemicals make them less attractive.

Aside from these synthetic chemicals and traditional methods, the use of non-chemical and low-cost technologies such as tapping the natural source of heat energy from the sun by the use of solar drying systems to heat the air that flows in the dryer, is a very effective, hygienic and efficient method for stored product protection. Solar dryers are specialized devices that control the drying

process and protect the agricultural product from being damaged by insects, pests, dust, rain and also from mould infection (Al-Juamily et al., 2007). According to Gatea (2009), the application of solar dryers in developing countries can reduce postharvest losses and significantly contribute to the availability of food.

Exposure to high temperatures to kill insects in stored food grains has long been known by farmers in developing countries where food grains are often laid in the open sun for thermal disinfestation. To achieve high mortality and destroy all life stages of insects, Hansen et al. (2011) reported that use of solar heating in excess of 50°C to disinfest stored commodities is possible. However, grain simply laid in the sun does not reach such high temperatures.

The present study describes the use of a developed mobile solar biomass hybrid dryer as a potential alterative for the eradication of insects in maize grains. Specifically, mortality of insects as affected by the high temperature and exposure period in the dryer was determined and compared to a control set-up using the open sun energy.

Materials and Methods

Experimental site and unit

The thermal disinfestation experiment was conducted using a developed 1-tonne capacity mobile solar biomass hybrid dryer (Fig. 1) designed and fabricated at the workshop of the Department of Agricultural and Biosystems Engineering, KNUST, Kumasi, Ghana.

Fig. 1 Developed mobile solar biomass hybrid dryer at KNUST, Kumasi, Ghana.

Description and operation of dryer

The mobile solar biomass hybrid dryer (SBHD) is based on a greenhouse structure design that utilizes locally available technology, materials and skills that make on-site construction possible. The dryer has two major parts; the drying compartment with overall dimension of 3 m x 1.8 m x 1.9 m totally enclosed with a 3 mm thick Perspex material. It has four layers of drying shelves (or racks) with a total holding capacity of 1 tonne. As shown in Fig. 2, the drying chamber is coupled to a biomass burner enclosed with a heat exchanger to raise the temperature of air that is blown into the drying chamber with a blower fan solely powered by an installed solar photovoltaic system which includes a back-up battery to store energy for off-peak operation and a DC bulb for night

operation. It integrates both solar and biomass energy to generate heat for drying crops or for thermal disinfestation of grain pests such as the MW. In operation, the dryer can rely on direct solar insolation during sunny days where trapped heat from the sun in the chamber is used for drying or disinfestation. Additionally, preheated air from the heat exchanger is forced/pumped into the chamber to affect drying or disinfestation (Fig. 2).
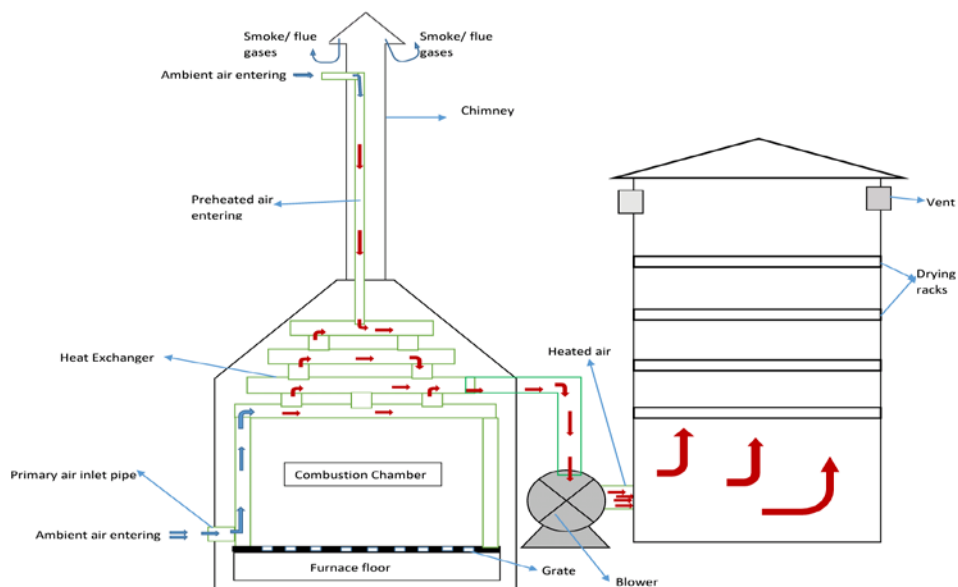
Fig. 2 Schematic view of the solar biomass hybrid dryer showing the air flow movement.

Methods

Culture of maize weevils

About 35 kg shelled maize (*Obatanba* variety) at 14% moisture content was obtained from the Agriculture Research Station at KNUST and used for the experiment. One kg of the dried maize grains was infested with adult *Sitophilus zeamais*. The infested grains were kept at room temperature for 10 days after which the insects were sieved from the grains. The sieved grains were thereafter kept in one litre Kilner jars at room temperature in the Entomology Laboratory of the Faculty of Agriculture at KNUST and cultured until the emergence of adult weevils. Emerged adult weevils served as the stock culture for the experiment.

Experimental set-up

The thermal disinfestation experiment was conducted on different days; 26th January 2017 (10:00 am to 16:00pm) where the heat source was from both solar and biomass energy (hybrid mode operation) and 7th March 2017 (11:00am to 17:00pm) where the heat for disinfestation was generated only from solar energy (insular mode). The experiment was set-up as a factorial experiment arranged in a Randomized Complete Block Design (RCBD). The effect of disinfestation period (3 and 6 hours) and heat source (solar and biomass combined; solar only) on weevil mortality were considered as treatments. Under each heat source application experiment, three replicate samples were set-up at each level in the dryer (four levels/blocks). The control samples were set-up in the open sun during the experiment. Under each experimental trial, 30 aerated cages were stocked with maize samples and the cultured weevils. Each level of the dryer had six cages (three

replicates for 3 hours of disinfestation period and the other three replicates for 6 hours). This brought the total cages in the dryer to 24 as shown in Fig. 3. The remaining six cages were set up in the open sun for the same thermal disinfestation period. The cages were fastened together and covered tightly to prevent any possible escape of the artificially introduced maize weevils after they were closed.

Fig. 3 Experimental cages for thermal disinfestation trials.

Disinfestation in solar biomass hybrid dryer

After stocking each cage with 500 g of maize, 20 of the cultured MW of different sexes and age were introduced into each of the 30 mesh-like 'cages' with forceps. The infested maize grains in the aerated cages were later placed on the drying racks/shelves in the dryer for thermal disinfestation at predetermined time intervals of 3 and 6 hours. The temperature profile in the dryer during the experiments under the different heat source applications (hybrid mode and solar only mode) was monitored using Tinytag data loggers (accuracy of ±0.01°C). The loggers were mounted at various levels in the dryer to record temperature conditions in the dryer and in the ambient environment. The loggers logged data at every 10-minute interval during the disinfestation period to account for weather fluctuations during the experiment.

Mortality rate

After the predetermined exposure periods (3 and 6 hours), the insects were separated by sieving the grains. The dead and live insects were counted and recorded. Similarly, the dead and live insects in the control set-up were also assessed. Inspected insects were confirmed dead when there was no response after pricking with the tip of the forceps. Mortality was estimated by counting the number of dead weevils and the mortality rate calculated using Equation 1 provided by Gazzoni (1998):

$$M_r = \frac{dw}{tw} \times 100 \dots\dots\dots\dots \text{Eqn. 1}$$

M_r = mortality rate

tw= total number of weevils

dw= number of dead weevils

Emergence of F1 progeny (First Filial generation)

At the end of the thermal disinfestation process, the sieved grains were placed in Kilner jars covered tightly with calico cloths. The containers were kept in the Lab until 30 days after which the number of insects that emerged from each replicate sample were counted after sieving and then recorded. Likewise, the number of insects that emerged from the control were also counted and recorded. This continued daily until there was no emergence of F1 progeny.

Data analysis

Values obtained on weevil mortality were subjected to Analysis of Variance (ANOVA) using Statistix 9. A significance level of 5% was used for all analyses. The Least Significant Difference (LSD) was calculated where a significance was found between treatment means.

Results

Temperature profile in the dryer under hybrid and insular mode compared to ambient temperature

Results of the temperature profile in the dryer relative to the position of the experimental cages during the different heat source applications are shown in Figs. 4 and 5. For the 6 hour disinfestation period, it was observed that temperature conditions in the dryer under both heat source applications varied from the bottom level (L1) to the topmost level (L4) in an increasing trend.
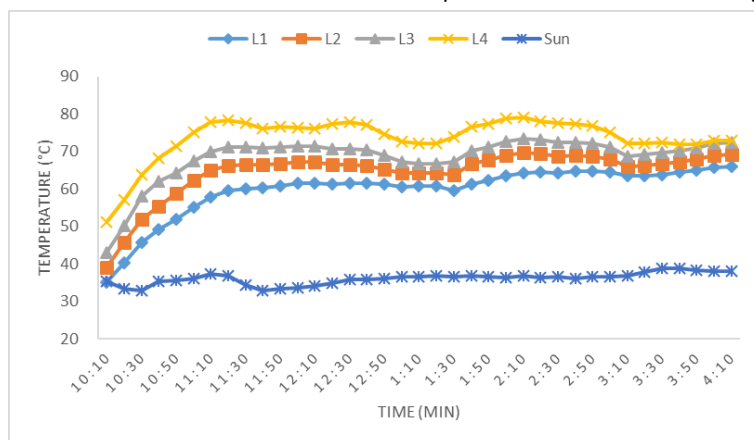
Fig. 4 Temperature variations in the dryer under hybrid mode test
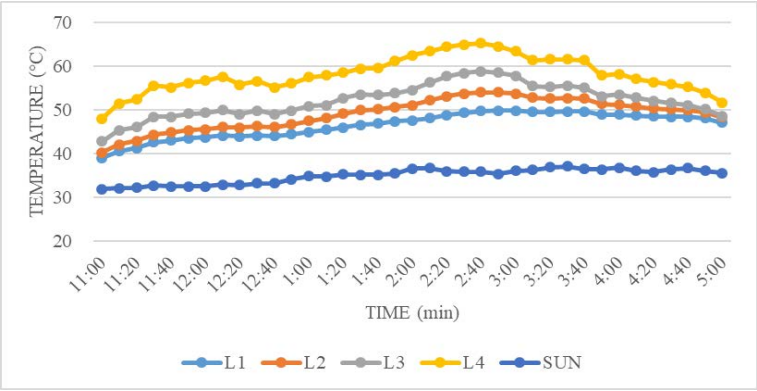
Fig. 5 Temperature variations in the dryer under solar only mode test

As presented in Tab. 1, mean temperature of 67°C was recorded in the dryer during thermal disinfestation using the combined heat source of biomass and solar (hybrid mode) while a mean temperature of 52°C was recorded using solar only as the heat source for disinfestation. Comparatively, the mean temperature inside the dryer was 31°C and 17°C higher than the ambient temperature during disinfestation under the hybrid and solar only modes, respectively.

Tab. 1 Mean temperature for insect disinfestation at 3 and 6 hour exposure periods.

Level	Mean temperatures (°C) at 3 and 6 hours (hybrid mode)		Mean temperatures (°C) at 3 and 6 hours (solar only mode)	
	3 hours	**6 hours**	**3 hours**	**6 hours**
L1	56.1	59.8	44.2	46.5
L2	61.6	64.5	46.5	49.1
L3	66.1	68.4	49.8	52.1
L4	72.4	73.6	56.5	58.1
Overall average in dryer	**64.1**	**66.6**	**49.3**	**51.5**
Sun	35.1	36.1	33.7	34.9

Mortality of adult *Sitophilus zeamais* and F1 progeny

Tab. 2 presents the results of mean mortality of MWduring thermal disinfestation for exposure periods of 3 and 6 hours under the hybrid and solar only mode tests. The mean emergence (F1 progeny) as affected by the temperature and exposure period in the dryer under the two experimental set-ups is also presented (Tab. 2).

Tab. 2 Effect of exposure period and temperature on mortality of *Sitophilus zeamais* and F1 progeny.

Exposure period and heat source	Mean temperature (°C)	Average mortality (%)	Mean adult emergence (F1 Progeny)
3 hours @ Solar + Biomass	64.1	84.9 ab	0
3 hours @ Solar only	49.3	65.4 c	0
6 hours @ Solar + Biomass	66.6	91.6 a	0
6 hours @ Solar only	51.5	78.2 b	0
3 hours @ Control (open sun)	35.1	13.3 d	6
6 hours @ Control (open sun)	34.9	41.2 e	5
LSD (5%)		**8.80**	

Within a column means followed by a different letter show significant difference (p<0.05).

Discussion

Effect of heat source on temperature trend

Temperature conditions in the dryer were significantly higher under the hybrid mode compared to when the heat for disinfestation was solely from the solar energy. However, there was a significant difference (P < 0.05) between the temperature conditions in the dryer under both heat source applications compared to ambient air temperatures and direct sun exposure. There was therefore a direct correlation between the energy input for thermal disinfestation and the temperature trend under the different modes of operation of the dryer. The increasing trend in temperature observed under the hybrid mode was due to the high energy input from both heating sources (solar and biomass). This was consistent with the work done by Okoroigwe et al. (2015) who reported that a hybrid heat source has an advantage over sole dependence on biomass or solar. Similar findings were also reported by Bolaji (2005), who designed and constructed a box type indirect solar dryer, where the drying chamber recorded a maximum temperature of 57°C at the time when the ambient temperature was 33.9°C. As clearly presented in Tab. 1, mean temperature conditions recorded in the dryer (hybrid or solar only modes) were above 50°C reported by Fields (1992) who suggested this temperature could cause death of insects within minutes of exposure.

Effect of exposure period and source of heat for disinfestation on mortality of adult *Sitophilus zeamais* and F1 progeny

From the results obtained, the exposure period of infested maize grains in the mobile solar biomass hybrid dryer is vital for thermal disinfestation of MW. Depending on the exposure period and the temperature profile in the dryer, insect infested grains could be controlled for long-term storage. There was significant difference (p < 0.05) in mortality of MW with respect to the exposure period and the source of heat used for disinfestation (Tab. 2) as compared to the control experiment.

During disinfestation under the hybrid mode, it was observed that (Tab. 2) mortality of adult weevils after 3 hours (10:10am to 1:10pm) and 6 hours (10:10am to 4:10pm) of exposure time showed no significant differences (P>0.05). There was, however, significant difference (P> 0.05) in adult weevil mortality during disinfestation relying only on solar energy as the heat source. Moreover, there was no significant difference (P<0.05) in MW mortality during thermal disinfestation at exposure periods of 3 hours (hybrid mode) and 6 hours (solar only). The highest mean mortality was achieved at the 6-hour exposure period in the hybrid mode although the results showed that thermal disinfestation under both experimental set-ups (hybrid and solar only modes) recorded a better effect on mortality compared to the control. Recorded mean temperatures were above 50 and 60°C in the dryer under both hybrid and solar only modes, respectively, and below 40°C for the control. Kitch et al. (1992) reported that exposing insects to temperatures above 45°C is known to be lethal to insects with most stored product insect pests known to succumb to death under such conditions. This agrees with Seidu et al. (2010) who reported that mortality of maize weevils exposed to varying temperatures and time in a conventional solar dryer required 120 minutes (2 hours) or more for achieve mortality. This suggests that shorter exposure periods of grains infested with the adult MW is required to achieve high mortality during thermal disinfestation in the hybrid mode while a longer exposure period of no less than 6 hours is required under the solar only mode to achieve the same efficacy.

Results on F1 progeny (Tab. 2) showed that disinfestation under both hybrid and solar only modes over 3 and 6-hour exposure periods was able to prevent the emergence of the MW by destroying all the developmental stages of the weevil during heat treatment in the dryer. However, there was some emergence of F1 progeny in the control under both 3 and 6-hour exposure periods. This is an indication that mean temperatures recorded in the dryer under both hybrid and solar only modes were high enough to kill adult weevils and destroy eggs they might have laid. This was evidence in the non-emergence of F1 progeny after the disinfested grains were stored in the lab for 30 days. However, there was re-emergence of F1 progeny in maize grains treated with the control set-up

(open sun disinfestation) where the ambient temperature had little effect on destroying all developmental stages of the MW including any laid eggs. This is corroborated by Agona and Nahdy (1998) who reported that the use of a low-cost solar dryer was effective in ensuring 100% mortality of adult beetles and the non-emergence of adults in solar treated cultures as well as eliminating all developmental stages of *Acanthoscelides obtectus* after exposure for 6 hours above 45°C. Similar trials have also recently been reported by Purdue University, USA researchers in which a similar method was used for killing cowpea weevils (*Callosobruchus maculatus*). They found that ambient temperature had little effect on the temperature developed inside the grain heater, provided that there was sunshine. Their results showed grain temperature of 62°C after 15 minutes of exposure and 100% insect kill after 3 hours. From the assessment of the hybrid dryer for potential use in thermal disinfestation, it was demonstrated that the dryer can be used in preventing damage by *Sitophilus zeamais* to maize grains. All life stages of the MW succumbed to death even at the lowest temperature achieved under the solar only mode within a disinfestation period of 3 hours and above. The successful use of the dryer for thermal disinfestation should be a motivation for farmers who could utilize the developed dryer for both drying their maize grains and controlling insects during storage to minimize postharvest losses and promote food security.

Acknowledgement

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Green Ecological Grain Storage Technology and Quality Control in China

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Abstract

Green ecological grain storage technologies (GEGSTs) are the means of controlling stored grain quality, and quality changes of stored grain are the basis of GEGSTs control. This paper introduces that GEGSTs are widely used in China, including monitoring and early warning of stored grain pest and mould, pest control by using food-grade materials, controlled atmosphere for pest control, ventilation for lowering and equalizing temperature, low and quasi-low temperature grain storage, treatment of hot spots, etc. And it introduces that grain processing enterprises' and market's request for grain quality, is called "quality control". It also clarifies that stored grain quality control is the purpose, and emphasizes that GEGSTs control is the process, so GEGSTs control should serve for quality control. Therefore, we propose that the technology application and the quality control of grain storage are equally important, and without the quality control, the technology application could be invalid, especially for sensitive areas in grain bulks. In the process of grain storage, special attention should be paid to quality changes in the sensitive areas, like real-time monitoring. Identify and utilize scientific and reasonable technology accordingly, including related technologies and equipment, to improve "overall" quality control level of stored grain bulks, and to gradually standardize them. By means of GEGSTs, positive ecological storage conditions are effectively utilized, which helps us achieve the purposes of safety, no pollution, high quality and nutrition during grain storage.

Key words: Storage Technologies, Grain consumption, Quality Control

Green ecological grain storage technologies (GEGSTs), based on the theory of grain bulk ecology, through the means of green ecological low-carbon, help us achieve the purpose of safety and quality control during grain storage. Grain storage technology control is a process, and grain storage quality control is the purpose, so grain storage technology control should serve for stored grain quality control. After harvest of grain, quality control in grain circulation involves three aspects, which are grain quality during consumption, warehousing and storage. Among them, stored grain quality is related to the warehousing quality and grain consumption quality, taking into account the two links of grain production and grain consumption. It is the key to do a good job in the convergence and coordination of these three aspects, to improve the technical level of grain storage management.

1. Green ecological grain storage technologies

GEGSTs are widely used in China, including pest control by using food-grade materials, ventilation for lowering and equalizing temperature, controlled atmosphere for pest control, low and quasi-low temperature grain storage, monitoring and early warning of stored grain pests and moulds, treatment of hot spots etc.

With the development of insect pheromones and different wavelength spectra to attract stored-grain pests, the density and insect situation of stored-grain pests in a granary could be monitored by using new trapping technology. Combined with the detection of grain condition, the population dynamics of stored-grain pests under different ecological conditions could be predicted, and thus the decision-making control technology was put forward. Integrated with grain storage information technology and other high-tech, a new core technology of comprehensive control of stored grain pests is formed.

Pest control technology by using food-grade materials is an upgrade of traditional inert-powder pest control technology with plant ash, diatomite and others. Insecticidal mechanisms of food-grade materials fall into the internode membrane of the insect body, which would lead to wear the

internode membrane during insects moving and adsorbing lubricating fluid and body fluids, thus resulting in pest death (Zidan Wu et al., 2011).

The gas composition in a sealed grain pile could be artificially changed, such as putting CO_2 and N_2 into the grain pile or reducing the oxygen concentration, so as to kill pests, inhibit the respiration and growth of the pests, prevent the occurrence of mold and delay the deterioration of stored grain quality. The main application technologies are related to natural hypoxia or artificial gas, which is essentially mechanical nitrogen-rich hypoxia process, to reduce oxygen in a granary.

Low temperature storage refers to the average grain temperature maintained at or below 15°C all year-round, and the partial maximum grain temperature is not higher than 20°C. Quasi-low temperature storage refers to the storage mode in which the average grain temperature is kept at and below 20°C all year-round, and the partial maximum grain temperature is not higher than 25°C. Grain warehousing temperature and moisture are the basis of low temperature and quasi-low temperature grain storage, which is generally divided into two cases: grain warehousing in high temperature seasons and grain warehousing in low temperature seasons. High temperature seasons are from May to October, and grain temperature is comparatively high when warehousing. In order to achieve low temperature and quasi-low temperature grain storage, cold ventilation technology must be employed, especially when the grain temperature is higher than 25°C. Once the granary is filled up, it is necessary to level off the grain surface, use horizontal ventilation technology to make outside air penetrate through the whole grain pile, or use uncovering-cloth ventilation technology for partial processing. These processes will help to not only eliminate the accumulated heat during grain warehousing, but also to eliminate the harmful gases released by the grain pile. Low temperature seasons are from November to March or April of the following year. During these times, grain temperature is low when warehousing ithe granary, so we just need to level grain surfaces.

2. Grain quality demand for grain consumption purposes

The quality demand of grain consumption varies according to the usage. The grain is eventually processed for humans, animals and industries in the market (Xiaohe Ma et al., 2008). Meeting the demand of the grain consumption market ensures grain storage rotation, higher prices for good quality, and good storage income and social benefits.

Human consumption (food grain) is the primary area of grain use, including mainly wheat and rice, and also maize and coarse cereals in small amounts. Food grain accounts for 50% of the annual grain consumption (Xiaohe Ma et al. 2008).

Requirements of the food grain are not only to eat enough, but also to eat well, to eat green, ecological and fresh food. In addition to providing products with good taste, color and smell, food grain processing must meet the standards of food hygiene, including prevention of heavy metals, mycotoxins, pesticide residues and other harmful substances in the product.

Grain provided for animals (fodder grain) is the second largest consumption area, supporting the development of the livestock and poultry industry and production of meat, eggs and milk. The fodder grain accounts for 33% of the annual grain consumption with consistent growth (Wei Jia et al., 2013).

In the process of development, the market demand for fodder grain quality is increasing and refining. Hygienical standards for feeds stipulate that the total number of mold in maize as well as wheat bran and rice bran is less than 40×10^3/g, and rules for maize with mold of $40 \sim 100 \times 10^3$/g is of limited use, and mold of more than 100×10^3/g is banned. Fodder corn standard stipulate that corn is divided into three levels, and the fatty acid value of the top-level corn is no more than 60 mg/100 g.

Grain provided for industries (industry grain), the third largest consumption area, accounts for 10% of the annual grain consumption, and the figure is predicted to be 13.9% in 2030. Industry grain is

used in a variety of products such as starch, modified starch, starch sugar, amino acid, organic acid, enzyme preparation, yeast and fuel ethanol, etc.

In recent years, some enterprises purchase and use poor quality grain in order to reduce the production cost of main raw materials, expecting to increase efficiency. But contrary to the expectation, low-quality grain leads to lower yield of main products, poor quality of by-products and poor market competitiveness.

3. Quality control requirements during takeover

Basic Requirements: "dry, full, clean"

Grain warehousing quality control is the source of grain circulation quality control. "Grain and Oil Storage Technical Specification" requires that quality of long-term stored grain should comply with the provisions of the Chinese national quality standards. Moisture content should not exceed the local safety moisture. Impurity content mixed with grain should not be greater than 1.0%, and when impurity content is higher, it should be cleaned out. At the same time, the stored grain quality indicators should comply with the "Stored Grain Quality Judgment Rules" and the relevant standards.

Quality Control Indicators

There are three main quality control indicators in the "Stored Grain Quality Judgment Rules", i.e., color and smell, fatty acid value, and taste score. Taste score, color and smell belong to sensory evaluation indexes determined in accordance with the conditions of the evaluation test by personnel with sensitive senses and identification ability usually in the laboratory environment. Fatty acid values are physical and chemical properties used for quantitative analyses.

Quality Control Key Points

The quality of newly stored grain is directly related to the appropriate storage degree, grain storage cycle and grain storage safety. On the basis of clearing impurities during warehousing to ensure "dry, full, clean", the fatty acid value of grain also needs to be strictly controlled.

A key link affecting fatty acid value changes is the process of grain drying. Because of the "labor problem", the number of post-harvest grain needing to be mechanically dried increases, and the number of natural air drying of grain systems decreases. The fatty acid value of mechanically dried grain is higher than that of naturally dried grain. The fatty acid value of newly harvested corn with natural drying is generally about 15 mg/100 g, and rarely exceeds 20 mg/100 g. The fatty acid value of mechanically dried corn reaches 29.7 mg/100 g ~ 45.3 mg/100 g, and among the samples with fatty acid values higher than 40 mg/100 g, there are more baked paste particles, more broken particles in the imperfect particles, and greater changes in the quality index in the mechanically dried corn than that with natural dryied (Chunlong Xia, 2008).

Technical Method of Quality Control

Before warehousing, we should pay more attention to controlling the fatty acid value. The lower the initial fatty acid value of grain is, the better. It is necessary for long-term storage of grain to take quasi-low temperature and low temperature control and other effective storage technology measures in order to control the rate of fatty acid increase and delay quality change; however, these measures will increase the cost of grain storage (Yurong Zhang et al., 2004).

In order to control the increase of fatty acid value, the drying process should be improved. In particular, the temperature of the drying air and the maximum temperature to which the corn is heated should be controlled to ensure the quality of the dried grain.

In addition, we should focus on developing grain drying technologies and devices that could be used and adopted easily by farmers. Farmers should be guided to carry out grain harvesting operations scientifically and reasonably, and do a good job at grain quality control.

4. Quality Control Requirements during Grain Storage

By taking advantage of good correlation between fatty acid and taste score, fatty acid value could be used as a sensitive indicator of daily monitoring of grain quality changes in order to monitor in a timely manner stored grain quality. We should pay special attention to parts of bulk grain, sensitive parts, and monitor in a timely manner, and to examine with reasonable scientific and technical measures, including related technologies and equipment, to improve "overall" quality control of stored grain.

5. Importance of Grain Quality Control

Guaranteeing grain quantity and quality are complementary. Guaranteeing grain quantity is relatively intuitive and tangible. However, maintaining quality, involving the biological and non-biological ecological environment of a grain bulk, is challenging. In order to control the physiology and biochemistry, molds, pests and other ecological factors of grain storage, it is necessary to strictly control grain quality during warehousing. At the same time, based on market oriented rules, we should strengthen the implememntation of proper grain storage technologies to achieve the requirementss of grain quality control, to meet the needs of grain consumption, and to ensure the high value of stored grain.

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A new approach to acoustic insect detection in grain storage

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Abstract

Insect pests in grain storages can cause severe financial losses. Infested grain needs to be treated and can be sold only with lower profit. Intense infestation can lead to contamination with mycotoxins and total loss of stock. Therefore, an early detection of insect storage pests is of great importance to farmers and storage keepers but is difficult to obtain in large amounts of grain.

Besides conventional detection methods such as insect traps and monitoring of temperature and relative humidity, acoustic monitoring can identify insect infestation. Insects in grain and other stored products produce sounds at a low level during movement and feeding activity. A new acoustic system was developed as part of the project "InsectTap" to increase the detectability of insect sounds. Highly sensitive microphones were installed inside a metal tube that increased the surface on which beetle signals could be detected. Additionally, the tube worked as a beetle trap recording all sounds from even one single beetle inside the trap.

The tube system was tested in 1 and 8 m³ boxes filled with wheat. Infestation could be detected at a very early stage about 8 weeks before a temperature rise, or beetles at the grain surface indicated an infestation.

In the next step, this "Beetle Sound Tube"-System will be installed in different grain silos aiming for automatic early detection and specific identification of infestation. The information provided to the farmer or storage

keeper allows early and specific treatment to reduce losses. Additionally, the introduction of parasitoids via the tube system will be tested to increase the efficacy of biological control.

Keywords: Acoustic Detection, Monitoring, InsectTap, Beetle Sound Tube, *Sitophilus granarius*.

Introduction

Early detection of insect storage pests is important to reduce losses and preserve high quality food. Recognition of infestation in large amounts of storage goods is difficult, and in many cases it is only noticeable when the amount of insects increases considerably and causes a rise of temperature and relative humidity. At this stage, mites and mould can lead to major secondary losses.

Treatment of insect infestation has become more difficult due to a decrease of available chemical substances for storage protection, an increase of organic farming that cannot use chemical agents and the increasing disapproval of consumers to chemical treatments. Therefore, early detection is crucial to have a choice between different non-chemical treatments that are not suitable for mass infestation.

Besides measuring temperature and relative humidity, using traps or sieving samples, the detection of feeding and movement sounds is another way to discover insects in stored goods. A great advantage of acoustics is that even the sounds of hidden stages of insects can be detected (Leblanc et al., 2009). But very low amplitudes of signals and sound insulation properties of grain make it difficult to detect the sounds at distances of more than a few centimetres (Hagstrum and Subramanyam, 2006).

Another difficulty that devices for acoustic detection of insects face are settlement sounds of grain that can be mistaken for insect sounds. Therefore, a permanently installed acoustic system could have advantages (Hagstrum and Subramanyam, 2006) compared to mobile probes or acoustic test containers.

Aim of the project "InsectTap" funded by the Federal Ministry of Food and Agriculture (BMEL) was the development of an acoustic early detection system that allows detection and specific identification of insect infestation. Experiments under controlled conditions showed as a first result discriminability of a number of adult beetle species by sound (Kirchner et al., 2016).

Another part of the project that will be described in this paper were pilot plant scale experiments in 1 and 8 m³ of stored wheat using high-sensitive microphones placed inside metal tubes to increase the detectability of insect sounds due to surface enlargement.

At the next step, this "Beetle Sound Tube"-System will be customized to the needs of farmers and keepers of small storage facilities and installed in different sized grain silos aiming at automatic early detection and specific identification of infestation. The information provided to the farmer or storage keeper allows early and specific treatment to reduce losses including the application of parasitoids via the tube system to allow easier access to the infestation.

Materials and Methods

Experimental set up

Two experiments were carried out using large wooden boxes of 1 or 8 m³ filled with wheat. The boxes placed inside an about 77 m² storehouse were equipped with 17 to 20 data loggers (EasyLog EL-USB 2) to record temperature and relative humidity and 3-4 microphones for acoustic measurements.

During the first experiment using the 1 m³ box (Fig. 1), three free field condenser microphones (PCB-378B02, PCB Piezotronics, Depew, USA) were used under different conditions. The microphones were either covered with a layer of PET rescue foil as dust protection and placed directly into the wheat or were suspended inside 0.75 m long galvanised steel tubes of 0.08 m diameter inserted into the wheat to focus sound signals from the surrounding substrate. While one of the tubes was a simple metal tube with a stainless steel lid at the bottom, the second tube was equivalent but with

a large number of 2.5 mm drilled holes and functioned as a beetle trap comparable to a WB probe trap (Barak et al., 1990) with a removable cup containing some grain at the lower end of the tube for accumulation of beetles. In the 8 m^3 experiment, four metal tubes of 1 m length and 0.1 m diameter were used from which one functioned as a trap.

The experiment in the 1 m^3-box was carried out between May and October 2016, while the 8 m^3 box was used from March to August 2017. At the beginning of both experiments, 200 wheat kernels containing larvae of the grain weevil (*Sitophilus granarius*) 25-28 days or 30-32 days after oviposition, respectively, were introduced at one position in the box. A data logger for temperature and relative humidity was placed directly above the position of insect infestation.
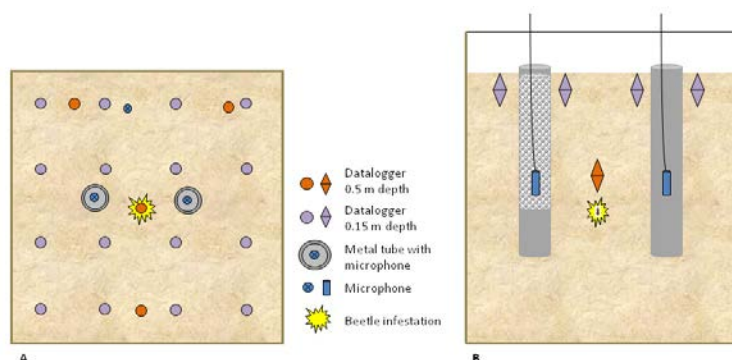
Fig. 1 Experimental set up of the 1 m^3 box. **A:** Top view of the box showing the positions of the three microphones, 20 data loggers and the position where the beetle infestation started. **B**: Lateral view with the two microphones in the two tubes (left tube functioned as a trap), data loggers and position of beetle infestation.

For the following months, temperature and relative humidity were logged every six hours. Acoustic data were recorded at the first 20 minutes of each hour using an IMC CS-3008-N High-resolution measurement device (imc Meßsysteme GmbH, Frankfurt, Germany) connected to a laptop using IMC Studio Pro 4.0 software. Additionally, the number of beetles in the trap was determined and the insects removed on a regular basis.

Acoustic evaluation

After it was checked that there was no daily rhythm in granary weevil activity, three times per day were chosen for acoustic evaluation (3 and 9 a.m., 9 p.m.). The times were chosen to include one recording during daytime, one during twilight and one during night-time. During daytime disturbance due to workers and traffic in the surroundings were common. In twilight there was less traffic, no working activity but natural sounds such as birds, while during night-time external noise was low unless the weather situation was rough.

The recordings were bandpass filtered (1000-12000 Hz using IMC FAMOS Professional 7.0) to reduce background noise. Four 15-second segments of the recording starting at minute 1, 6, 11 and 16 were acoustically evaluated by a trained person, counting the number of insect signals. In case of strong external disturbances the section for evaluation was moved to the next 15 undisturbed seconds of the recording. Therefore, 12 periods of 15 seconds were evaluated each day and the number of signals added up to a daily activity figure with standard deviation. Tab. 1 gives an overview about the duration of both experiments and the evaluated times.

In case of very frequent insect signals (more than 2.3 signals/second) an accurate count of signals was not possible and in those cases the result was given as >35 for the 15 second section. Results that are based on at least one 15-second section with more than 35 signals are indicated in the result section.

The increase of beetle activity inside the box as indication for increasing number of insects over time was evaluated at intervals of 1-11 but mainly 3-4 days.

Tab. 1 Overview of experimental duration and acoustically evaluated days.

	Duration of the experiment	**Evaluated days**
1 m³ box	148 days ≈ 21 weeks	24 days (72 hours)
8 m³ box	166 days ≈ 24 weeks	26 days (78 hours)

Modelling of beetle population

To estimate the size of the beetle population during the course of the experiment, the computer model "SITOPHEX" was used (Prozell et al., 2004). This model calculates the number of beetles and development stages over time considering temperature, relative humidity, reproduction rate and mortality rates of different stages.

Results

Temperature

In the first weeks of the experiments the temperatures inside the boxes rose slowly depending – with some delay - on the temperatures outside the box. The temperature outside the box and inside the storehouse was largely dependent on the ambient temperature.

After 12 weeks, the temperature inside the 1 m³ box just above the beetle-infestation rose quickly. During a period of 12 days, the temperature increased by 11°C, while the temperatures outside the box and the meteorological data remained at a lower level. The temperature increase inside the box was therefore not caused by external temperatures but biological activity (Fig. 2).
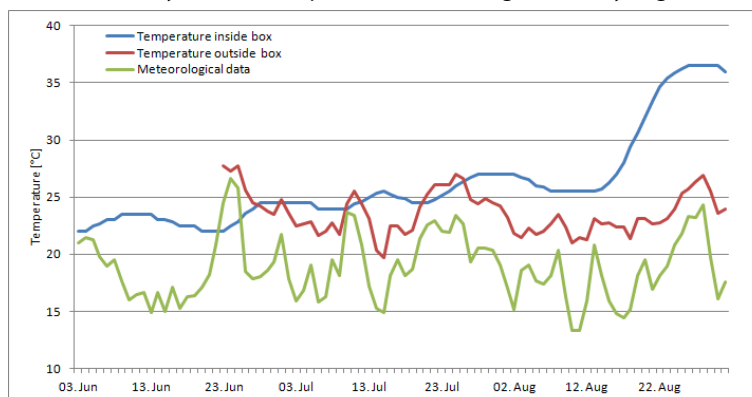
Fig. 2 Daily average temperature inside the 1 m³ experimental box above initial beetle infestation point compared with temperatures outside the box and data from a nearby meteorological station at about 1 km distance from the building.

The increase of temperature was most pronounced in the area where the beetle larvae were placed at the beginning of the experiment (Fig. 3) indicating a proliferation of weevils and larvae in the area.
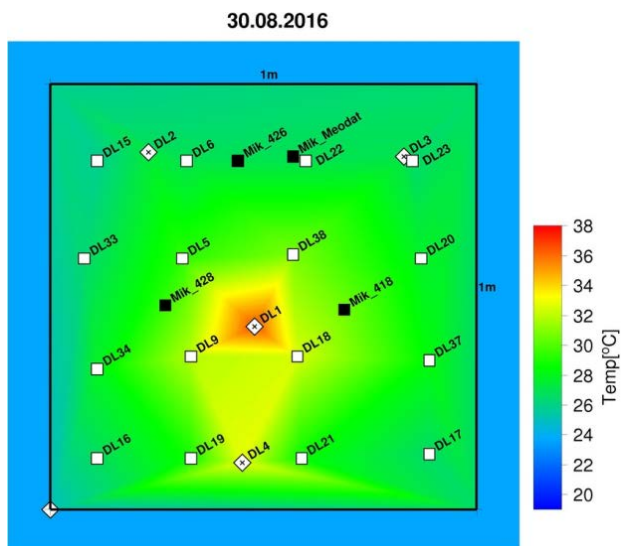
Fig. 3 Temperature in and outside the 1 m³ experimental box measured with 21 data loggers on the 30th of August. DL1 is the data logger just above the centre of beetle infestation.

An increase of relative humidity could be observed simultaneously with the rise of temperature. While relative humidity rose by 7 percentage points in the first 10 weeks of the experiment, it increased steeply another nearly 3 percentage points in 5 days. Afterwards the relative humidity decreased again but stayed at a higher level.

The results of the 8 m³ box were comparable, but the increase of temperature started after 122 days and therefore more than 5 weeks later compared to the 1 m³ box. The reason for this delay is the much lower temperature in the second experiment. While the first experiment in the 1 m³ box started in May with wheat temperatures of more than 20°C in the box, the second experiment in the 8 m³ box started in March during very cold weather. Start temperature of the wheat was about 16°C and it took until the middle of May before the wheat reached a comparable temperature as at the start of the first experiment. This led to delayed development of beetles and therefore a later increase of temperature as an indicator for beetle infestation (Fig. 4).
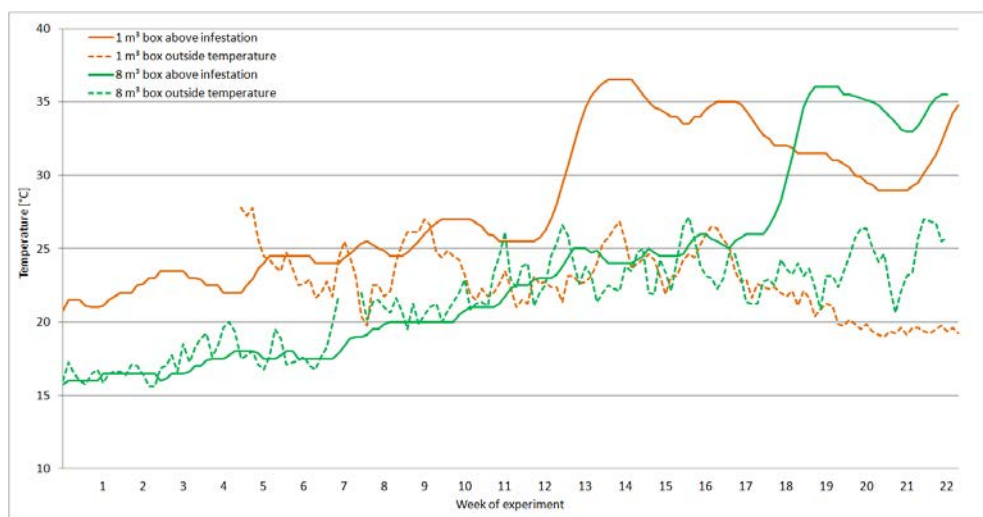
Fig. 4 Comparison of temperature in both experiments (1 and 8 m³) over time. A temperature increase indicating insect infestation in the 1 m³ box became obvious at week 12 while it took until week 18 in the 8 m³ box due to lower wheat temperatures.

Modelling of beetle population

The population size of adult *S. granarius* calculated using "SITOPHEX" showed large differences between both experiments (Fig. 5). While the population rose from 200 to nearly 400000 adults in the 1 m³ box in 21 weeks, it only reached about 150000 in 24 weeks in the 8 m³ box due to the lower temperatures and therefore slower insect development.
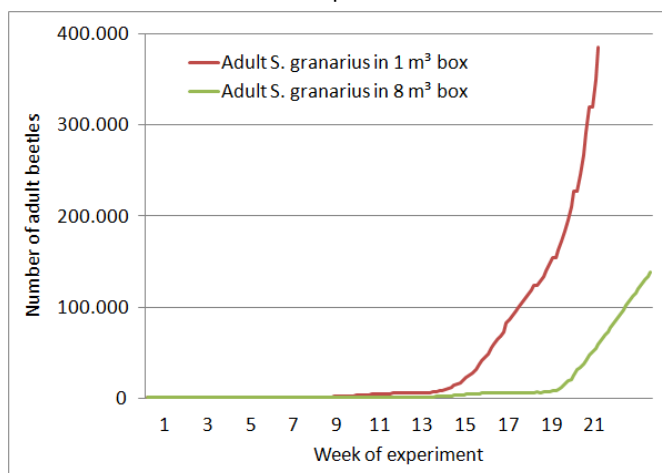
Fig. 5 Calculated numbers of adult *Sitophilus granarius* during the course of the experiments in 1 and 8 m³ wheat based on the software "SITOPHEX".

Experiment in the 1 m³ box

The evaluation of the recordings started before the first beetles were expected to hatch and ended at the end of August after the temperature increase indicated infestation. From the end of July (experimental day 69) onwards the number of signals picked up by the microphones in both metal

tubes exceeded the maximum countable number of 140 signals/minute, continuously. At the microphone placed directly into the grain, this point was reached after the 2nd of August.

Eleven days after the first beetles were expected to hatch, the first weak acoustic beetle signals could be detected with the microphone inside the tube trap at 0.22 m distance from the infestation start point while after 23 days the signals were strong and easy to detect. **Fehler! Verweisquelle konnte nicht gefunden werden.** shows the number of beetle signals during the course of the experiment from the day, when the first signals were detected, to the day, when the number of signals exceeded the maximum countable number of signals on all three microphones on the 6th of August.
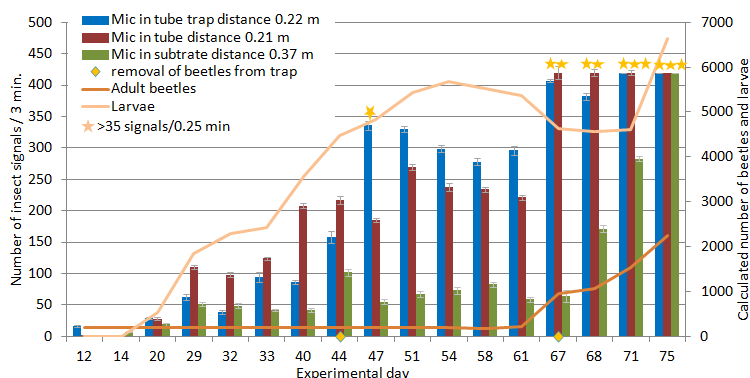
Fig. 6 Beetle signals recorded with three microphones inside the 1 m³ box. The columns show the sum of signals of twelve 15-second periods during an experimental day with standard deviation. Days on which the maximum countable number of 35 signals per 15 seconds was exceeded in at least one period are marked with an asterisk. Additionally, the calculated number of larvae and adult beetles inside the experimental box and the days on which beetles were removed from the trap are given.

All microphones showed an increase of signals with time corresponding to the increasing number of beetles and larvae inside the box. Already three weeks after the beginning of the experiment and about two weeks after the first adult beetles hatched signals could be counted regularly at all microphones.

A decrease of numbers of larvae was observed between experimental day 54 and 71 which resulted from the fact that all introduced larvae had the same age. Therefore, they pupated all at about the same time which led to a decrease of larvae before the number of adults increases. Afterwards, the number of larvae increased steeply after the young larvae of the next generation started to hatch.

Experiment in the 8 m³ box

First beetle signals could be detected inside the tube trap at the beginning of May more than 8 weeks after the first beetles hatched. Fig. 7 shows the number of beetle signals during the course of the experiment from the day when the first signals were detected to mid-August, when the experiment ended.

For more than 7 weeks, the microphone inside the tube trap was the only one recording signals. The high number of signals inside the tube trap was caused by few beetles inside the trap. After removal of three beetles from the trap on day 69, the number of detected signals decreased from 267 before to seven signals after removal. The next beetles were trapped in the tube causing the next peak on day 79. After the next generation of beetles hatched and the number of beetles increased inside the box, the trap-effect became negligible and the removal of beetles from the trap did not cause a clear decrease of signal numbers due to insects moving and feeding in the surrounding of the tube.

The microphone inside the tube at 0.62 m distance from the infestation recorded the first signals nearly 16 weeks after the first beetles hatched. The number of signals increased with the number of larvae and beetles in the box.

After nearly 21 weeks the first beetle signals were detected at a distance of 1.04 m, while it took only another 2 weeks until signals were recorded at 1.48 m distance from the infestation.
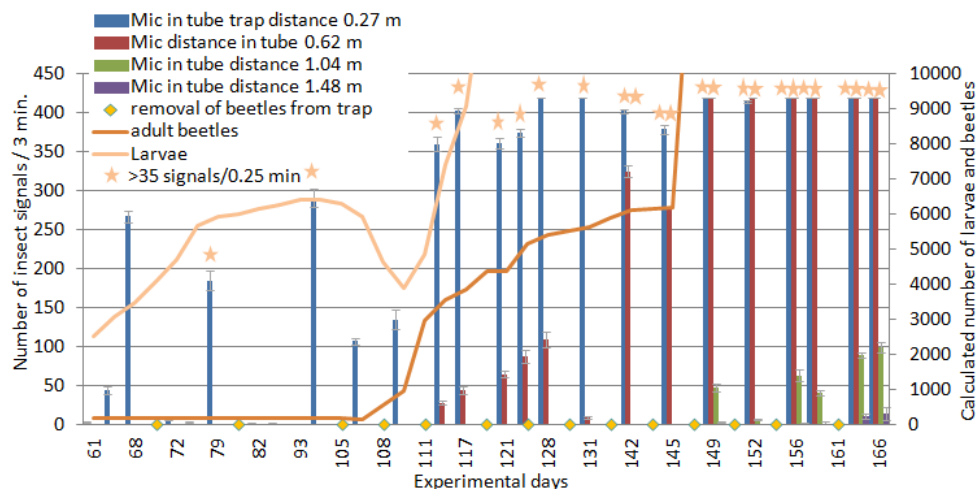
Fig. 7 Beetle signals recorded with four microphones inside the 8 m³ box. The columns show the sum of signals of twelve 15-second periods during an experimental day with standard deviation. Days on which the maximum countable number of 35 signals per 15 seconds was exceeded in at least one period are marked with an asterisk. Additionally, the calculated number of larvae and adult beetles inside the experimental box and the days on which the beetle were removed from the trap are given.

Comparison of temperature, insect detection and acoustic signals in both experiments

The results of both boxes are comparable apart from the fact that the development of beetle infestation was slower in the 8 m³ box due to lower temperatures. Fig. 8 shows the temperature above beetle infestation in both experiments as already given in Fig. 4 but time-displaced for better comparison. The day, when first signals were detected on the different microphones is displayed in the figure, showing that in both experiments an infestation of beetles could be discovered at least 8 weeks before an increase of temperature directly above the infestation was measurable and at least 6 weeks before beetles appeared on the surface of the substrate.
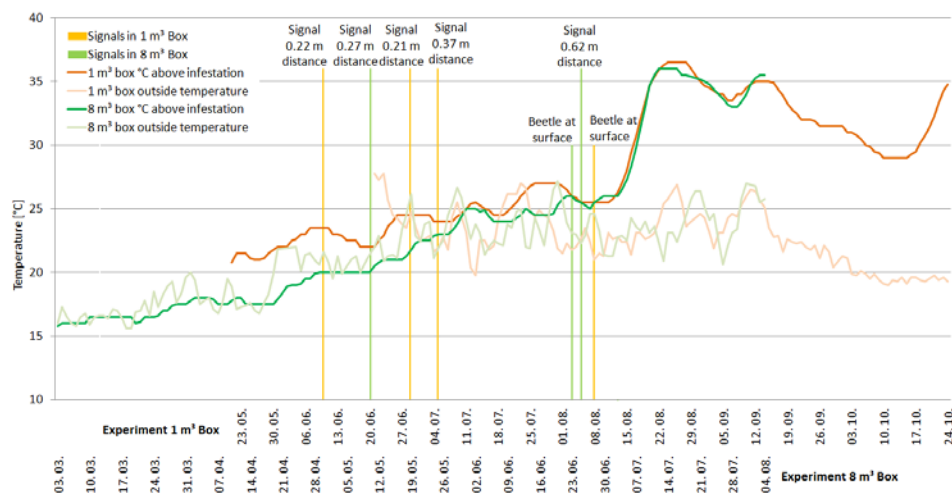
Fig. 8 Comparison of temperature in both experiments (1 and 8 m³) displayed six weeks time-displaced. Both experiments show a very similar temperature curve related to the beetle infestation. Additionally, given are the times of the first signals recorded by microphones at different distances and of the first observation of beetles at the substrate surface in both experiments.

Discussion

Aim of the project was the development of a permanent acoustic early detection system for farmers and smaller storage keepers. The results showed that acoustic monitoring can provide much earlier detection of beetle infestation compared to conventional methods such as temperature measurements or surface traps. But it must be considered that in professionally managed storages the detection with conventional methods such as traps might be possible at an earlier stage as shown in the experiments and that in those cases the gap between acoustic and conventional detection might be smaller. But farmers and keepers of smaller storages often do not have the time for close inspections or the wheat is stored in silos that are not easy to access. In these cases, one could therefore benefit from early acoustic detection. In both tests, acoustic detection was possible many weeks before temperature rose. Of course, the distance between the initial point of infestation and the first acoustic device would determine how much earlier acoustic detection is possible in comparison to temperature probing or traps.

On the other hand, in the experiments the position of the infestation was known and the temperature measurements were taken exactly at the right position to detect an increase of temperature as quickly as possible. Under real-life conditions the temperature increase would likely be detected at a later stage due to a less perfect position of the sensor. Thus, temperature monitoring could be even slower than recorded here.

The beetles were detected at an early stage at distances of 0.22 to 0.27 m from the infestation and even at distances of 0.62 m from the infestation acoustic detection was earlier than by temperature.

During the 1 m³ box experiment, the microphone inside the tube detected more signals than the one placed directly inside the wheat. This might be due to the larger surface of the tube that bundles the signals from a larger area. However, it could also be because the microphone in the tube was closer to the release point of beetles (microphone in tube 0.21 m, microphone in substrate 0.37 m). Additionally, it is not known how evenly the beetles spread from the position of the initial infestation and therefore how many beetles were close to which microphone when signals were detected. But since a microphone directly inside a stored grain mass would be very susceptible to

dust and tractive forces during grain loading and unloading, the tube would be useful to provide protection for the highly sensitive equipment and might also have acoustical advantages.

The tube trap greatly increased the detection as long as the number of beetles was small and even one beetle in the trap caused strong signals. At a later stage of infestation, the trap function was negligible, with still high numbers of signals after removal of beetles from the trap.

The calculated number of beetles for the experiments was important to get an impression about the population size and the differences between the two experiments. Since the program was not developed for experiments like the one described above, there is an important flaw. While it is possible to enter the number of beetles at the start of the experiment as a basis for the population, it is not possible to subtract the number of beetles removed from the trap. Especially in the first weeks of the experiment with only 200 adult beetles in the box, even small numbers of removed beetles will alter the size of the developing population. Therefore, the population size given in the results is likely to be overestimated.

The results indicated that the described acoustic system might be a suitable method for early detection of insects in storages. In a next step, the developed "Beetle Sound Tubes" will be installed in silos and tested with automatic signal detection software to provide farmers and storekeepers with detailed information about infestation and possible treatment.

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Controlling insects in stored grain by disturbing the grain

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Abstract

Insects can cause damage to stored grain, especially on smallholder farms in the tropics. *Sitophilus zeamais* (maize weevil, MW) and *Rhyzopertha dominica* (lesser grain borer, LGB) are often involved. Our objective was to determine, by four experiments, if physical disturbance of grain can control these pests. In Experiment 1, 2.6-L unsealed recycled coffee cans were each loaded with 1 kg of maize and 25 live adult MW/kg. Every 12 h, disturbed treatment cans were manually rolled through one circumference. After 160 d, live MW numbers had been reduced by 93% compared to undisturbed cans. In Experiment 2, MW-infested maize was placed in 20-L plastic cans and stored by farmers in Tanzania. Each farmer had three cans. Two were disturbed by shaking morning and evening and the third was left undisturbed. After 90 d, MW populations had increased in the undisturbed containers, but had decreased to zero in every disturbed container. In Experiments 3 (and 4), maize (wheat) infested with 25 adult MW/kg (LGB/kg) was placed in six boxes. Three of the boxes were disturbed every 12 h by use of Sukup motor-driven grain stirrers; the other three were undisturbed. After 120 days, MW numbers in undisturbed boxes had increased, but were zero in stirred boxes. In Experiment 4, 80-d samples showed increased numbers of LGB in undisturbed boxes but reductions of over 98% in stirred boxes. Quality of disturbed grain was similar or better than that of undisturbed grain. This work suggests that grain disturbance may be an effective non-chemical, non-hermetic physical approach for control of stored grain insects.

Keywords: maize, wheat, maize weevil, lesser grain borer, grain disturbance, postharvest loss

1. Introduction

About 70 million Mg of maize and 25 million Mg of wheat are grown in Africa each year (FAOSTAT, 2014; USDA, 2018). Postharvest dryweight losses for maize and wheat in Africa for 2016 are estimated at 18.8 and 13.6%, respectively (APHLIS, 2018). Without proper management, losses for an individual producer can reach 100%. A large contributor to the postharvest loss in maize is *Sitophilus zeamais*, the maize weevil (MW). Female maize weevils deposit eggs in holes bored into the grain and seal each hole with a protective gelatinous plug (Danho et al., 2015). Upon hatching, larvae feed on the endosperm of the kernel, and leave as adults through an exit hole. Maize weevils will over time totally destroy stored maize. One of the main contributors to postharvest loss in wheat is *Rhyzopertha dominica*, the lesser grain borer (LGB) (Government of Canada, 2013). Female grain borers deposit up to 500 eggs loosely onto kernels of grain and the egg stage lasts about 32 days. Larvae then eat into the wheat kernels where they complete their development. Adults emerge by chewing through the outer grain layers and can live up to 240 days (Akol et al., 2011). LGBs feed on the grain and leave behind empty husks and flour. Hermetic storage and use of insecticides are effective approaches to prevent or control insects in grain stored on smallholder farms, but each has their issues. Maintaining hermetic conditions in a container is difficult. Purchase of insecticides is a troubling recurring cost, toxic effects to people are possible due to misuse or residue, insect resistance can develop, effective insecticides may not be available, and fumigants may have environmental effects. Another approach that can be effective for smallholder farmers and others is physical disturbance that is an action such as tumbling or stirring that causes kernels to change position. This disturbance does not involve use of chemicals and it can be accomplished many different ways. Quentin et al. (1991), working with common beans infested with the common bean weevil, *Acanthoscelides obtectus* (Say), investigated the effect of disturbance by bean tumbling to control these storage insects. They hypothesized that when beans are physically disturbed numerous times, weevil larvae die due to exhaustion before gaining access to the cotyledon. The experiment consisted of tumbling storage containers loaded with beans and bean weevils every eight hours. A 95% or greater overall mean reduction in bean weevil population was achieved due to storage container physical disturbance. This paper describes four experiments carried out with the objective of determining the effectiveness of disturbance for control of maize weevils in stored maize and for control of lesser grain borers in wheat. Grain quality parameters (moisture content, fine material and test weight) were measured as part of each of the experiments, but only insect mortality is discussed in this paper.

2. Materials and Methods

Experiment 1 Materials and Methods

Recycled 2.6-L plastic ground coffee containers were used to hold the maize and weevils (Fig. 1). The containers had two internal baffles at approximately 120^0 apart as part of the container design. A third 1.5-x-1.5 x 10-cm wood baffle was affixed in by means of screws to ensure thoroughly mixing. One 10-cm diameter round hole was cut through each lid and screen was glued over the holes with silicon glue to allow air circulation while preventing escape of weevils. The lids with screens were held on the containers by two rubber bands per container. Commercial comingled bulk maize used in the experiment was purchased from West Central Coop Elevator (1095 T Ave, Boone, IA 50036).

Fig. 1. Experimental containers for Experiment 1 (Bbosa et al., 2014).

Each plastic container was loaded with 1.00 kg of 13.6% (w.b.) moisture maize that left approximately a quarter of the container volume unoccupied for thorough mixing while being turned. Maize weevils for this experiment *(S. zeamais)* were obtained from a supply maintained in maize of 10-14% (w.b.) moisture content at 27°C by the Department of Agricultural and Biosystems Engineering at Iowa State University. The experiment consisted of two treatments: undisturbed (control) containers and disturbed containers with three replications of each container, and four different storage times (40, 80, 120 and 160 days), totaling 2x3x4=24 containers. Twenty five live unsexed adult weevils were loaded into each of the containers, which were then randomly laid longitudinally in a chamber maintained at 27^0C. Humidity was not controlled in the chamber. Every 12 h, the disturbed treatment containers were manually rolled through one circumference (15.6 cm diameter or 49 cm). At 40, 80, 120 and 160 d, three undisturbed (control) containers and three disturbed containers were picked randomly from the experimental chamber for data collection. Weevil mortality and grain quality parameters were determined. A two-way ANOVA was performed and Tukey's means comparison was used to detect statistical significance in treatments at α=0.05 using JMP Pro 10.

Experiment 2 Methods and Materials

Experiment 2 was conducted over a three months period in three maize-producing regions (Manyara, Dodoma and Morogoro) of Tanzania. For each region, one major maize-producing district was selected. Then one ward was selected and from each ward, and three small-holder maize farmers were randomly chosen. Each farmer was given twelve plastic containers—nine for treatments and three for control. The study consisted of two treatments: disturbed and control. A total of 108 clean 20-L plastic containers (36 per region) were used. Each container was loaded with 10 kg of fresh white maize and 0.50 kg of white maize infested with mixed-aged adult *S. zeamais*. The initial numbers of *S. zeamais* were determined (Tab. 1). The disturbed containers were disturbed twice a day (12 hours apart), whereas the control containers were not disturbed until the end of the

study. At the end of each storage time (30, 60 and 90 days), three treatment containers and one control from each farmer were randomly opened and the number of live and dead *S. zeamais* were determined. Data collected were analyzed using the Statistical Analysis System (SAS) software using α = 0.05.

Tab. 1 Initial numbers of *S. zeamais* in each region per 0.5 kg of infested maize for Experiment 2 (Suleiman et al., 2016).

Storage Time (days)	Dodoma		Morogoro		Manyara	
	Control	Disturbed	Control	Disturbed	Control	Disturbed
30	89	53	28	21	75	30
60	52	54	25	27	73	41
90	74	51	23	20	120	86

Experiments 3 & 4 Materials and Methods

These two experiments used the same equipment and procedure. Grain infested with 25 insects/kg was loaded in six 104 cm x 13 cm x 76 cm boxes in a 27^{0}C laboratory. Experiment 3 used maize and maize weevils; Experiment 4 used wheat and lesser grain borers. Three of the boxes in each experiment were disturbed by use of commercial Sukup electric motor-driven grain stirrers, i.e., one stirrer per box (Sukup Manufacturing Co., 2014) every 12 hours; the other three control boxes in each experiment were left undisturbed. Every 40 days, all the boxes were sampled using a grain probe.

Samples were analyzed for presence of live insects and for grain quality parameters.

3. Results

Experiment 1 Results

At 40 d, the live maize weevil mean declined from 25 to 11±1 in the undisturbed, and to 6±3 in the disturbed treatment, however this difference between treatments was not statistically significant (Tab. 2). By 80 d, the undisturbed population rebounded to 15±2, while the disturbed population dropped further to 1±2, where it remained through 120 d. The disturbed treatment population reached 3±2 at 160 d. It is unclear whether this slowly increasing trend would continue if the maize were stored longer. For 120 and 160 d storage periods, undisturbed containers showed a continued increase in the number of live weevils whereas in the disturbed containers numbers remained low. Live weevil means were not significantly different at 0 and 40 d between treatments but were significantly higher for the undisturbed treatment at 80 (p=0.0016), 120 (p=0.0030) and 160 d (p=0.0006). After 160 days, live weevil means in the disturbed containers were 7% of those in the undisturbed containers. An analysis of the results with time was also done for each treatment (Tab. 2). In the undisturbed treatment, there were no significant differences between 0, 40 and 80 days. Live weevil means were not significantly different between 120 and 160 days, but these values were significantly higher than those for 0, 40 and 80 days. The live weevil means in the disturbed treatment were significantly lower at all times after 0 day.

Tab. 2 Comparison of means of live weevils over time for disturbed versus undisturbed (control) treatments for Experiment 1 (Bbosa et al., 2014).

Item	Treatment	Storage Time (days)				
		0	40	80	120	160
Number of live weevils/kg	Undisturbed	25±0[Ab]	11±1[Ab]	15±2[Ab]	40±8[Aa]	44±5[Aa]
	Disturbed	25±0[Aa]	6±3[Ab]	1±2[Bb]	1±2[Bb]	3±2[Bb]

Each value within the table is the mean ± standard deviation of three replicates. Means not followed by the same upper case letter between treatments or not followed by the same lower case letter within each treatment indicate significant difference at the 0.05 level.

Experiment 2 Results

Tab. 3 shows the number of live insects throughout the study. For all control containers, insect numbers increased significantly between 30 and 60 days, and between 60 and 90 days. For the disturbed containers, there were no live weevils in any containers in any region after 90 days. Weevil numbers did not decrease significantly after 30 days in any region except Dodoma.

Tab 3. Number of live *S. zeamais* in maize for Experiment 2 (Suleiman et al., 2016).

Storage Time (days)	Control containers			Disturbed containers		
	Dodoma	Morogoro	Manyara	Dodoma	Morogoro	Manyara
30	20 ± 8^c	9 ± 2^c	12 ± 4^c	10 ± 2^a	2 ± 1^a	3 ± 1^a
60	68 ± 31^b	49 ± 35^b	77 ± 44^b	2 ± 1^b	5 ± 1^a	0 ± 0^a
90	109 ± 22^a	119 ± 35^b	152 ± 36^a	0 ± 0^b	0 ± 0^a	0 ± 0^a

Each value within the table is the mean ± standard deviation of three replicates. Means not followed by the same lower case letter in each column indicate significant difference at the 0.05 level.

Experiment 3 Results

After 40 days, live MW population means in unstirred control boxes decreased significantly to 1.7 per kg of maize but then rebounded significantly to 18 after 80 days (Tab. 4). No live MW were found in any of the stirred box samples after 40 or after 80 days. The experiment was terminated after 80 days. Stirring greatly reduced or eliminated maize weevils in the stirred boxes.

Experiment 4 Results

The 40-day samples of wheat from the three control boxes all contained multiple LGB, while there was a total of one LGB in the stirred box samples (Tab. 5). The mean of the control group was significantly greater than that of the stirring treatment. At 80 days, stirring was stopped and the stirred boxes were undisturbed for the next 40 days to see if eggs and larvae would emerge as adults. There were not significant differences found between the stirred and control treatments, although control box means are far higher than stirred box means. This is presumably because of the high standard deviations among the control replicates at both 80 and 120 days. Further analysis of these data is underway. Discarding of one or two outlier data points may be justified and may result in significant differences between treatments at 80 and 120 days.

4. Discussion

Assuming further analysis concludes there are significant differences between treatments after 80 days for Experiment 4, there is evidence from these four experiments that disturbance is effective in controlling maize weevil in stored maize and lesser grain borer in stored wheat. Further research will be needed to determine how disturbance can be carried out in larger grain containers. One untested possibility is to use grain stirring machines in conventional steel grain bins to carry out disturbance.

Tab. 4 Means comparison of live weevils for stirred versus unstirred (control) containers in Experiment 3 (Rau et al., 2018).

Item	Treatment	T=0 d	T=40 d	T=80 d
	Control	25 ± 0^{Aa}	1.7 ± 0.6^{Ab}	18 ± 4.0^{Ac}
Number of live weevils per kg maize				
	Stirred	25 ± 0^{Aa}	0 ± 0^{Ba}	0 ± 0^{Ba}

Each value within the table is the mean ± standard deviation of three replicates. Means not followed by the same upper case letter between treatments or not followed by the same lower case letter within each treatment indicate significant difference at the 0.05 level.

Tab. 5 Comparison of means of live lesser grain borers over time for disturbed versus undisturbed wheat in Experiment 4.

Item	Treatment	T=0 days	T=40 days	T=80 days	T=120 days
Number of live lesser grain borers/kg	Control	25 ± 0^{Aa}	11 ± 2.5^{Aa}	131 ± 110^{Aa}	91 ± 99^{Aa}
	Stirred	25 ± 0^{Aa}	1 ± 1.6^{Bc}	2 ± 1.7^{Ac}	8 ± 1.7^{Ab}

Each value within the table is the mean ± standard deviation of three replicates. Means not followed by the same upper case letter between treatments or not followed by the same lower case letter within each treatment indicate significant difference at the 0.05 level.

This technology is currently available. In all four experiments, quality of disturbed grain was similar or better than that of grain in undisturbed containers, except fine material in the stirred boxes which was higher than in the undisturbed boxes. In three of the experiments, we observed a drop in live insects from initial numbers in the control containers during the initial storage periods. Bbosa et al. (2014) also observed this decrease in an experiment with steel barrels. This decrease probably happens because some adult weevils die before adult weevils from eggs deposited in this new environment begin to emerge. All of these experiments employed a 12-hour disturbance interval, although we did not have a solid reason for choosing this interval. Quentin et al. (1991) found an eight-hour interval to be effective for control of bean weevils in stored beans. Additional research is needed to understand why disturbance is effective and to identify an optimum disturbance interval.

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The Adoption of Thermosiphon Powered, Ground Level Phosphine Application Systems in Australia.

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Abstract

Safe storage of grain on Australian farms requires a sealable silo to exclude grain insects and enable effective fumigation to avoid the development of insect resistance to phosphine.

A sealable silo must also be fitted with a pressure relief system that allows air to enter the silo rapidly, to avoid damage to the silo fabric, in the event of a sudden temperature drop and subsequent contraction of the internal atmosphere.

The most effective pressure relief system allows air into the headspace by a pipe attached to the silo wall, which is connected to an oil bath valve at ground level to facilitate servicing. The oil bath valve prevents grain insects entering and will allow air to bypass when the internal pressure exceeds or falls below 30 – 40 Pascals.

The addition of a pipe connecting the headspace pipe to the base of the silo creates a gas recirculation loop. Ambient temperature influences the air within the external pipe and Thermosiphon currents are created, circulating the internal atmosphere.

In 2004, a silo manufacturer in Western Australia proposed such a recirculation loop adding an aluminium phosphide (AlP) reaction chamber into the circuit at ground level. A ground-level application system removes the need to climb the silo, making fumigation simpler and safer.

Initial experiments in 2005 revealed that the phosphine gas would be extracted from the reaction chamber by Thermosiphon air movement, without building to dangerous concentrations.

Seven silo manufacturers across Australia have adopted the Thermosiphon recirculation system linked to a ground level AlP reaction chamber, producing nearly 12,000 transportable silos of 80 – 100t capacity in that period.

The development of the recirculation system and effectiveness of Thermosiphon gas distribution is discussed in this paper.

Keywords: sealed silo, Thermosiphon, recirculation, Aluminium Phosphide, Phosphine.

1. Introduction

Storing grain in Australia is characterised by the challenges of grain insect attack, similar to all warm climate countries in the world. The grain is harvested warm in early summer and remains warm in the silos unless aeration is used but this has limitations in the ability to cool the grain until winter occurs some 3 – 4 months later. Stored grain insects are endemic in the environment, and present a constant threat to stored food products.

To prevent insect attack and enable effective fumigations, sealed gas-tight grain stores have been a feature of the Western Australian grain industry since the 1980's when the central storage operators, Cooperative Bulk Handling of Western Australia, decided to seal all permanent grain storage (Newman, 2006). A 'nil-tolerance' of stored grain insects in grain delivered to the central system was established.

To meet this standard, higher quality of grain storage on farms was needed and assistance was co-opted from local silo manufacturers to produce sealable grain silos (Newman, 1997). The most common types of grain silos in Western Australia are <100t capacity, assembled in a factory and delivered on hydraulic trailers in one piece to the farm, ready to be set up for storage and fumigation. The factory construction process enables a high quality product to be manufactured and sealed to a gas-tight pressure test standard (AS 2628 - 2010).

A sealable silo must be fitted with a pressure relief system to avoid damage to the silo fabric. It allows air to enter the silo rapidly in the event of a sudden temperature or atmospheric pressure drop and subsequent contraction of the internal atmosphere.

The most effective pressure relief system allows air into the headspace via a pipe attached to the silo wall, which is connected to an oil bath valve at ground level to facilitate servicing. The oil bath valve prevents grain insects entering and will allow air to bypass when the internal pressure exceeds or falls below 30 – 40 Pascals. One silo manufacturer in Western Australia (WA) Moylan Silos, based at Kellerberrin, created an efficient pressure relief system using a 90 mm PVC pipe to the headspace coupled to a PVC oil bath valve at ground level (Fig. 1). Many thousands of these silos were produced and remain in use on farms.

Fig 1.   Fig 2

To initiate fumigation in these silos, fumigators attach a safety harness, carry the required amount of AlP to the top of the silo, fit on a personal air-purifying respirator, spread the solid formulation onto a wide tray in the headspace of the silo and close and seal the top hatch (Fig. 2).

2. Ground level phosphine application system proposed

In 2004, Mr Don Bird, owner of Bird's Silos, Popanyinning, WA proposed a recirculation loop, connecting a 90 mm headspace pipe to the base of the silo as a conduit for gasses and adding an AlP reaction chamber into the circuit at ground level (Boland, 1984). Ambient temperature influences the air within the external pipe and Thermosiphon currents are created, moving the gas up or down the pipe depending on the temperature gradient with the commodity. The ground level phosphine application system make the fumigation safer for the fumigator, removing the need to climb the silo. Simultaneously in 2004 a company in WA created a translucent, diesel resistant, Linear Low Density Poly Ethylene oil bath pressure relief valve (PRV) to fit to a 90 mm headspace pipe (Fig. 3). This enabled instant inspection of the oil levels in the valve to ensure the air entering will by-pass at a safe pressure and can also be used as a manometer for pressure testing.

Fig 3.

Fig. 4

3. Methods

In January 2005, a 90.9 m^3 elevated silo was prepared for a pilot trial of the Thermosiphon ground level phosphine application system on a farm at Yornaning, WA. A phosphine reaction chamber was constructed consisting of a metal box with a shelf to hold the AlP tablets and 32 mm internal diameter flexible tubing entering each side of the box to allow air to flow through. This phosphine reaction chamber was placed underneath the silo and the flexible tubing was connected into the base of the silo and to the headspace pipe (Newman et al., 2006). The gas concentrations were measured with a Spectros Non-dispersible Infrared Phosphine Monitor (Supplied by Fosfoquim of Chile), which could record phosphine concentrations up to 10000 ppm. The Thermosiphon pipe attached to the silo wall connecting the headspace to the phosphine reaction chamber was constructed of white PVC and included a translucent PRV.

Phosphine tablets at a rate of 1.5 g/m^3 (#130) were spread on solid trays in the phosphine reaction chamber. Peaks ranging up to 7000 ppm in the phosphine chamber were observed when the air ceased to move in the headspace pipe. This happened twice daily as the airflow direction reversed due to the change from diurnal or nocturnal ambient conditions and air moved up or down the pipe. There was a concern that the flexible tubing connecting the phosphine reaction chamber to the Thermosiphon pipe was restricting the airflow allowing higher concentrations of phosphine to occur.

The next experiment on the same farm in a similar silo incorporated a black painted PVC Thermosiphon pipe and an application rate of 1.1 g/m^3 (#100) using the same phosphine reaction chamber. The silo experienced lower peaks of up to 2300 ppm, which was due to a lower rate of application and faster air movement in the black Thermosiphon pipe.

In February 2005, airflow monitoring was conducted on a black coloured 90 mm PVC Thermosiphon pipe attached to a 90.9 m^3 capacity silo. The pipe to the headspace was connected to a 40 mm steel pipe into the base of the silo. Measurements were taken over 24 hours of the air flowing through the Thermosiphon system using a Kurz hot wire anemometer. The results showed the air moving

under favourable warm ambient conditions and stopping completely when the ambient and commodity temperatures were similar (Tab. 1).

Table 1.
Thermosiphon air speed test, February 26th 2005, E Popanyinning WA
Silo pressure test >180s, Barley @ 9.6% m.c. and 29°C

Weather	Time	Ambient °C	Ambient wind speed kph	Airspeed tube m/s (32 mm orifice)	Metres/s 90 mm pipe	Litres / min 90 mm pipe	m/3/hr
Fine	12:30	31	7.2	0.55	0.068	25.95	1.55
Fine	13:30	34	8	0.6	0.074	28.24	1.69
Fine	14:30	36	11	0.45	0.056	21.37	1.28
Fine	15:30	35.5	22	0.48	0.059	22.521	1.35
Fine	16:30	36	13	0.45	0.056	21.37	1.28
Cloud	17:30	35	16	0.29	0.035	13.6	0.8
Cloud	18:00	34	18	0.29	0.035	13.6	0.8
Cloud	18:30	33.5	15	0.21	0.026	9.9	0.59
Cloud	19:00	33	7	0.19	0.023	8.7	0.53
Cloud	19:30	32	6	0.11	0.014	5.3	0.32
Cloud	20:00	31	5.5	0.09	0.011	4.2	0.25
Cloud	21:21	30	0	0		0	
Cloud	22:00	29	0	0		0	
Cloud	23:00	28	0	0		0	
Part cloud	0:00	26	3	0		0	
Part cloud	2:00	24	6	0.04	0.005	1.9	0.11
Part cloud	4:00	24	5	0.06	0.007	2.67	0.16
Part cloud	6:00	23	0	0.1	0.012	4.6	0.27
Cloud	7:00	26	2	0.05	0.006	2.3	0.14
Cloud	8:00	28	8	0.08	0.01	3.8	0.23
Cloud	9:00	30	15	0.19	0.023	8.7	0.53
Cloud	10:00	31	20	0.15	0.018	6.8	0.41
Rain	11:00	27	9	0.04	0.005	1.9	0.11
Rain	11:30	27	7	0		0	

A fumigation was commenced in the same silo the following month when AlP tablets at a rate of 1.5 g/m³ were placed on the sealing plate (Fig. 4) at the base of the silo. The space between the seal plate and the grain control 'butterfly valve', provided adequate space as a phosphine reaction chamber. In this experiment gas concentration reached a maximum of 3500 ppm in the phosphine reaction chamber and up to 1750 ppm in the headspace (Fig. 5). A test in a similar silo the following summer produced similar results with the characteristic high and low peaks in the phosphine chamber and even concentrations in other parts of the grain mass.
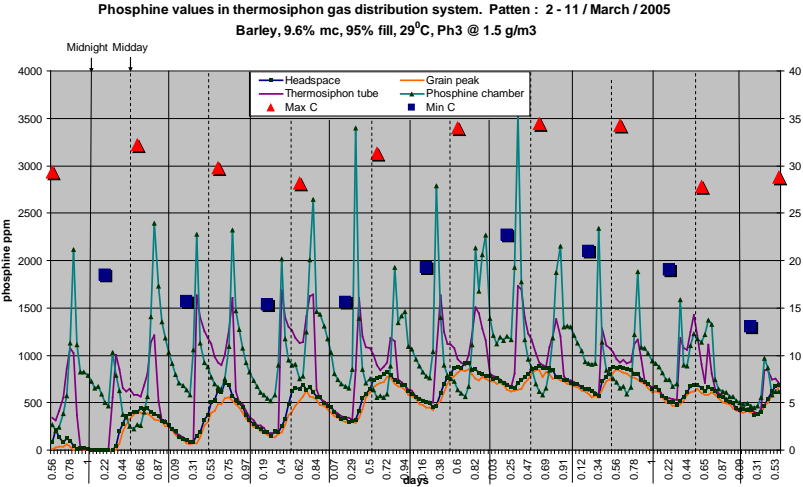
Fig. 5

The low concentrations in the phosphine reaction chamber shown in Tab. 2 are caused by the air flowing rapidly up the Thermosiphon pipe in the morning as the sun heats the pipe. The high concentrations in the evening are a result of the air moving down the pipe as the ambient temperature falls below that of the stored commodity. The even concentrations of gas in the grain mass demonstrate the mixing effect of the Thermosiphon induced air currents. This is in contrast to a 'top loaded' AlP fumigation where there is an initial high concentration in the headspace, which reduces over time as convection currents and diffusion carry the gas to the lower parts of the silo.

The results of these experiments demonstrated to Bird's Silos that the system was safe, efficient at moving released phosphine gas into the grain profile and removed the need to climb the silo to apply AlP into the headspace. The company modified the production line to produce all silos from their factory with the Thermosiphon powered ground level phosphine application system.

An important modification to the new silos was a deep bowl phosphine reaction chamber to hold a 'Bag Chain' formulation of AlP or a removable perforated steel plate in the base to hold the tablet formulation of AlP and allow the powder to drop through as phosphine gas is generated (Figs. 6a & 6b). In addition, the top lid on the silo was fitted with a cable and winch device, which is operated at ground level to open, close and seal the top lid also without having to climb the silo.

Fig. 6a &6b

4. Ground level phosphine application system adopted by other silo manufacturers

A silo manufacturer in South Australia showed interest in the system and a cooperative arrangement with Bird's Silos was established to share the technique. A silo company in Victoria also became interested in the system and came to an agreement with Bird's Silos to share the information. This company was part of a larger corporation controlling three independent manufacturing plants in three other Australian states who also adopted the ground level application system.

Modifications to the phosphine reaction chamber and connections have been made by silo factories across the country but retain the principle of the system with a 90 mm headspace pipe connected to the base of the silo. Fig. 7 shows one of the variations of the phosphine reaction chamber at ground level.

Six silo manufacturing plants around Australia have now adopted the translucent PRV and statistics provided by the manufacturing company indicate that up to December 2017 approximately 6500 silos have been fitted with the Thermosiphon powered ground level phosphine application system. In addition, Moylan Silos in WA, who first fitted the 90 mm headspace pipe, have also created a ground level phosphine application system but retained the PVC, PRV (Fig. 8) and retrofitted it to all silos produced since 2009. In that period, they have produced approximately 5400 silos. Across Australia there are now approximately 12,000 silos fitted with the ground level phosphine application system with Thermosiphon distribution.

Fig. 7

Fig. 8

5. Inert atmosphere application

The simple addition of three ball valves into the lower Thermosiphon pipe allows purging of the silo atmosphere at ground level through one of the valves while loading gas such as nitrogen (N_2) or carbon dioxide though one of the other valves (Fig. 9). The purging valve provides a convenient point at which to measure the composition of the internal atmosphere. When using inert gasses in a sealable silo, the halving pressure test must be elevated to a minimum of 5 minutes to avoid oxygen (O_2) ingress over the longer fumigation periods required.

Application of N_2 using a 30 m^3 per hour Pressure Swing Absorption N_2 generator (Fig. 10) into a 90.0 m^3 silo takes approximately 2.5 hours with an additional 0.5 hour the following day to remove the oxygen desorbed from the grain and retain the O_2 concentration at 1% (Newman, J – personal communication). Measurements over the succeeding 28 days showed even concentrations at all points as the gas was recirculated by the Thermosiphon pipe with a slight decay to ~3% O_2.

6. Testing of the Thermosiphon system at Kansas State University (KSU), USA

In 2015, a 63 m^3 Bird's Silo was transported from Popanyinning, WA to KSU, to conduct detailed analyses of the Thermosiphon ground level phosphine application system. The silo was shipped in pieces and assembled on site by a group of people including Mr. Don Bird and the author. Sealing on site was more complicated and the standard achieved in the factory could not be emulated in the field. In addition, a locally manufactured 71.9 m^3 SCAFCO silo was assembled on the site and sealed as it was being constructed; however, it was not designed to be sealed and required considerable innovation on site to achieve a seal (Fig. 11). The result was that both silos were sealed to a lower standard than required in Australia under AS 2628 (5 minutes halving pressure for a newly constructed silo).

Mr. S. Cook commenced experiments in August 2015 as part of a Master of Science degree (Cook, 2016). The experiments were conducted with solid formulation AlP, gaseous phosphine and sulfuryl fluoride. AlP tablets were applied in the ground level phosphine reaction chamber, gaseous

phosphine was applied via the Thermosiphon pipe and sulfuryl fluoride was applied through one of the monitoring lines directly into the grain. The silos were set up to include a ball valve in the lower pipe so that the gas recirculation could be studied with and without Thermosiphon.

Fig. 9

Fig. 10

Fig. 11

Experiments showed the Thermosiphon effect moving phosphine gas upward into the headspace during the period when the sun was warming the external pipe and concentrations rising in the lower parts of the silo in the cool evening as the air flow reversed and moved released gas out of the phosphine reaction chamber. When the Thermosiphon was turned off, the phosphine was forced to move upwards through the grain mass taking some time to reach lethal concentrations in

all parts of the silo, relying only on thermal convection currents. Air speed velocities in the Thermosiphon pipe were between 0.02 – 0.08 m/s under sunny conditions and 0.01 – 0.02 m/s in partly cloudy conditions (Cook, 2016).

7. Discussion

The development of the Thermosiphon ground level phosphine application system has made Australian grain silos safer for the fumigator and grain manager. Experiments in Australia on silos up to 1200 t have demonstrated that a Thermosiphon system provides effective recirculation without the use of electrically powered fans (Newman, 2012).

The addition of a Thermosiphon pipe to any silo ensures continuous mixing of the internal atmosphere and has been shown to be effective when used as the delivery conduit for a gaseous phosphine application. The gas is injected into the silo and the aeration fans operated with all seal plates in place to circulate the gas for 60 – 90 minutes, after which the aeration fans are turned off. The gas continues to circulate as powered by Thermosiphon alone, producing even concentrations throughout the silo for the remainder of the fumigation period (Ball, S personal communication).

Research at KSU demonstrated the effectiveness of the Thermosiphon powered ground level application system in distributing phosphine rapidly throughout the grain bulk. In that experiment, turning off the Thermosiphon air currents demonstrated the slower incorporation of phosphine by thermal air currents and diffusion alone. In comparison, with the Thermosiphon operating there was rapid mixing of the phosphine gas throughout the silo.

Future developments that could be explored to reduce the need to climb the silo include using the headspace pipe as conduit for extracting grain odours or carbon dioxide to determine grain quality and presence of mould or insects. Custom-made pheromone traps inserted into the headspace pipe at ground level would attract grain insects, providing a decision tool to initiate a fumigation procedure.

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Lessons learned for phosphine distribution and efficacy by using wireless phosphine sensors

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Extended abstract

1. Introduction

Phosphine is by far the most commonly used fumigant for disinfestation of stored grains, pulses etc. and also of dry processed commodities (Fields and White, 2002; Opit et al., 2012). For instance, approximately 80% of the grain production in Australia is fumigated with phosphine (Collins et al., 2001). It is a colorless, odorless and flammable toxic gas (Chaundhry, 2000). Phosphine is generally cheap, easy to apply for most durable commodities and it is effective for all life stages for nearly all the major insect pests, whereas it leaves minimal residues (Chaundry, 2000; Hasan and Reichmuth, 2004; Wang et al., 2006; Nayak and Collins, 2008). However, the extensive use of phosphine, in conjunction with low concentrations and poor sealing, has raised resistance issues and may lead to serious fumigation failures (Zeng, 1999; Collins, 2009). Currently, resistance by various storage insect populations is a reality in several parts of the world (Collins et al., 2002; Daglish, 2004). There are many traditional techniques available for monitoring gas concentration such as digital monitors that are placed outside of the treated area and glass tubes that are used to quantify concentration by sucking air from the treated substrate. Both methods are difficult in their use, often inaccurate and they need specialized personnel. Despite the fact that there are different types of electronic equipment that can be used to estimate phosphine concentration, the majority of them cannot be placed inside the treated area, due to the corrosiveness caused by this gas.

Recently, phosphine wireless sensors that can be placed inside the treated area have been developed and evaluated with success in storage facilities in Greece (Athanassiou et al., 2016). This initial work clearly indicated that gas concentration is uneven in the treated area, and that further experimental work is needed to evaluate its distribution. Moreover, it has been reported that inside a flour mill in the Czech Republic phosphine concentration varied remarkably, and the main factors for these variations were temperature and relative humidity gradients (Aulicky et al., 2015). Phosphine distribution in silos has been modelled by Isa et al., (2016) but there is still inadequate information regarding the effect of different biotic and abiotic factors towards this direction. At the same time, there are not many data available for the distribution patterns and spatio-temporal movement of phosphine in other commercial storage formations and facilities, such as containers, warehouses, silos and shipholds. Thus, the purpose of this study is to evaluate wireless phosphine sensors by estimating both gas concentration and kill rates of major stored product insects in "real world" tests.

2. Materials and Methods

2.1 Test insects

Adults of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), were used in the trials. The insects used were reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Protection and Rural Environment, University of Thessaly, at 25°C, 65% relative humidity (r.h.) and continuous darkness. For each of the above species, two

populations were used in the experiment, one field and one laboratory population, namely GA6 *R. dominica*, ASC11 *O. surinamensis*, laboratory *R. dominica* and laboratory *O. surinamensis*. The laboratory populations are being reared for more than 20 years under laboratory conditions. The field populations were collected from different storage facilities from Greece and were characterized as tolerant to phosphine. From the above species, *R. dominica* was reared on whole wheat kernels, whereas *O. surinamensis* on oat flakes.

2.2 Experimental procedure

Plastic cylindrical vials (3 cm diameter and 8 cm in height) were the experimental units for the tests; the vial neck was covered with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent insects from escaping. Each vial was filled with 10 g of commodity, i.e., whole wheat grain for *R. dominica* and oat flakes for *O. surinamensis*. Then, ten adults of each species and population were introduced into each vial (separate vials for each species and population). In each fumigation trial, the vials were placed in different locations within each facility. For each species and population three vials were prepared per location and per facility. Separate vials with insects, placed in untreated areas of each facility served as controls. Then the vials were transferred to LEAZ and adult mortality was assessed. The vials were kept in incubators set at 25°C, 55% r.h. in continuous darkness and progeny numbers were recorded 65 d later. Phosphine concentration monitoring was performed by the use of wireless sensors (Centaur Analytics Inc. CA, USA), and wireless signal amplifiers and receivers were connected to computers. The sensors were placed at various locations inside the treated area, including all the locations where insects had been placed.

2.3 Data analysis

All data, separately for each trial and insect species were submitted to Independent t-test, with insect mortality as the response variable. To determine the effect of location for each trial, data were subjected to an one-way ANOVA with insect mortality as the response variable and location as the main effect. Control mortality was generally low, so the data for control mortality were not used in the analysis. The same approach was also followed in the case of progeny production counts. Means were separated by using the Student's *t* and Tukey-Kramer HSD test at 0.05, whenever this test was considered necessary.

3. Results

Figures below show the results according to the fumigation treatment at different facilities, i.e., a warehouse, a container, two shipholds and two silos, with wireless phosphine sensors which were located in the fumigated area (Figs. 1, 2, 3, 4, 5, 6). In all cases, the mortality of control was generally low for all insect species and populations and did not exceed 10%. Regarding the fumigation which was carried out in the warehouse, complete control was detected only for the *O. surinamensis* laboratory population in contrast with the other three populations tested (Tab. 1). In that facility, the maximum level of phosphine concentration was 80 ppm for less than four days (Fig. 1). On the other hand, in the fumigated container, mortality reached 100% for all tested populations, while the concentration of phosphine was 2000 ppm for five days (Fig. 2). Furthermore, at the fumigated shipholds, where no forced recirculation system (J-system) was applied, mortality was complete (100%) only for the laboratory population of *O. surinamensis*. Moreover, progeny production in the treated substrate was lower when the J-system was applied, but parental survival could not be avoided. In these treatments, the concentration of phosphine reached 300 ppm for two days (Fig. 4). Regarding the fumigation which was carried out in the silo, the concentration of phosphine ranged between 200 and 600 ppm (Fig. 5), which clearly indicated that phosphine could not be distributed normally in the treated grain mass. The use of J-system in a silo showed that the phosphine concentration gradually increased (Fig. 6).
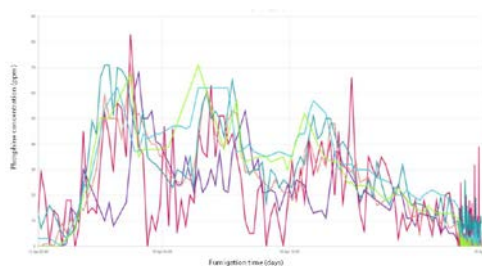
Fig. 1 Phosphine concentration during the fumigation inside a warehouse with six different wireless sensors (shown with different colors) placed at different locations.
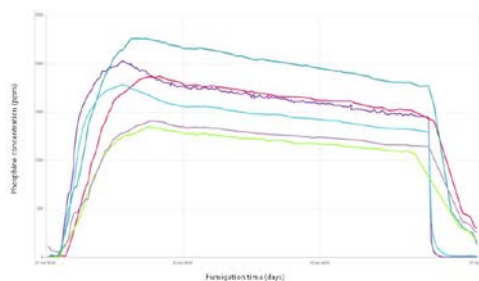
Fig. 2 Phosphine concentration during the fumigation inside a container with six different wireless sensors (shown with different colors) placed at different locations.
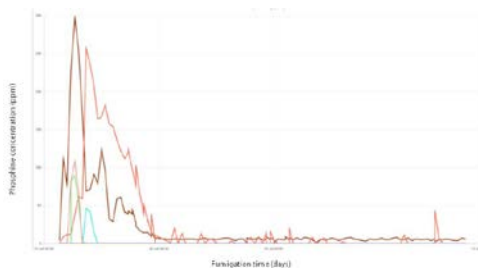
Fig. 3 Phosphine concentration during the fumigation inside a shiphold with five different wireless sensors (shown with different colors) placed at different locations.
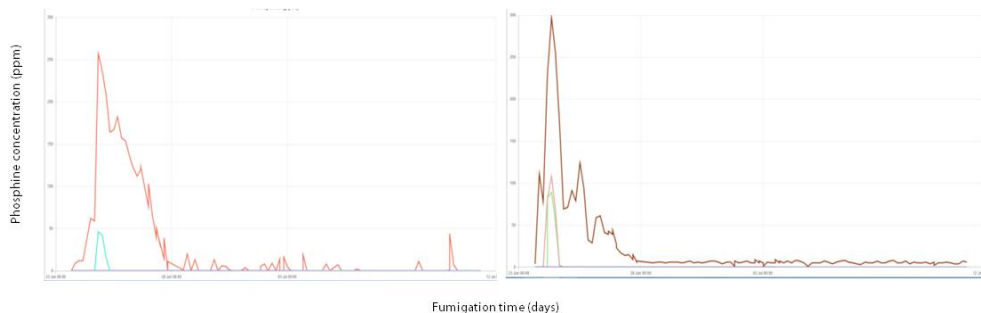
Fig. 4 Phosphine concentration during the fumigation in a ship hold with no use of recirculation system (left) with two different wireless sensors and in a ship hold with the use of a recirculation system (right) with three different wireless sensors (shown with different colors), placed at different locations.
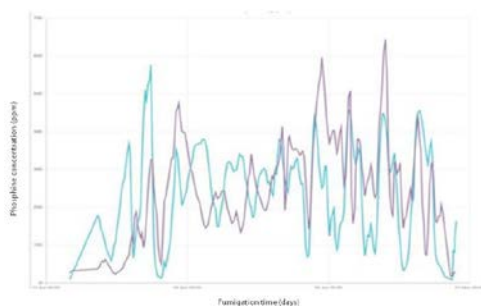
Fig. 5 Phosphine concentration during the fumigation inside a silo with two different wireless sensors (shown with different colors) placed at different locations without using forced recirculation system.
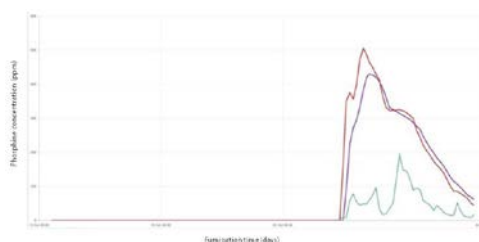
Fig. 6 Phosphine concentration during the fumigation inside a silo with three different wireless sensors (shown with different colors) placed at different locations by using forced recirculation system.

Tab. 1 Mortality (% ± SE) of parental adults for field and laboratory populations, in different facilities in which phosphine had been applied and the respective progeny production (number of adults per vial ± SE) 65 d later.

Facility	Insects	Mortality	Progeny production
Warehouse	ASC11 *O. surinamensis*	100 ± 0.0	0.0 ± 0.0
	Lab *O. surinamensis*	100 ± 0.0	0.0 ± 0.0
	GA6 *R. dominica*	100 ± 0.0	2.0 ± 0.7 a
	Lab *R. dominica*	100 ± 0.0	0.0 ± 0.0 b
Container	ASC11 *O. surinamensis*	34.2 ± 3.3 a	65.1 ± 11.4 a
	Lab *O. surinamensis*	100 ± 0.0 b	0.0 ± 0.0 b
	GA6 *R. dominica*	6.6 ± 2.2 a	48.4 ± 3.8 a
	Lab *R. dominica*	75.7 ± 4.1 b	12.6 ± 3.3 b
Shipholds	ASC11 *O. surinamensis*	not measured	0.3
	Lab *O. surinamensis*	not measured	0.0
	GA6 *R. dominica*	not measured	52.7 a
	Lab *R. dominica*	not measured	1.0 b

Within each trial and each species, means followed by different letters are significantly different. Where no letters exist, no significant differences are noted with Student's test at 0.05.

4. Discussion

In the fumigation treatments, we found high survival percentages of exposed adults and a considerable number of offspring in all cases, with the exception of the fumigations in the containers, in which complete control (100% mortality) was detected. This was partially due to the short duration of fumigation (approx. three to four days), in combination with low concentrations of phosphine in the warehouses, silos and shipholds. Phosphine leakage and sorption by the treated commodity are highly responsible for gas losses during fumigations (Bell, 2000, Aulicky et al., 2015). As a consequence, there was a sufficient number of insects that survived fumigation, and this number could gradually lead to resistance development. On the other hand, the fumigations in containers, which were the "best case scenario" here, clearly suggest that, if applied properly, phosphine can definitely lead to 100% efficacy levels. In the current trial, the container fumigation resulted in complete parental mortality, in conjunction with extremely low numbers of progeny production. In this context, for the same reasons noted above, fumigations in shipholds and silos are likely to fail due to increased leakage, which cannot be detected and quantified easily with the majority of phosphine detection techniques. In this regard, wireless phosphine sensors can be a valuable tool towards this direction (Athanassiou et al., 2016). Based on our results, in large areas, such as silos, distribution of phosphine was rather limited and thus, there were large areas within the grain mass that did not get enough gas in order to achieve a satisfactory insect mortality. The

adoption of a recirculation system in these cases can improve fumigation results. Summarizing, our tests clearly indicated that phosphine sensors were quite effective in measuring phosphine concentrations and can play an important role in the future in IPM-based programs during the post-harvest stages of agricultural commodities. Hence, sensors can be used as a "precision fumigation" tool and provide real-time estimates for insect control.

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Use of a 3D Finite Element Model for Post Fumigation Phosphine Movement Analysis

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Abstract

Phosphine is a dangerous gas commonly used in fumigations throughout the world. Grain that has not fully released the phosphine it absorbed during fumigation may continue to desorb phosphine into the headspace of a storage structure. U.S. OSHA standards for handling phosphine state the acceptable limit at 0.3 ppm. If this limit is exceeded grain handling may become dangerous. It is important to understand the process of phosphine

venting and desorption in order to ensure safe handling of fumigated grain in silos and during shipments. In order to achieve this, the venting and release of phosphine was studied on location in a well-sealed grain silo in Lake Grace, Western Australia, to serve as a set of data for verification of a computational model. This situation was then modeled using a 3D finite element model and compared to the real world results. Results were calculated using two fumigant desorption models based on previous literature, a reversed sorption model and an air-grain equilibrium model. Simulations reproduced accurate trends of desorption but did not accurately reproduce the quantity of fumigant, with a 55.5% error for the model based on reversed sorption equations and 86.3% error for the air-grain equilibrium based model. For both models, simulations were conducted to compare the effectiveness of existing grain venting regulations at producing grain that is within safe handling limits. These results highlight the necessity for continued desorption research and the importance of following venting guidelines.

1. Introduction

A successful fumigation relies on exposing each insect within a grain mass to the specific concentration of fumigant for a specified amount of time needed to kill all insects present at all life stages. There is a significant amount of literature available on the bio-efficacy of fumigants such as phosphine against a range of stored product pests at multiple life stages (Chaudhry, 2000; Price and Mills, 1988), however, information on the fumigant activity within the commodity during a fumigation is very limited. Therefore, modeling the behavior of gas fumigants in the interstitial air volume of the stored grain mass is helpful in determining what factors may cause fumigation failures, and how those factors can be affected by environmental conditions. A previous attempt at developing such a fumigation model was made by Isa et al. (2016) using the program Fluent instead of an independent computer code. They also simulated vertical gas flow in a silo using Fluent and Comsol (Isa et al., 2011). While using any of the available fluid dynamics software packages has several advantages, such as ease of use and ease of visualizing results, it has disadvantages as well. Their fumigation model simulates both sorption and leakage losses, but the leakage losses are not influenced by weather condition or operational variables which is not realistic. Since the boundary conditions are set inside Fluent, loss prediction was implemented with point losses only. The amount of loss was then controlled only by pressure half loss time. This strategy may be insufficient not only for fumigant loss that is affected by weather, but also in its inability to consider the combined effect of many small leaks over the entire external surface of the silo.

The M-L 3D finite element ecosystem model was previously developed to investigate stored grain environments and has the capacity to monitor chemical concentrations throughout the grain mass (Lawrence, 2010; Lawrence and Maier, 2011). In order for this model to accurately predict fumigant concentrations the model had to be improved with the added capacity to account for fumigant loss. The primary sources of fumigant loss are fumigant leakage from the silo and fumigant sorption into the grain.

Sorption of gas by grain was listed as one of the factors most likely to cause inadequate fumigation conditions in Australia (Darby, 2011). Wheat at higher temperature sorbed a greater amount of phosphine than lower temperature wheat. After 96 hours in a container with initially 1 mg/L phosphine, the fumigant concentration in the interstitial airspace of stored wheat at 35°C was below 0.1 mg/L, whereas in wheat at 15°C it was around 0.5 mg/L (Darby, 2011). That result was supported by Reed and Pan (2000), Sato and Suwanai (1974) and Dumas (1980) who reported phosphine sorption increased with higher grain temperature and moisture content. An increase of temperature also caused faster rates of sorption of phosphine in wheat independently from moisture content. An increase from 24°C to 35°C caused the sorption rate constant to increase from 0.0064 to 0.186 (Banks, 1986; Berck, 1968). Increased adsorption of phosphine to the surface of cereal grains with increasing temperature was also shown in Sato and Suwanai (1974).

There are a number of factors that may deter the efficacy of a fumigation where enough fumigant was applied to theoretically control the insects. According to Banks and Annis (1984) these factors are excessive overall loss of fumigant, inadequate fumigant dosage in localized regions, excessive

delay between application and fumigant reaching particular regions, or a combination of these factors occurring simultaneously. To observe whether any of these effects were occurring in a fumigation would be difficult and would require excessive monitoring of fumigant concentrations at a number of locations in the silo. Even with such controls, there could be regions that are not monitored and experience problems, or environmental conditions that are abnormal or unforeseen. How environmental factors and operational procedures influence a fumigation can be more easily and thoroughly investigated using a fumigant model that incorporates sorption and fumigant loss. A better understanding of such influences would allow applicators to take more effective corrective actions to prevent fumigation failures.

2. Materials and Methods

2.1 Effect of Sorption

To estimate sorption loss of phosphine gas in a grain silo, an equation for concentration as a function of time was obtained from Daglish and Pavic (2008). The equation is valid at a 1 mg/L application, and 0.75 fill ratio, resulting in an R^2 value of 0.96 at 25°C and 55% relative humidity. The equation presented in the literature was adjusted to fit the time step and units in the code, i.e., an hourly time step and units of kg/m^3. Additionally, to calculate the amount of phosphine lost due to sorption, the equation was modified by taking the derivative with respect to time. The resulting baseline sorption equation was:

$$C = 0.0000026e^{-0.0017t} \qquad\qquad \text{Eq 1}$$

where,

C = fumigant concentration lost [kg/m^3], t = time [h]

Fumigant sorption also varies due to other factors that are important variables in our experiment, such as temperature and moisture content of grain. To account for these variables, Eq [1] was multiplied by factors dependent on temperature and moisture content. The effect of temperature on phosphine sorption was studied by Darby (2011) who determined sorption losses at 35°C were about five times as large as losses at 15°C, at a constant equilibrium relative humidity of 65%. Therefore, this result can be modeled with an exponential equation dependent on temperature, where the value at 35°C is five times the value at 15°C. The value for this expression is set to equal one when the temperature is at 25°C, because that is the temperature of the baseline equation from Daglish and Pavic (2008). This means that when the temperature equals that of the baseline equation, the overall equation should be unchanged. The effect of moisture content on the sorption of phosphine was studied by Reed and Pan (2000). They determined fumigant loss for several temperatures at two values of wheat moisture content, i.e., 11% and 13.5%. The sorption at the higher moisture content was 1.8 times greater than the sorption at the lower moisture content at 25°C. This was modeled with an exponential equation which was set to 11.5%, the equilibrium moisture content of the wheat from the baseline Daglish and Pavic (2008) equation. The resulting equation for fumigant loss due to sorption into the grain mass when modified to account for changing temperatures and moisture contents is therefore:

$$C = 0.0000026e^{-0.0017t} * 0.13365e^{0.0805T} * 0.067e^{0.235M} \qquad\qquad \text{Eq 2}$$

where,

C = fumigant concentration lost [kg/m^3]

t = time [h]

T = temperature [°C]

M = moisture content [%], wet basis

Implementing this equation into the fortran computer code required that the fumigant concentration lost due to sorption is subtracted from the current fumigant concentration at each node for each time step but only if the current fumigant concentration at that node is higher than the sorption amount to be subtracted. If the phosphine concentration at a node is less than the concentration that would be lost to sorption, the phosphine value at that node is set to zero instead.

2.2 Effect of Silo Leakage

To estimate the amount of fumigant lost due to leakage from the silo, estimates for individual sources of leakage were taken from Banks and Annis (1984) along with additional information from the other sources to extrapolate estimates of fumigant loss as a summation of losses from various sources. The most significant sources of fumigant loss result from: (1) concentration differences between the inside of the grain silo and the ambient conditions, (2) chimney effects due to temperature differences, (3) chimney effects due to concentration gradients, and (4) wind effects.

To implement these equations into the computer code, the calculated fumigant concentration lost is subtracted from the current fumigant concentration at each node along the vertical silo wall at each time step but only if the fumigant concentration at that node is higher than the leakage amount to be subtracted. If the phosphine concentration is less than the amount to be subtracted, the concentration is set to zero.

The final equation to predict fumigant leakage from the silo due to effects of fumigant sorption and loss, and modified for changed environmental conditions is therefore:

$$C = \frac{5}{x} * \frac{Nn}{Nb} * (0.0002233e^{0.4621Sw} * Ci + 0.0000248\, e^{0.1386Tc} * Ci + 0.0000326e^{0.1386Td} * Ci + 0.0029962Ci^2) \qquad \text{Eq 3}$$

Where,

C = fumigant concentration lost [kg/m^3]

X = pressure half loss time (minutes)

N_n = number of nodes

N_b = number of boundary nodes

Once equations were developed to predict phosphine loss from sorption and leakage from the fumigation in question from factors such as the effects of weather, they could be used to determine the sensitivity of fumigations to changing environmental conditions. The original 10-day fumigation was conducted from Aug 31 to Sept 9, 2015 in Manhattan, Kansas. Weather data for that time period was acquired from the Kansas State University Mesonet database (http://mesonet.k-state.edu/). This weather data was modified by changing hourly values of each key parameter (wind speed, ambient temperature, relative humidity) by +/- 25% and +/-50%. The modified simulations were compared to a base case that used the original weather data to simulate the fumigation described in Cook (2016).

2.3 Simulated Fumigation

A mesh with 2,587 nodes was created in the Abaqus finite element software for the simulation based on the dimensions of the silo supplied. While the dimensions were modeled precisely, the major discrepancy is that this silo contains a cone shaped bottom, and our model is limited to a flat bottom silo. For this reason, the cone was left off, but the extra distance may have provided space for more mixing of the fumigant before it arrived in the region of the simulation. For this reason, fumigant was applied across the entire lower boundary of the simulation, excluding boundary nodes. Weather data for the period in question was taken from the Kansas State University Mesonet database (http://mesonet.k-state.edu/). As phosphine cannot be directly input into the model, a

phosphine application method was implemented in which the base nodes of the silo, excluding edge nodes, were set at a starting amount that was held for 24 hours to approximate the phosphine release time in the experiment (Cook, 2016).

3. Results and Discussion

3.1 Simulation Accuracy

With model parameters such as loss and leakage quantified, the model was verified by comparison of the simulated fumigant concentrations to the experimental values measured by Cook (2016) with a gas release period of 24 h. Both the experimental and simulated results indicate a rapid increase of phosphine at the beginning of the fumigation, followed by a loss of phosphine that continually slows until the end of the fumigation, as seen in Fig. 1. While the trends are similar, the maximum average concentration is greater when only considering the points available in the experimental data. This effect demonstrates the potential for over predicting phosphine concentration when not measuring points along the sidewall of the silo.

The quantitative comparisons between the measured and predicted values are based on results reflecting the same locations and time readings. The root mean square error of this verification was 47.5 ppm, the average difference was 0.1 ppm, and the average of the absolute values of the differences was 38.6 ppm. The overall average experimental fumigant concentration was 283.3 ppm, therefore, the average percentage error compared to predicted values was 13.6%. The major discrepancy between the results seems to begin the evening of the first day of fumigation, around 1830 to 1930, when the increase in phosphine in the experimental data begins slowing and the predicted data do not. This coincides with the beginning of a decrease in night time temperature. This may be similar to the night time phosphine drop noted in Australian experimental data that was made available to the authors by the PBCRC (data not shown). This culminates in the largest difference between experimental and predicted data, at 1100 the next morning, after which the experimental phosphine readings begin to climb back to levels predicted by the simulation. The low temperature that night was 21.8°C, the afternoon highs for August 31st and September 1st were around 33°C. A similar effect appears to happen at a smaller scale the next night, with the values dropping slightly and then rebounding in the morning of September 2nd. The temperature that morning was around 25°C. After the second night, night time concentration drops are not seen in the experimental data, either because they did not happen, their effect was smaller due to lower phosphine values, or they were missed due to lack of night time phosphine sampling. The net effect, however, is that the predicted values appear to be a few hours ahead of the actual values. The night time decrease may also explain why the simulation slightly over predicted the amount of phosphine reported. This, along with an over prediction of the low phosphine levels seen late in the experiment, comprise the major differences between the simulation and experiment.

3.2 Temperature of Ambient Air

Shown in Fig. 2 are the predicted average concentrations of phosphine for five simulations with a varying ambient temperature, expressed as percentages of the ambient temperature for the model verification (i.e., 100%) in degree Celsius scale. As expected, when ambient temperatures were increased, total average phosphine concentrations in the silo decreased. The effect of increasing temperatures is not directly proportional, as the effects of temperature changes decrease as the temperature increases. When temperatures were decreased, total average phosphine concentrations increased by a higher amount. Halving the ambient temperature resulted in a phosphine concentration that when averaged over all locations and times was 26% greater than the concentration from the verification. At 1.5x the ambient temperature, overall average phosphine concentration was 27% less than the overall average concentration from the verification. Fumigations with lower ambient temperatures achieved higher maximum phosphine

concentrations (416, 410, 400, 380, and 364 for 50-150%, respectively). The scale of the effects builds with time, becoming larger as the simulation progressed (Fig. 1). By the end of the simulation the percentage differences from original were 102%, 57.3%, -47.1%, and -67.9%, for the 50%, 75%, 125%, and 150% ambient temperature situations, respectively. The temperature decreases caused 57% and 102% increases in phosphine concentration for the 75% and 50% temperature cases, respectively.
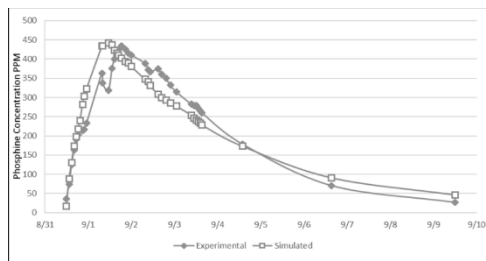
Fig. 1 – Comparison between average experimental and predicted phosphine concentration (ppm) results considering only data at the same locations and times from which data were recorded by Cook (2016) between August 31 and September 9, 2015, with a 24h fumigant release.
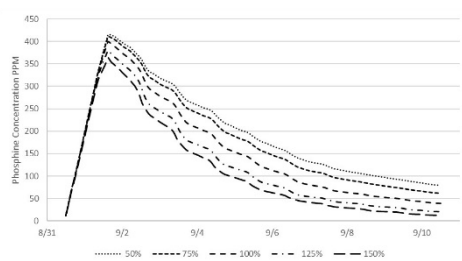
Fig. 2 – Overall average phosphine concentration (ppm) for five temperature conditions, expressed as a percentage of the temperatures (100%) used in the verification conducted between August 31 and September 9, 2015.

Increasing the ambient temperature by a factor of three (i.e., from 150% to 50%) decreased the overall average Ct-product by 71.5%, i.e., from to 47500 ppm-h to 27700 ppm-h (Table 1). Doubling the ambient temperature of the silo (i.e., from 75% to 150%) decreased overall average Ct-products by 39.1%, from 43800 ppm-h to 27700 ppm-h.

Table 1 – Cumulative average Ct-products (ppm-h) and the difference from original (%) for five ambient temperatures, expressed as a percentage of the ambient temperatures (100%) used in the verification conducted between August 31 and September 9, 2015.

	50%	75%	100%	125%	150%
ppm-h	47500	43800	37700	31400	2,700
Percent Difference	26.0	16.1	0.0	-16.6	-26.6

The primary reason for this relationship is found in the fumigant loss equation and its reliance on temperature. Based on baseline equations developed in Banks and Annis (1984), fumigant concentration loss increases as a function of grain temperature along the silo wall, and with increases in the difference between the silo temperature and ambient temperature. Additionally, temperature increases also increase the fumigant loss due to sorption as detailed in Daglish and Pavic (2008). Increasing and decreasing ambient temperature does not have an equal influence on overall fumigant concentrations. This is due to the previously discussed effect on increasing leakage from the silo. As the leakage rates increase at high temperatures, the effect diminishes because the leakage effect due to temperature difference comprises only two terms in the overall leakage equation, which is additive. This can be seen clearly in Table 1, as increasing from the 100% case to 125% had a larger effect than increasing from 125% to 150%, and decreasing from 100% to 75% had a larger effect than decreasing from 75% to 50%.

The effect of temperature is of particular interest in subtropical grain growing regions such as Australia, where temperatures are high and grain is commonly fumigated in the summer. Higher temperatures cause increased gas leakage, making sealing even more important. While high temperatures cause a decrease in phosphine concentrations in the grain mass, phosphine is more effective against insects at higher temperatures (Bond, 1989; Sun, 1946) in large part due to their increased activity and higher respiration rates. If, however, the silos were well sealed, increased

leakage caused by higher temperatures would be mitigated and insect susceptibility to the fumigant would be maximized.

3.2.3. Wind Speed

Shown in Fig. 4 are the predicted average concentrations for phosphine for five simulations with varying wind speeds, expressed as percentages of the wind speeds from the model verification (i.e., 100%). As expected, phosphine concentrations were higher for silos with lower wind speeds. Halving wind speed resulted in a phosphine concentration that when averaged over all locations and times was 10.4% greater than the concentration from the verification. At 1.5x, the same overall average phosphine concentration was 13.3% less than the overall average concentration from the verification. Fumigations with lower wind speeds achieved higher maximum phosphine concentrations (407, 404, 400, 393, and 386 for 50-150%, respectively), as leakage begins taking effect before the maximum values are reached. Percentage changes resulting from the five simulations are shown in Fig. 5. By the end of the simulation the percentage differences from original were 25%, 14%, -15%, and -29% for the 50%, 75%, 125%, and 150% wind speed cases, respectively.
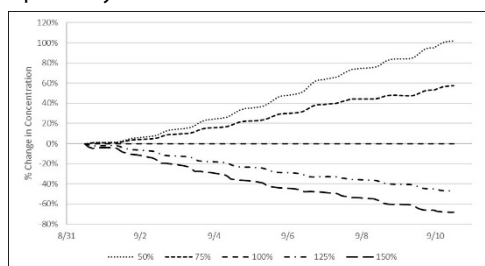
Fig. 3 – Change in overall average phosphine concentration (%) for five temperature conditions, expressed as a percentage of the temperatures (100%) used in the verification conducted between August 31 and September 9, 2015.
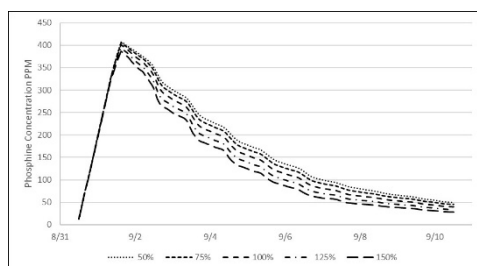
Fig. 4 – Overall average phosphine concentration (ppm) for five wind speeds, expressed as a percentage of the wind speeds (100%) used in the verification conducted between August 31 and September 9, 2015.
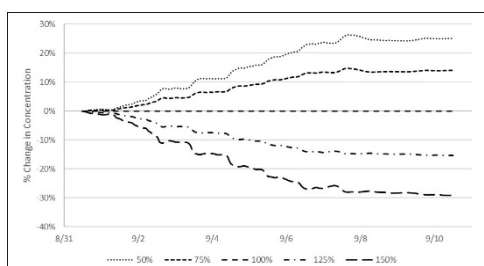
Fig. 5 – Change in overall average phosphine concentration (%) for five wind speeds, expressed as a percentage of the wind speeds (100%) used in the verification conducted between August 31 and September 9, 2015.

Increasing the wind speed by a factor of three (i.e., from 50% to 150%) decreased the overall average Ct-product by 27%, i.e., from 41600 ppm-h to 31700 ppm-h (Table 2). Doubling the wind speed of the silo (i.e., from 75 to 150%) decreased overall average Ct-products by 21%, from 39900 ppm-h to 31700 ppm-h.

Table 2 – Cumulative average Ct-products (ppm-h) and the difference from original (%) for wind speeds, expressed as a percentage of the wind speeds (100%) used in the verification conducted between August 31 and September 9, 2015.

	50%	75%	100%	125%	150%
Ct-product	41600	39900	37700	35200	31700
Percent Difference	10.4	6.0	0.0	-6.7	-13.3

For temperature and leakage effects, any change in conditions that resulted in an increased loss of fumigant had a smaller proportional change as higher amounts of loss were reached. In this case, the effect of increased wind speeds did not decrease at higher wind speeds, and in fact had a slightly larger impact on phosphine concentrations. The value in the exponent of the wind speed equation adapted for the model in Plumier et al. (2018) is more than three times the exponent in the temperature equations. Thus, the exponential effect of increasing wind speed will be much more pronounced than changes in temperature or leakage as seen previously. The exponential increase in fumigant concentration loss due to higher wind speeds was slightly more than enough to overcome the diminishing returns of increasing leakage, and will be more relevant given the likelihood that weather events can cause wind speed changes greater than those tested, which is unlikely for temperature. These results also agree with the results of Chayaprasert et al. (2015), which demonstrated increasing wind effects at higher velocities. The influence of wind speed on a fumigation is often more variable than the influence of ambient temperature, due to the overall percentage changes that occur. While the temperature effect continued to increase consistently over the course of the simulation, the wind effect was more dependent on varying weather conditions. The low wind speed conditions that were observed beginning on September 8 caused the effects of changing wind speed to level off, as seen in Fig. 5. These results indicate that weather events that cause high wind speeds are capable of having a large disruptive impact on phosphine concentrations in a silo. This points to the importance of best fumigation management practices such as seal testing a silo before a fumigation and monitoring gas concentrations during a fumigation. Monitoring phosphine concentrations helps detect increased fumigant loss due to sudden increases in wind speed.

4. Conclusions

- The verification demonstrates that the model effectively predicted the trend of phosphine concentrations, and predicted the overall Ct-product of the fumigation reasonably accurate.
- The accuracy of this fumigation model was found to be sufficient to use the model as a tool for conducting future simulations on predicting fumigant concentrations as a function of environmental conditions and operational variables.
- Increasing temperature and wind speed decreased phosphine concentrations, with temperature changes having a more significant impact overall than wind speed changes at tested levels. However, given the larger variability of wind effects possible beyond tested levels and the greater impact of increasing wind speeds relative to temperature, high wind weather events such as thunderstorms have the potential for substantial disruptive impact on phosphine concentrations.

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A Novel Engineering Design of Small Scale Metallic Silo for Food Safety in Rural India

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Abstract

Wheat is an essential component of the human diet for most of the world. In India wheat is an important staple food crop and it is used for the preparation of a diversity of products like *roti*, *parantha* (semi fried), *puri* (fried), bread, pasta, noodles, buiscuits etc. It has been reported that ~60-70% of wheat produced is stored at home or farm levels for domestic consumption. In order to understand the rural grain storage system, an extensive field study was carried out in villages of Haryana state (India). The field study revealed that ~95% of families store their grains in metallic silos of different sizes (300 to 2000 kg) and only Aluminium phosphide tablets (locally called sulfas) are used to protect grains from storage pests. Aluminium phosphide (AlP) tablets are used in an unscientific manner to control insect pest infestation, resulting in residues in stored grain. An experimental study of 12 months was carried out to identify the problems associated with pest management in conventional metallic silos. The storage period was divided into two parts, i.e., summer and winter, of 180 days each. Ambient temperature and relative humidity (RH) were recorded continuously for the entire period and temperature inside the silos was also recorded at different locations. The emergence of 'hot spots' was found during May-June when the temperature ranged from 37.6 to 42.7°C inside the silo during the summer season. During this period ambient temperature and RH ranged from 22.6-44.2°C and 37-82%. At this stage, convection current caused moisture migration at the top and bottom of the silo, whereas in the winter season moisture migration inside the silo was observed only at the top layer. Wheat samples from the topmost layer, in the vicinity of the "hot-spot" and from the bottom layers were collected and analyzed for various quality parameters.

The wheat samples near the "hot-spot" emergence were found most deteriorated in every aspect, for instance, in terms of protein content (decreased by 21.77%), fat content (decreased by 64.05%), germination capacity (decreased by 84.06%), thousand kernel weight (decreased by 22.09%), ash content (decreased by 41.96%), acidity (increased from 3.07-6.23 mm/gm) and insect-damaged kernels (increased by 80%). The results confirmed that even in a very small silo of 100 kg capacity if grains are stored without any fumigation treatment,

there exists the potential for moisture migration because of temperature fluctuation causing hot-spot formation, leading to grain quality deterioration.

Keeping in view the above aspects, an integrated engineering design of a double wall metallic silo with the special provision of a vertical perforated metallic tube in the centre was designed and fabricated. Tri-layer materials were tested for their thermal properties for fulfilling the needs of thermal insulation in the double wall silo. Wheat straw was found to be the best material in terms of thermal conductivity with a value of 0.040 W/mK. The special provision consisted of a removable string fitted with plates for keeping AlP tablets. To understand the function of the perforated tube in the centre of the silo for preserving stored wheat quality, 100 Kg of wheat (HD2733) was filled in this silo and after 12 months storage, wheat samples (at different depths inside the silo) were collected with the help of grain probes and mixed properly for quality parameter determination. Seed germination was determined before and after storage. It was found that germination decreased from 96% to 84%. Moisture content increased during storage from 9.8 to 12.7%. The initial kernel damage observed was 2-3% whereas after storage it was in the range of 13-15%. The initial lipid content recorded was 2.08% whereas after storage it was 1.4%. Also, the protein content decreased by 9.01%. Other parameters also showed quality degradation with time. The results were compared with the control (conventional) silo and it was found that the newly designed silo was better in terms of preventing insect infestation and quality deterioration. Also, the newly designed silo had a special provision for keeping AlP tablets suspended in the perforated tube to better control insects.

Future vision

The gap in technology transfer in India is increasing the chemical load on stored grain which can be minimized by incorporating small changes in the existing design of silos. To avoid the unnecessary repetitive use of AlP tablets, scientific knowledge should be developed and adopted, for example, on suitable wrapping/packaging material for AlP release at a slow rate over longer periods for effective control of insect pests in stored grains.

Keywords: Wheat, thermal conductivity, AlP, insect trap, design.

Food industry practices affecting Integrated Pest Management

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Abstract

Manufacturers of dry food products have a real challenge to exclude pests everywhere along the food chain because of the rather complex and different environments of food industry buildings. Current practices that influence pest presence and development in food industry facilities have been identified in the stages of food plant design, food ingredient reception and storage, processing or conditioning of finished food, and marketing. The preventive pest control measures in the food industry may be ineffective because of a non-observance of simple rules of good manufacturing practice (GMP), such as permanent control and monitoring of critical points or the ban of unsafe practices favourable to pest entry and infestation in food plants. The underutilization of methods for rapid assessment of pest presence and movement within food industry facilities, as well as the inability to rely on pest monitoring data for the economic damage threshold (EDT), are also underlined. Practical tools for processing data from pest monitoring systems should improve pest presence detection and alert. More realistic EDTs need to be proposed with direct links to decision-making support. More practical predictive models are also required for predicting the long-term efficacy and resilience of corrective control methods in food processing buildings, which should render the implementation of complex IPM programs easier.

Keywords: pests, food industry, manufacturing practices, food processing, IPM program

1. Introduction

Pest management practices in food industries are facing an important need to protect durable food products against pest infestation as many markets have very low pest-induced damage tolerance and are also subject to increasingly intense scrutiny through external inspections and audits. There are somewhat antagonistic trends such as less reliance on the use of residual pesticide treatments

and the demand for perfect food products, free of pesticide residues, which is becoming today one of the main challenges faced by the food industry in the field of pest management. However, food facilities typically are large complex structures with many locations vulnerable to insect pest infestation. They differ from each other in their activity or function (warehouse, mill, food processing plant, retail store, supermarket), in the concerned commodity (cereals, legums, animal-based materials, spices, dried fruits, cocoa), in the type of product generated (whole grain, flour, human food, pet food, confectionery, feed, etc.), in structure type (old or new, with variable construction material), in their equipment, in their geographic location and surrounding landscape, etc. This makes generalization about the pest infestation risks extremely complex and difficult: pest situation of a particular food industry facility has very specific characteristics for a given location.

Pest management in food facilities is a prerequisite for achieving food safety and food hygiene considering the scope of global quality assurance systems (HACCP). Recent regulations (EU Hygiene package and US Food Safety Modernization Act of FDA) aim at enforcing the application of HACCP systems to all food chains and in all plants, distribution centers and grocer's or retail stores. The main objectives of this paper consist in the analysis of the practices in the food industry affecting the risk level for both pest infestation risk and decision-making process for IPM related to food hygiene HACCP system conception and implementation, specifically adapted to the dry food industry sector.

2. Pest exclusion measures and sanitation in food industry facilities

Most buildings provide three main attractions for pests: shelter, food and warmth. It is commonly assumed that older buildings are more prone to infestation, but new buildings with enclosed roof spaces, suspended ceilings, wall cavities, panelling, raised floors, service ducts and lift shafts provide a large number of harbourages – with many interconnections – allowing a wide range of internal movement for pests. Most pests actually require very small amounts of food – an adult mouse for example, can survive on as little as 3 grams a day. A few degrees increase in temperature may be sufficient to encourage infestation, particularly in winter months. A master sanitation schedule is a vital component that influences pest management in the food industries. Importance of sanitation programs, and constant requirement for training personnel to implement sanitation practices are essential.

Elimination of pest refuges and pest colony "nests"

Harbourage of insect colonies

Constant monitoring of insects with different techniques and particular attention on behalf of staff prevent heavy infestations. This was accomplished by limiting Lepidoptera and Coleoptera populations by intensive trapping with pheromone and food traps, by examining tracks on dust left on floors or machine supports, substituting wooden structures with metallic ones, sealing cracks and crevices in walls and floors and replacing screw conveyors with pneumatic (fluid-lift) conveyors. Some elements in building structures and machinery needed to be changed or replaced (*e.g.*, gaskets). Crevices in which debris could accumulate must be sealed, wall edges and column floor junctions shoud be modified to avoid food particles accumulation.

Cleaning and hygiene maintenance

The removal of debris is more efficient than any localized chemical treatment. Only by controlling the entire processing cycle, from the purchase of raw material to the distribution of the finished product, will it be possible to reduce the risk of infestation. Nowadays, a few quality managers of food industries consider the problem of maintaining proper hygienic conditions as really important, although it represents the first step in reducing pest infestations. However, in many cases, standard cleaning procedures were modified but staff was not trained to clean the least accessible areas that are generally neglected. Therefore these are sure to be sources of infestation, and thus being considered as a potential critical control point. The most vulnerable points may be identified by

visual inspection of trained personnel, or better by an external audit carried out by a sanitation specialist. The attention of all staff should be drawn to the importance of cleanliness as it is their duty to adhere to these recommendations.

Influence of physical condition control

Site location and structure type design

Knowing that some pest infestation risks can originate from the proximate environment of any food plant, the perimeter around all structures and between structures should be kept free of vegetation and better with a concrete pavement of minimum one meter wide. The basement walls of food plant buildings should be "insect proof" at the junction with the steel cladding of the building wall. The repair of these damages creates critical entry points for pests that need to be quickly achieved and visual inspection of the exterior of the buildings should be easy all around. Where a new construction is being considered, an assessment of activities and the environment in the proximity to the proposed site must be made. Landfill sites, watercourses, marshlands, derelict sites, farms are examples of activities that regularly generate pest activity. When an old industrial building is re-used, the previous use of the site and its pest history must be considered. Thus, buildings that have previously been used in the food industry are most likely to have a pest history. Retrospective repair is far harder to accomplish once production has started and is running and when the construction company no longer has a presence on site. As a formal rule, no food should be allowed on to the site being constructed.

Temperature and air-conditoned manufacturing units

The population dynamics of stored product insect pest such as *Plodia interpunctella* or *Tribolium* spp. - which are common species in food industry facilities - is at their optimum in the range of 25-30°C. In factories producing cooked products (such as biscuits or bread), ambient temperature may be in this range all the year, especially in the rooms where cooking ovens produce heat. These areas have an increase risk of insect pest presence such as *P. interpunctella* which may lay eggs on the product after cooking. As an example of risky situations, when a belt covered with cooling biscuits stops (because of a technical issue), cooled biscuits are available to *P. interpunctella* female for egg deposition. One solution to this issue is to cool the food production areas with unprotected food flow to below the lower threshold of moving activity of flying insects (*P. interpunctella* or *Ephestia* spp. or *Stegobium paniceum*), *i.e.,* below 15°C. Below this lower limit, insects remain quiet and do not lay eggs on the produce before wrapping (*e.g.,* biscuits) and packaging. Consequently, air conditioning production areas to temperatures of 14-15°C or lower is a recommended practice that inhibits insect movements.

Internal and external lighting of the buildings

The type of lighting at a premise will, to a certain extent, determine the attractiveness of the site to flying insects. Most attractive types are mercury-vapour lamps and special fluorescent lamps used for perfect colour rendition. Next come "ordinary" commercial and household fluorescent tubes. The warmth of infrared light is also attractive to insects, although the area of attraction surrounding the source will probably extend only for a few meters. High-pressure sodium-vapour lamps, however, emit very little UV or IR and are currently thought to be the least attractive to insects. Unfortunately, these lamps give an orange light and cannot be used where the recognition of colours is important. It is recommended that an absolute minimum amount of lighting is physically attached to the building. Instead, position lights 5 or 6 meters away and direct lighting towards doorways. Apart from the obvious benefits of attracting insects away from the building, there are also benefits to be obtained in making the building less attractive to geckos, bats and birds that often roost and nest on such lighting structures due to their warmth. Lighting just inside doorways and in loading bays should be high-pressure sodium-vapour or low wattage incandescent bulbs.

White or light yellow surfaces of buildings should be avoided due to their ability to reflect UV light. Darker blue or green colours are preferable.

Exterior environment of food industry buildings

Perimeter security fences are generally of chain-link, wire mesh, weld-mesh or metal railing construction. These should be set into concrete footings to prevent mammals gaining entry under the fence. In the immediate building perimeter, concrete pathways are preferable to gravel pathways as gravel could be burrowed into by rodents despite of the ability of gravel to back fill on itself. Paving slabs are often laid on sand, which is conducive to infestation by ants and allows mole gallery digging.

Water drainage

Pooling water from overflow will encourage various pests, particularly flies. A readily available source of water is also a requirement for successful rat populations. Good drainage of land is required to avoid waterlogged soil. Certain insect pests (*e.g.,* cockroaches) rely on a water source for breeding. Grids should be designed so that waste materials can pass through easily and they can be removed easily for cleaning.

Increased risk of infestation by exterior environment

It is not advisable to plant trees or bushes near a food facility that will result in direct contact of tree leaves and branches with the exterior wall of the facility. This should be systematically avoided, because foliage provides excellent harbourage for many pest species. At a respectable distance from the walls, preference should be given to plants that shed the least seeds and fruits. Seeds and fruit may initially attract and then support insects, rats and mice, and various pest birds. Shrubs and trees should be of a coniferous type (releasing flavor repulsive for a range of food industry related insects). Leaf fall from deciduous trees that accumulates in guttering will restrict the run-off of rainwater and may give rise to localised infestations of insects that rely on standing water to breed, for example midges and mosquitoes. Leaves that accumulate along foundations provide harbourage and sheltered runs for rats and mice. Tree limbs and branches should be at least 2 m away from building exteriors (3 m if squirrels are a problem). Plants should not be planted too densely. Dense ground cover will provide cover and harbourage for rodent pests. Access in between shrubs is important for pest control inspection. Vegetation should not encroach within 5 m from any outside wall of a building. Rural vegetation can aggravate both rodent and insect pests. Climbing plants should not be planted against the walls of buildings. These could create entry routes for pest rodents, harbourage for pest bird species and entry routes for some insect pests. Grass should be kept closely cut at all times. Long grass will offer cover and harbourage for rodent pests. Raining water downspouts are easy ways for rodents to climb near the roof of the buildings to reach access to the space between the roof and the wall existing in numerous buildings.

Risks related to building structure design

Wall foundations must be taken down to a solid bottom at least 80 cm below ground level and concrete laid between the walls to prevent rodents burrowing into the building. The addition of a concrete curtain wall to a depth of 60 cm will protect the foundations against rodent ingress. It may be appropriate to apply a band of "non-friction" material 1 meter above ground level to prevent rodents climbing external walls. Airbricks supply ventilation to walled cavities but they also may allow mice and insect pests access. Pre-formed corrugated cladding should be avoided as corrugations are difficult to seal adequately against pest entry at the point where they meet conventional walling. An epoxy-resin type material should be considered. The external surface of walling should have no ledges because ledges may provide suitable day or nighttime roosts for pest bird species. For the same reason, over-developed external wall fascia should be avoided. The internal surface of walling should have no ledges. Ledges provide suitable areas for product residues

to accumulate and are difficult to access for cleaning. All drains should be accessible (from visit 'openings') and facilitate flushing and rodding. Special attention must be given to vertical ducts that pass between floors. Ducting may also allow rodent and insect pests free movement between floors (Troller, 1993).

Interior design of food plants and stores for pest-proofing

Floor, walls and ceilings design and colour

Tiled flooring is not recommended. All expansion joints should be well sealed and sealing material should be made from a material that allows for movement. Flooring under equipment (sur-elevated from the floor) should be completely smooth to allow thorough removal of waste material. Covings at wall to floor junctions reduce the accumulation of debris and facilitate effective cleaning. All cracks and crevices should be sealed to prevent the accumulation of product residues that provide insect breeding sites. Buildings are often designed in a way hard to reach for regular cleaning, for example roofs or very high ceilings, accumulate dust and debris and serve as a harborage for pests. So, one of the key industry practices that affect pest management is the building design.

Available access of pests to food and/or water inside food facilities

As rodents, birds and cockroaches rely on a supply of drinking water, sources of free water should be avoided. Any pools on concrete floors or on flat roofs have to be removed. Drainage channels should be sufficiently wide to accommodate expected volumes. They should be fitted with drainage grills that do not clog with waste and are easily removed for cleaning. The ends of drainage channels should be buttressed so that waste does not accumulate. Rainwater down pipes should be fitted externally; rodent entry into a down pipe from the ground can be prevented by the use of a back inlet gully. Pipes and cables, *i.e.,* gas, electric and water, must be tightly sealed where they pass through walls as rodents may gain entry via this route. Ducts can be sub-divided to prevent rodents gaining access along their length.

Doors, windows and portal apertures

Exit doors should be a good fit and self-closing; with a sensor to detect if the door has been properly open. Rats and mice can move around within a building via gaps that exist below doors. Roll-up doors should be fitted with a flexible bottom "seal" and T extensions to fit rail tracks. The use of strip curtain doors or rubber flap-back doors around external wall door openings should be avoided. Automatic high-speed roller doors are preferable but their timing needs to be adjusted so that they are open for the minimum amount of time. Vehicle loading ports should be adequately sealed once trailers have docked, and the port doors should not be opened until trailers are completely in position. Open loading ports equipped with lights will attract night flying and daytime flying insects. Installing doors that have hollow frames is not a recommended practice. Mice may use hollow doorframes as harbourage. Insects can breed in the accumulated food debris inside the base of the frame. Although opening windows can be adequately screened against flying insect ingress, air conditioning with light positive pressure inside the building is preferable. Nevertheless, a useful device to protect buildings from flying insect entry is the air curtain. Especially points of lorry loading openings, where doors are not very tightly closed, can be effectively protected from flying insects by this device. Outside air containing flying insects can be drawn into buildings that have negative pressures. Pest birds may use window ledges as day or nighttime roosts. Ceiling voids are potential harbourages for pests. Enclosed voids can also make inspection for pests difficult. GMP compliances for point of entries and common sense practices can eliminate pest infestation.

Storage of food products above ground level

Racking should be used to keep all goods off the floor. Raising goods will also allow effective cleaning. Adequate space around racking should be allowed. This will facilitate good pest control

inspection and allow for thorough cleaning. The pillars supporting the rack for pallets of raw food commodities are often protected from shocks by metallic shields that may house dust and food ingredients. These pods of pallet racks must be regularly cleaned to avoid insect colonies to establish in such protected locations. Adequate space between racking bays should be provided. This will allow for good pest control inspection and allow for thorough cleaning. Good stock rotation methods should be enforced. A minimum quantity of ingredients/packaging should be kept in stock; it is preferrable to have suppliers who are flexible enough to supply on demand. The use of pallets constructed of wood should progressively be replaced by the use of plastic pallets. Storage shelving should not have concealed cavities. If spillages cannot be cleaned easily, pests may make use of them to conceal their harbourages. Cleaning floors and wall basis must be regularly carried out and if possible each day.

Organization of food product chain

Food processing chain organization

The major principle of product flow organization in a food processing plant is that raw material and processed or finished produts should not be in close proximity. The strict separation of raw and processed product is essential to avoid contamination of any kind. The GMP recommendation for product flow direction in the process area is in compliance with the "go forward" principle, without raw ingredients that never cross processed or semi-processed food line. Because insect pest development cycles last a minimum of one month in indoor conditions, raw food commodities must be kept a minimum period of time in storage workshops. So, in all storage rooms, the product flow must comply with the "first in, first out" principle so that the stock rotation should be as short as possible. On the line for dry food product processing, there is a critical need to ensure a sanitary environment.

Cleaning material and equipment

The cleaning should focus on ingredients and dough fragments that have fallen down and have accumulated below the conveying belts or are sticking on belt support rollers. All residues in machinery should be removed at a regular interval (*e.g.,* each day) and the whole machinery should be thouroughly cleaned after each change in product type or before long shut-down periods. The food products waiting on a stopped conveyor for more than half an hour should be immediately removed and should not be stored in open containers close to the processing chain. Equipment which is to be taken out of production for a long period of time must be thoroughly cleaned to remove all food residues. All these cleaning practices are part of GMP and comply with the principles of proper sanitation in the food system sustained by recent regulations such as the Food Safety Modernization Act, enacted in 2011, or the EU Food Hygiene regulation package (Anonymous, 2004).

Underused packaging and food materials

Little used ingredients and packaging material are more likely to have pest activity develop in them and to be used by pests as harbourage. As an example, corrugated cardboard material temporarily stored in a food processing area may be a perfect refuge for migrant larvae of the Indian meal moth.

Isolation and treatment of infested commodities and out-of-use material

The construction of a quarantine building is recommended for the isolation of infested commodities or commodities that are being received from a suspect supplier. Returned goods should be stored in their own quarantine area away from ingredients, packaging and finished goods - ideally in a separate building unconnected to main production and warehousing areas. When food processing or packaging material is out of use, this equipment always remains attractive from food product or food dust deposit inside, which may attract flying pests. This "out-of-use" equipment should be

rapidly disposed off from workshops containing raw ingredients or processed food.

Packaging defaults (imperfect insect proofing)

Finished food produced from food processing plants is susceptible to quick infestation all along the marketing channels if packaging material is permeable to food flavor. This permeability to food flavor is a common weakness of a lot of cheap packaging films that are used to package finished food products. The result of such permeability generally is a rapid localisation of appropriate feed substrate by flying insects (*e.g., Plodia interpunctella*) or by rodents. Additionally, certain types of package (cardboard cassette and boxes with flexible pouring spout, or bags with wide aperture without resealing system after first use) are no more preventing insect entry after first aperture and the first pick up of food.

3. Early detection of pest presence and monitoring insect pest density

Identification of vulnerable situations for pest in food industry

Visual inspection "corridor" between products, machinery and walls

In storerooms, stacking of goods should be about 30 – 50 cm away from walls to allow free access to the area behind for inspection and cleaning. Strict segregation is required between raw materials, food processing areas, finished food products, and packaging zone to prevent cross-contamination. Plant and other equipment must be free of infestation before being brought on site. Rubbish storage areas must be kept tidy, using only close-fitting containers regularly emptied.

Management of waste

Waste areas should be sited more than 10 m away from the main building in order that any pests that may be attracted are kept at a distance. All waste bins should have tight fitting lids which must be kept closed at all times. If individual bins or skips are not covered, then the area should be enclosed within a mesh cage to prevent access by birds. Waste skips should be placed on a concrete pad to prevent rats burrowing underneath and be situated on rails of a height that will allow for thorough cleaning below.

Factors limiting IPM strategies implementation in the food industry

Full implementation of the IPM approach requires more effort than other types of control programmes, but once in place, it can be used to make more reliable pest management decisions. Unfortunately, many of the studies reported in the literature have been achieved under laboratory conditions, so there is limited information on their integration under field conditions. The IPM strategy is based on corrective intervention in dependence on EDT.

Self-determination of EDT and decision support tools use

Relationship between monitoring data and pest infestation level prediction

Many of the components of an IPM programme are known and are available for use, but our understanding of how to optimally integrate and target these tactics as part of IPM is limited. An IPM program is an evolving process that applies local intelligence and responds to changing needs (Pinniger and Child, 2002). Adoption has also been hindered by: i/ a poor understanding of pest population displacement in the spatially and temporally complex landscapes where food is processed and stored; ii/ the difficulty of evaluating pest populations; iii/ the limited information on structure treatment efficacy, and iv/ how to optimally select and combine management tools. Many questions remain about the use of these tools: from the very practical issues such as how many traps are needed and which types work best, to fundamental issues concerning the relationship between trap captures and pest population density, distribution and level of infestation.

- **Strengthening pest monitoring programs for food industry**

Insect monitoring is a primordial component of pest management in food processing plants (Campbell et al., 2002). Economic losses due to insects and unnecessary pest management expenses can be avoided using insect monitoring and decision-making tools related to risk prediction by the assessment of EDT, predictive models of pest populations density changes over time, and expert systems to determine the best time and way to suppress pest populations (Arthur and Phillips, 2003; Fleurat-Lessard, 2011). Computer simulation models can be used to compare the effectiveness of different pest management methods, alone or in combination, for stored-product insects. These models can also be used to evaluate the effectiveness of different implementation options, and to optimise the timing of pest management programs for stored-product insects (Fleurat-Lessard, 2011; Campbell et al., 2012). Currently, computer simulation models are available primarily for insect pests of stored grain, but in the future such models should be particularly useful in decison-making for pest management strategies for dry food processing and marketing chains (Trematera, 2013).

4. Modern tools to be integrated in IPM programs for pest risk minimization

As stated by Adam et al. (2006) in the case of implementation of IPM in stored-grain, many quality managers of food plants have not yet adopted IPM practices for many reasons: additional cost or personel implication, minimum required knowledge, difficulty to adopt a new technology by the managers, pressure of pesticide supplier or fumigation company, etc. Limited acceptance of IPM in food facilities is partially explained by a combination of the costs of corrective pest control interventions, difficulties in sampling properly, unreliable data, and difficulties encountered in the calculation of meaningful EDT. Precise treatment thresholds and economic injury levels have not been completely established for operational practice, and standards and rejection criteria are inconsistent and difficult to apply. As a result, treatments based on an economic threshold are not typically performed and control strategies are often applied preventatively, even when using tactics that do not have any residual effect. In current practice, many locations still rely on calendar-based pesticide applications and have little understanding of the basis of pest management. Nevertheless, most of the risks of infestation of food industry plants by noxious pests listed above may be controlled by customized application of IPM programs covering the four components of dry Food Quality and Safety Assurance from raw commodities to finished food products (Tab. 1). Combining and integrating different management tools and careful selection and timing of different approaches, together with an understanding of pest behaviour and ecology, should result in a greater effectiveness and more accurate solutions to pest presence in finished food.

Peculiarities of bulk-stored commodities

For bulk-stored commodities, and particularly in commercial elevators, it is often difficult to adequately monitor large grain bulks due to the need to directly sample the large volume of grain and detect relatively low densities of insects. Collecting samples may only give information on insect presence when relatively high densities are present. The lower limit of density that can be expected from bulk sample examination is evaluated at one insect per 2 kg of raw material (as grain) (Fleurat-Lessard, 2011). This is already a high level of infestation and much higher than most of the tolerable EDT (more often fixed at one insect per 5 kg of grain). As grain products move from bulk storage to processing and milling facilities, then through distribution and marketing channels to consumers, the concept of EDT becomes more difficult to apply. When there is 'zero tolerance' for insects, controls become more preventative, but it is not very realistic. More often with bulk raw commodities, there are no precise damage thresholds or injury levels, and it may be difficult to adequately determine pest levels or to estimate all of the direct and indirect costs of corrective interventions. In this context, there is reluctance or lack of interest on the part of the food grain storage and handling industry to move away from calendar-based pesticide treatments to a more integrated approach, based on prevention rather than control after EDT is reached. This is due, in

large part, to a justifiable concern about making mistakes with pest control in an industry with an extremely low pest threshold requirement.

Difficulty of applying biocontrol agents in the food industry buildings

The artificial nature of food chain environments and low tolerance in many situations for the presence of insects, means IPM relies less on promoting population regulation using natural enemies and puts greater focus on modifying the environment to make it less favourable for pest establishment and persistence. The exception to this is bulk storage, where biological control shows more potential for success since some insects can be tolerated in many situations and natural enemies can be cleaned out of the material before processing (Schöller and Flinn, 2000; Phillips and Throne, 2010).

A summary of the more promising modern tools that may be integrated to IPM programs for the food industry is described in Tab. 1. The IPM concept is a whole system based on risk prevention, monitoring and prevision including pest resistance management, use of selective chemical treatments, use of corrective intervention thresholds, and promoting environmental sustainability.

5. Further research needs for larger implementation of IPM in the food industry

Research should optimise or further develop other semiochemicals (attractants and repellents) to aid in the monitoring of some stored-product insects and to provide new biocontrol tools. In this regard, future stored-product protection combinations of repellents and attractants may also find use in push-pull tactics (Cook et al., 2007). Push-pull strategies involve the behavioural manipulation of insect pests and their natural enemies via the integration of stimuli that act to make the protected resource unattractive or unsuitable to the pest (push) whilst luring them towards an attractive source (pull) from where the pests are subsequently removed. Deterrent or repellent semiochemicals can be used to discourage pests from entering a premise, while at the same time, attractants or stimulants can encourage pests to congregate in an adjacent area where they can be controlled more effectively and safely by chemical pesticides or biocontrol agents. Computer, smartphone and touchpad applications affording a practical and user-friendly support in building IPM specific programs and on-line advice for risk assessment and prevention should become accessible to food industry quality managers in the near future.

Tab. 1 IPM more recent tools that may be integrated in IPM programs for the food industry.

IPM component	Actions for risk management	Alternative tool	Main advantage	Main constraint
Identification of critical pest entry points in food industry facilities	Identification of critical points by which insect pests can penetrate into the facility	Interpretation of trap network data with geographical positioning system (GPS)	Accurate detection of the core of infestation	Each trap bar-coding and GPS positioning of each trap
		Localisation of loci of pest infestation by contour mapping from trap catches	Accurate localisation of infested goods	Weekly trap data processing
Pest exclusion measures for risk prevention	Sanitation measures especially at pest entry points and regular inspection and surveillance of identified CCP. Regulation of physical conditions	Low temperature and RH in working areas when free-access food is on the chain	Corrective treatment never needed	Air conditioning of all rooms
		Pest-proof packaging film and structure for finished food products for sale	Protection from pests in the marketing channels	Bioassay to carry out for food bag or box testing insect proof properties
Permanent monitoring for risk prediction	Identification of infestation locations inside the building, processing equipment and machinery	Enhanced strategies of pheromone use: mass-trapping and attract-and-kill strategies	Effective means of surveillance for flying insects	Not adapted to limit crawling beetles populations
		Permeation of food facility atmosphere with pheromone for mating-disruption or auto-confusion	Effective against flying insects	Slow reduction of pest population expensive renewal of dispensers
		Use of electronic devices detecting very low level of insect density in bulked commodities	Early detection especially for grain insect pests	Only useful for insect detection in bulked commodities
		Prevision of pest density changes over time by predictive models from physical-chemical parameters or conditions	Calculation of safe storage time before EDT reaching	Collection of daily data of temperature and RH for model feeding
Application of pest control measures (when EDT is reached)	Selection of non-chemical solutions rather than chemical disinfestation means develop the use of biocontrol or beneficial agents	Pheromone trap use for auto-inoculation-release of a microbial pesticide	Self-function device	Expensive and slow in action
		Improvement of efficacy of registered pesticides by combination with mineral products or biorationals	Lower risk of chemical residues in food	Preventive action; weak curative effect
		Replacement of surface or space treatments with chemicals by bio-control agents or biopesticides	Targeting more specific pest species than chemical pesticides	Difficulties to register for use in food processing plants
		Use of physical treatment as alternative to fumigation (microwave heating, temporary freezing, controlled- and modified-atmospheres)	Complete disinfestation process with neither persitence nor residual effect	Competitive only for high value commodities (e.g., medicinal plants and spices)
		New fumigants for whole structure, plant or warehouse disinfestation (SO$_2$F$_2$, methyl iodide, ethyl formate ...)	Complete disinfestation of food plants or stores in a sigle fumigation	Minimal airtightness of buildings required; manager reluctance
		Use of natural pesticides of microbial or fungal origin, a vegetal extract or an essential oil (EO)	Short period of remanence (activity and smell) for the most volatile EO	Difficulty to register fomulations from a few number of active substances
		New formulation or conditioning of phosphine controlled-release phosphine gas by automatic equipment	More practical implementation and control of fumigant doses	Managers reluctance to regularly use toxic gas at a high concentration
		Replacement of fumigation of food-processing plants by heat disinfestation	Complete disinfestation through one application	Stop of the working activity during one day minimum

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Abbreviations

EDT=Economic Damage Threshold; EU=European Union; FDA US=Food & Drug Administration; GMP   =Good manufacturing practices; IPM=Integrated Pest Managment; IR=Infra-red radiation; HACCP=Hazard Analysis Critical Control Point; IMM=Indian meal moth; UV=Ultra-violet light

Static and Dynamic Stress Analysis of Flat Bottom-Bamboo Reinforced Concrete Silo for Rough Rice Storage

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Abstract

Concrete silos are one of the most robust and reliable structures for grain storage in tropical countries. This study analysed the structural behavior of a low cost, flat bottom bamboo-reinforced concrete (BRC) silo for rough rice storage. This research included the design and development of a BRC silo in accordance with the guidelines mentioned by the Indian Standard (IS) codes. The Finite Element Method was employed to develop the stress profile in the silo walls under "grain at rest" and "grain filling" conditions. The results obtained were further compared with experimental results, classic silo theories (Janssen's theory) and standards of different countries in the world. The numerical technique gave stress magnitudes very close to those of the experimental results. The classic theories as well as the standards of different countries predicted an over estimation of the magnitude of stresses in the BRC silo. This would result in extra cost of construction of BRC silos. The study also suggested that the vertical stresses were most predominant under static and filling conditions. Maximum stresses were developed at the silo bottom. This study is expected to aid the development of economical silos with minimum wall thickness and material requirement which are ideal for on-site construction and use by smallholder farmers.

Keywords: Bamboo reinforced concrete silos, rough rice, finite element method, stress profile

1. Introduction

Food grain silos are highly efficient in storing bulk grains for a long period of time and safe from deteriorating agents such as rodents and insect pests. Modern silos are generally made of materials such as galvanized steel, reinforced cement concrete, painted Aluminum, plastic etc. If designed properly, these structures provide hermetic conditions ideal for safe storage of food grains, ensuring

minimal storage induced losses and improved food security. Bamboo based grain storage structures have been popular among Indian farmers since time immemorial. These include traditional bamboo baskets, mud plastered bamboo bins, bamboo-reinforced concrete bins etc. Bamboo based concrete structures have gained popularity in the present time and have been judged as an environmental friendly and sustainable technology (Holani, 2001).

In spite of several studies being performed on grain storage silos, the structural behavior of certain designs is still unclear. The complexities associated with different types of grain silos can be mainly attributed to the nature of the bulk material being stored (moisture content, internal friction, bulk density, grain shape etc), the nature of the containment structure (material, shape, dimensions etc) and the interactive effects between the stored material and the structure walls. Classic silo theories such as Janssen's and Reimbert's theories fail to address the effect of these critical factors. International standards for silo designs such as the Indian Standard codes, Eurocode and Australian Standards are also designed based on these classic theories. While experimental trials are the most accurate method of studying silo behavior during grain storage, the high cost associated with their construction and automation discourages researchers from utilizing them.

Recent studies have reported numerical methods such as finite element method (FEM) and discrete element method (DEM) as reliable techniques for modelling of grain silo phenomena such as silo filling and discharge (Gallego et al., 2015), granular flow patterns (Wang et al., 2013), buckling (Zaccari and Cudemo, 2016) and development of innovative silo shapes (Anand et al., 2008). While continuum based FEM is ideal for the prediction of stresses developed on silo walls, the discretization based DEM has been found to be suitable for modelling granular flow of grains in and out of silos (Rotter et al., 1998). FEM is highly efficient in developing a realistic elastoplastic behavior of stored grains as a continuum medium and predicting its effect on the silo body. More recent research has occurred applying FEM for modelling stresses developed in silos with different planforms (square, rectangular), construction material (steel, concrete, polymethylacrylate), eccentric discharge, and flat or hopper bottoms. Advances have been made in simulating the interactive effects between grain-silo walls from node-node contact to surface-surface contact algorithms.

This study aimed at designing and developing a bamboo reinforced concrete silo of 1000 kg capacity to be used for rough rice storage at the farm level. A 3D FEM model was developed for the prediction of wall pressures in this intermediate slenderness silo, under static and filling conditions of rough rice. The predicted pressure values have been further compared with the results of classic silo theories, standard codes as well as experimental values.

2. Materials and methods

2.1 Stored granular material

The paddy to be stored inside the BRC silo was procured fresh from the experimental farm of the Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur. A high amylose, long variety of paddy, IR-36, was chosen for the present study, considering its popularity and ease of availability in the region. The paddy was thoroughly cleaned to ensure the best quality. The paddy was tested for its moisture content prior to storage.

2.2 Experimental determination of FEM input parameters

The results of the finite element method is highly dependent on the input parameters associated with the particles/bodies involved in the system concerned. The physical properties of the granular material was directly obtained from the works done at the Central Institute of Agricultural Engineering, Bhopal (Reddy and Chakraverty, 2004). The poisson's ratio of the paddy was determined from the K_o test (Moya et al., 2002), wherein, K_o, is the lateral pressure ratio. The modulus

of elasticity of the paddy grains were obtained from the triaxial test described by the same group of authors. The values of various parameters used in this study have been tabulated below:

Tab. 1 Input parameters for paddy.

Material parameter	Values	Source
Grain unit weight (kN/m³)	5.638	Experimental procedure
Angle of repose (˚)	42·35	Reddy and Chakraverty (2004)
Wall friction coefficient	0.5	IS 4995: 1974- Part 1
Modulus of elasticity (kPa)	10,000	Moya et al. (2006)
Poisson's ratio	0.2	Moya et al. (2006)

Tab. 2 Input parameters for concrete and bamboo reinforcement.

Material parameter	Values	Source
Concrete (M20 grade)		
Concrete unit weight (kN/m³)	22.55	IS 456:2000
Modulus of elasticity (kPa)	2.23×10^7	IS 456:2000
Poisson's ratio	0.18	IS 456:2000
Bamboo		
Bamboo specific weight (kN/m³)	8.825	Agarwal et al. (2014)
Modulus of elasticity (kPa)	2.44×10^7	Agarwal et al. (2014)
Poisson's ratio	0.28	Agarwal et al. (2014)

2.3 Finite Element Method

Material models

The stress profile developed on the BRC silo walls was developed in ANSYS software (ANSYS, 2018) reproducing the real silo dimensions and geometry. The reinforcing bamboo strips were generated in the design modeler and their properties were assigned. A linear elastic material model was used to describe the concrete as the non-linearity arises only under high levels of stresses. The granular material stored in the silo was described using an elastoplastic material model, wherein the elastic portion was described using the isotropic linear elastic model, while the plastic portion was described by the Drucker-Prager model (Drucker and Prager, 1952). The material parameters involved in these models have been selected according to Gallego et al. (2010). The Coloumb friction model was selected to model the interaction between paddy grains and the silo walls, wherein the wall friction coefficient was the most relevant parameter.

Types of ANSYS elements

The ANSYS elements are highly effective in modelling the physics of the problem and in the present study different elements have been assigned to the individual silo system components. The silo body, made of concrete was assigned the element type SOLID186 while the reinforced bamboo strips were modelled using LINK180 elements. The SOLID186 element is a 3D homogenous structural solid with 20 nodes supporting features such as plasticity, stress stiffening, and mixed formulation capability for simulating deformation of nearly incompressible elastoplastic material. The LINK180 element on the other hand is a 3D spar, tension-compression element with nodes having three degrees of freedom each. The grain body was assigned the element type SOLID187, a 3D 10 node element with the ability to model irregular meshes. The contact algorithm between the silo walls and grains were described using pair based contact elements (CONTA173 and TARGE170). The CONTA173 can model contact and sliding between a 3D rigid body and a deformable body.

Boundary conditions

Only two boundary conditions were employed for modelling the stress profile in BRC silo walls:

- • Fixed support at the silo bottom (constrained rigid body motion) and
- • Bulk unit weight of the stored paddy.

During the filling process, all nodes at the silo bottom were restrained from motion in any direction (Gallego et al., 2010). Prior studies by the same group of authors had reported that progressive filling yields better results than en masse filling. The grain filling process was modelled using the sequential activation of subsequent grain layers through the birth and death feature of ANSYS (Gallego et al., 2015). The Newton Raphson procedure was adopted to solve the set of nonlinear equations during the entire process.

3. Results and discussion

3.1 Description of silo design

An intermediate slenderness, bamboo-reinforced concrete (BRC) silo of 1000 kg capacity as shown in Fig. 1 was developed at the Indian Institute of Technology Kharagpur. It was comprised of a domical roof, silo body and a base support. The grain is loaded from the top opening (40 x 40 cm) on the domical roof and the discharge done through an inclined chute (15 cm φ) at the bottom of the silo wall. The domical roof was made of concrete of 8 cm thickness. The cylindrical silo body was made of a bamboo framework reinforced in cement concrete. The frame work consisted of vertical and circumferential bamboo strips with trapezoidal cross section (2 x 1 x 0.5 cm). The bamboo strips were coated with a layer of araldite and sand spray to ensure better adherence with concrete. The thickness of the silo wall was limited to 12 cm. The entire silo was supported on a circular base of 40 cm height and a diameter of 2 m. This ensured protection from water seepage and inaccessibility to rodents.
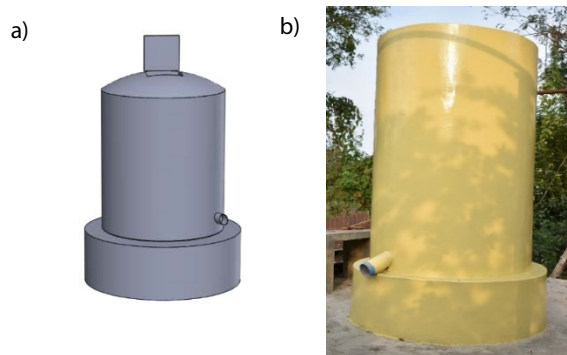
Fig. 1 Bamboo-reinforced concrete silo for rough rice storage: a) 3D representation b) Silo erected on the field.

3.2 Lateral and vertical pressures along the BRC silo walls under static condition

The lateral and vertical pressures prevalent in the BRC silo under static state are depicted in Fig. 2. The wall pressure values predicted by the FEM model were lesser than those estimated using the design codes or Janssen's equation. This could be due to the differences in the input parameters of the material models used. Several studies have reported that the material model adopted has the least effect on the pressures developed in a flat bottom silo. However, the poisson's ratio (ν), lateral pressure ratio (k) and wall friction coefficient (μ) employed in the models significantly influence the pressure profile obtained. For instance, the k values adopted in the Indian standard code and Eurocode (EN1991-4) are 0.5 and 0.63, respectively, while one adopted in the numerical model was 0.47. The pressure values were normalized in order to reduce the redundancy in the data values. Finite element analysis suggested a peak pressure at the silo bottom, which was not accounted for in any other method of estimation. Varying the mesh density at the silo base did not affect this peak

pressure. The results suggested that the wall thickness of 15 cm for concrete grain silos, suggested in the Indian Standard codes, is an over estimation and would result in additional costs for economically constraints farmers. A wall thickness of 10 cm was sufficient to take the grain loads, static and dynamic, which were well within the permissible strength of concrete. However, this finding has to be supplemented with studies related to the life cycle assessment of the BRC silo.
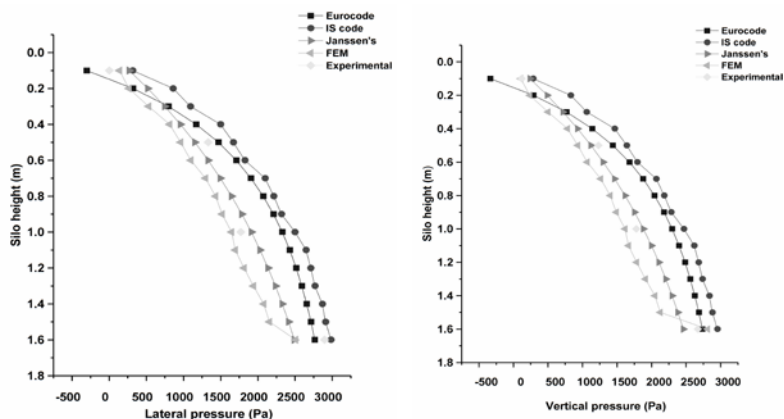
Fig. 2 Lateral and vertical pressures acting on the BRC silo wall under static conditions.

3.3 Lateral and vertical pressures along the BRC silo walls under filling conditions

The pressure profile of the silo walls is illustrated in Fig. 3. Significant distortions were observed in the pressure values predicted by FEM. These values were normalized and the modified values were represented in the chart. These distortion could be a result of numerical issues, roughness of meshes, or differences developed in the subsequent grain layers during filling. Previous studies have reported similar kind of leaps in FE values during grain filling in metal silos (Gallego et al., 2010). These issues needs to be addressed in depth to obtain better pressure distribution profiles in grain silos. The study also suggests that during the filling of grains the vertical stresses are more predominant than the normal pressures.
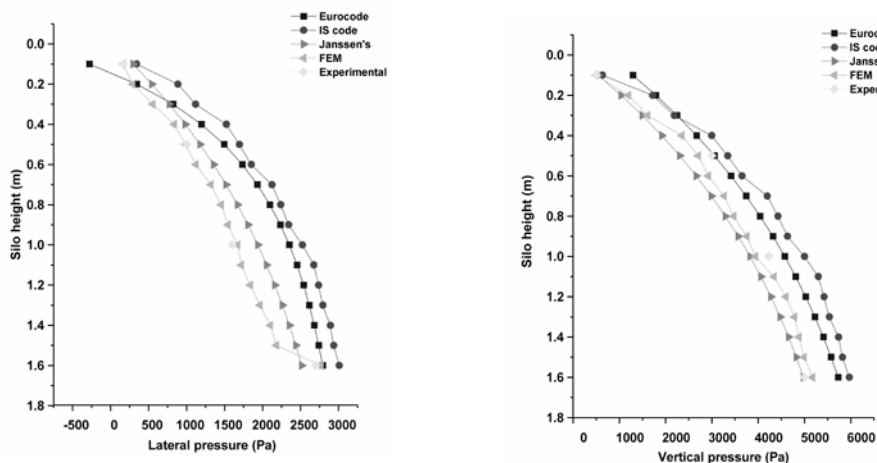
Fig. 3 Lateral and vertical pressures acting on the BRC silo wall during progressive filling of grain.

4. Conclusion

The study dealt with the development of a low cost bamboo reinforced concrete silo for farm level storage of rough rice. The structural safety of the developed structure was analyzed using the finite element method and the results were compared with Janssen's theory and design codes. The numerical method has been adjudged as one of the most reliable and versatile tools for development of innovative structures of various scales. The selection of input parameters for the FE model is a critical process affecting the prediction of the method. Dynamic pressures developed during filling of grains have been quantified in this study.

Acknowledgements

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Increase of Paddy Moisture with Automatic Aeration in a Warehouse Guided by Adsorption Equilibrium Absolute Humidity Equation

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Abstract

An automatic bulk monitoring and aeration controller was programmed with an adsorption equilibrium absolute humidity (CAE)equation and was used to aerate paddy with the aim to increase moisture content (MC)

and preventing fissuring. The ventilation control window for rewetting paddy was developed according to two conditions: (i) the average grain bulk temperature (t_g) is higher than the dewpoint temperature (DPT_a) of the atmosphere; and (ii) the equilibrium absolute humidity (EAH_g) of grain moisture content plus 1 percentage point is lower than the absolute humidity (AH_a) of the atmosphere. The ventilators were turned on when the atmosphere state point was within the ventilation windowand turned off outside that window. In a humid subtropical monsoon climate, during Oct. 8th to Nov. 1st, 2013, the system was used for a paddy depot of 1035 t in Dianjiang, Chongqing province. The natural humid air was introduced into the paddy bulk by negative pressure suction aeration during the 10-12 h night time period and allowed to equilibrate with grain kernels during the 12-14 h day time period. Aeration increased grain MC by 0.6 percentage points with two 1.5 kW axial flow ventilators and power consumption of 209 kW·h. The unit energy consumption was 0.336 KW·h (1% moisture·t)$^{-1}$. The broken milled rice percentage was decreased by 2-3 percentage points. In the warm temperate semi-humid monsoon climate, during April 13th to June 16th, 2017, the system was used to rewet japonic paddy in a 2489 t depot in Qihe, Shandong province. The conditions for running two 0.85 kW axial flow fans were: (i) when the atmosphere relative humidity (RH_a) is ≤80% and its temperature (t_a) is <28°C, t_g>DPT_a, and EAH_g<AH_a; and (ii) when RH_a >80% and t_a <28°C. Whenever t_a was >28°C, the two fans were switched off. This rewetting aeration increased grain MC from 13.5% to 14.0%, and the unit energy consumption was 0.455 kW·h (1% moisture·t)$^{-1}$. The percentages of average head rice yield and damaged grains after aeration were 71.7% and 7.7%, respectively.

Keywords: Paddy, EMC, moisture adsorption, increasing moisture, automatic aeration.

1. Introduction

Rice is the staple food for approximately 65% of the Chinese population. China is the world's largest rice producer with annual production over 144 million metric tons (FAO, 2014), and due to its large population, about 40% of its production is assigned to store for two years in the form of paddy with deterioration controlled largely through moisture content (MC) and temperature. For improving physical control in paddy storage, sound knowledge of the relationship between equilibrium moisture content (EMC) and equilibrium relative humidity (ERH) is essential (Jayas & Mazza, 1991; Sun, 1999; Li et al 2010; Li & Jiang, 2014). After two-year storage, paddy in China usually has moisture losses over 1.5-2.0% wet basis (w.b.). In order to increase the head rice yield and milled rice quality, rewetting up to 14% w.b. 1-3 months before retrieving from storage is needed. The present study investigated increasing paddy moisture with automatic mechanical aeration in a flat warehouse based on an adsorption equilibrium absolute humidity equation (CAE) with the aim to determine suitable aeration rewetting conditions.

2. Materials and Methods

2.1. Using the CAE equation in a computer controlled grain aeration system

In a computer controlled grain aeration system (Wu, 1987; Wu and Li, 1994), the parameters known as the CAE model for paddy adsorption (Li et al., 2014) was used to make curve graphs for determining the equilibrium relative humidity (ERH_g) of paddy kernels with particular MC at certain temperature. The following equation (1) was used to make the curve graphs for determining the equilibrium absolute humidity (EAH_g) of paddy kernels with particular MC at certain grain temperature and dewpoint temperature (DPT_g) of grain at this absolute humidity:

$$EAH_g = \exp\left\{\frac{\left[\frac{D}{222}\left(\exp\left(\frac{B_1-MC}{A_1}\right)-\exp\left(\frac{B_2-MC}{A_2}\right)\right)+0.9845\right]\cdot\left(1737.1-\frac{474242}{273+t_g}\right)+D\left[1-\exp\left(\frac{B_1-MC}{A_1}\right)\right]-68.57}{87.72}\right\} \quad (1)$$

where EAH_g is grain bulk equilibrium absolute humidity (mm Hg), MC is grain moisture content (% w.b.), t_g is grain temperature (°C). A_1, A_2, B_1, B_2, D are five parameters of the CAE equation.

The dewpoint temperature (DPT_g in °C) of the grain bulk was calculated by equation (2):

$$DPT_g = \frac{474242}{1872.7-89.1\lg(EAH_g)} - 273 \quad (2)$$

The atmosphere absolute humidity (AH_a) and dewpoint temperature (DPT_a) were respectively calculated with equations (3) and (4):

$$AH_a = 100\exp\left\{\frac{87.72\lg(RH_a)+0.9845\left(1737.1-\frac{474242}{273+t_a}\right)-270.57}{87.72}\right\} \quad (3)$$

$$\text{DPT}_a = \frac{474242}{\frac{474242}{273+t_a} - 89.1\lg(\text{RH}_a) + 410.34} - 273 \qquad (4)$$

where AH_a is atmosphere absolute humidity (mm Hg), RH_a is atmosphere relative humidity (%), and t_a is atmosphere temperature (°C), DPT_a is atmosphere dewpoint temperature (°C).

The relative humidity or absolute humidity in equations (1) - (4) was calculated on the basis of sea level atmospheric pressure. The values of DPT_g and DPT_a were used in characterizing whether dew condensation would occur with a decrease in temperature.

2.2. Aeration window controlling ventilator operation

The ventilation window for increasing grain moisture was constructed according to two conditions of aeration control: (i) the grain bulk temperature (t_g) is higher than the dewpoint temperature (DPT_a) of the atmosphere; and (ii) a condition in the Grain Industry Standard LS/T 1202-2002 of the PRC was modified as follows: the equilibrium absolute humidity (EAH_g) of grain moisture plus 1 percentage point is lower than the absolute humidity (AH_a) of the atmosphere, and not the grain moisture content plus 2.5 percentage points. Whenever both conditions were true then the axial flow ventilators were switched on. Fig. 1 shows the aeration rewetting window. If the grain state (13.5% MC) has an adsorption equilibrium absolute humidity of 10 mmHg and grain temperature of 15.8°C, and the atmosphere has an equilibrium absolute humidity of 12 mmHg and dewpoint of 14°C, airflow with lower temperature and higher humidity could increase the moisture of the paddy bulk, and thus the axial flow ventilators would be switched on.

The aeration controlling system included the hardware such as ventilator-controlling module, digital humidity transmitter, new type temperature measuring cable, and protective filter cover for humidity sensors. This system automatically detected grain bulk temperature and the air temperature and relative humidity of the headspace in the warehouse every 15 min, and the atmosphere temperature and relative humidity outside of the warehouse every 5 min. An aeration window was constructed by the curves of paddy adsorptive equilibrium absolute humidity and the saturation absolute humidity. When the atmosphere state point lied within the aeration window, the axial-flow ventilators were turned on to increase paddy MC. When the atmosphere state point was outside the aeration window, the axial-flow ventilators were turned off.

2.3. Two in-situ experiments for remoisturizing aeration in flat warehouses

2.3.1. The remoisturizing aeration in a warehouse in Dianjiang, Chongqing

The first experiment was carried out at the Dianjiang State Grain Reserve Depot, Dianjiang, Chongqing, China. Dianjiang lies in a basin (30°N, 107°E, 450 meters of average altitude) with a subtropical humid monsoon climate. The experimental No. 12 warehouse made of steel frame, concrete wall and tile roof, is 31.4 m in length and 14.12 m in width. It has six ground cage-channels equipped with two axial-flow ventilators, each ventilator responsible for three channels. The gap between two channels is 4.7 m, the percent of aperture in each cage-channel at the beginning and the end are 25% and 35%, respectively. The ratio of longest to shortest pathway of air is 1.5. The indica paddy of 1330 tonne was garnered in January 2012 and had a 5.12 m bulk height and 0.6% of foreign material. During April to September 2013, 295.5 tonnes of paddy was sold and the rest of the 1034.5 tonnes of paddy were used for the rewetting experiment starting on October 7th. The warehouse doors were closed, and its four windows in the sides above the grain surface were opened. Aeration used negative suction pressure to draw air into the warehouse through the windows then passed downward through the layers of the grain bulk, and was exhausted from the ground-level ventilators. The two ventilators (SFG4-2 type, 1.5 kW power) generate 320/220 Pa of full/static pressure, 11000 m^3/h of air volume, and 2800 r/min of rotational speed, thus the calculated airflow rate is 10.6 $\text{m}^3\text{h}^{-1}\text{t}^{-1}$. In order to accurately determine the electricity consumption, an intelligent electricity meter was used for aeration manipulation. The actual power consumption was 0.567 kW·h (1%moisture·t)-1.

2.3.2. Rewetting aeration in a flat warehouse in Qihe, China

The second experiment was carried out at Shandong Grain Reserve Depot for Army Provision, Qihe, Shandong province, China. Qihe lies in a basin (36.8°N, 116.8°E, 20 meters of average altitude) with warm temperate semi-humid monsoon climate. The experimental No. 13 warehouse is 39.8 m in length and 20.4 m in width. It has five U-shaped air channels equipped with two axial-flow fans, each fan is connected to 2.5 U-shaped air channels. The ratio of longest to shortest pathway of air is 1.4. The japanic paddy of 2489 tonne from the northeast China was garnered in December 2016, with 5.0 m bulk height, 14.0% MC and 0.6% foreign material. After levelling the grain bulk surface, the equalizing-temperature aeration decreased the average grain bulk MC to 13.5% during January 2017. Aeration was negative suction pressure suction with air entering the warehouse through five vents and then passed upward through the paddy bulk layers, and finally exhausted from the two fans fixed on the windows in the roof structure. The warehouse doors were closed, its five vents in the warehouse side bottom were opened, and the two axial-flow fans fixed on the windows at opposite sides above the paddy surface were opened. The two fans (FTA-75 type, 0.85 kW power) have 320/220 Pa of full/static pressure, 13800 m^3 h^{-1} of air volume, and 2300 r min^{-1} of rotational speed, thus the calculated ventilation rate is 8.9 m^3 h^{-1} t^{-1}. The No. 13 warehouse of paddy was used for the reweeting experiment with natural humid air during April 13th to June 16th, 2017.

2.3.2.1. Protocol for rewetting paddy in No. 13 warehouse

Firstly, the local daily 24-h data of atmosphere temperature and RH during April to June in 2015 and 2016 were collected from Qihe County Bureau of Meteorology. A paddy desorption equilibrium moisture equation (eqn. 5) was used to predict paddy static moisture content near the warehouse vents:

$$EMC_p = 36.953 \cdot RH^3 - 48.528 \cdot RH^2 + 30.791 \cdot RH + 0.03859 \cdot RH^2 \cdot t + 0.006744 \cdot RH \cdot t - 0.08611 \cdot t + 5.089 \qquad (5)$$

where EMC_p is the predicted EMC (%w.b.) of paddy, RH and t are the relative humidity (%) and temperature (°C) of the atmosphere, respectively.

Secondly, the aeration channels in the paddy warehouse were used for automatic rewetting aeration. The temperature of the grain bulk was similar to the atmoshperic temperature thus the atmospheric RH should be 25% higher than the RH of the grain bulk. Thirdly, the grain bulk temperature and grain moisture near the vents and at the bulk surface were checked regularly. The percentage of head rice yield and damaged grains from sampling sites were determined.

3. Results

3.1. The rewetting aeration in No. 12 flat warehouse in Dianjiang, Chongqing

3.1.1. Change in the BCDE area of rewetting aeration window
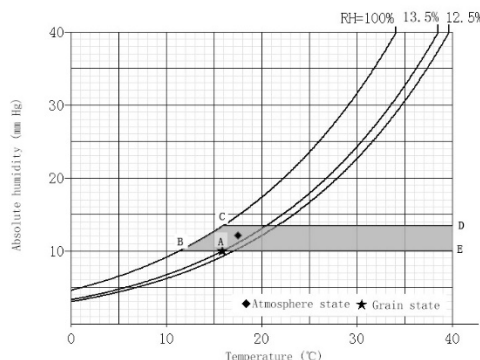
Fig. 1 The operating window for aeration to rewet paddy rice.
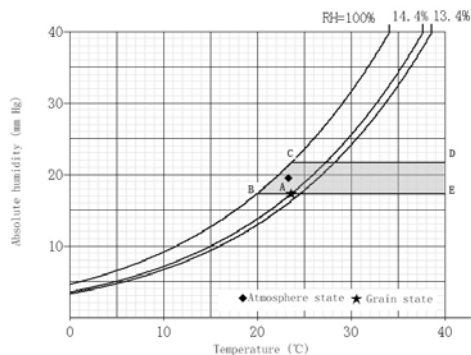
Fig. 2 The ventilators were running at 23:31 on October 8th, 2013.

At 23:31 on October 8th, 2013, the automatic detection system showed average grain bulk temperature (t_g) of 23.6°C in No. 12 warehouse, atmosphere temperature of 23.3°C, and atmosphere RH of 92%. The moisture of grain bulk was determined to be 13.4% using a LSKC-4B type moisture meter (Wuhan Electronic Devices Second Factory, China). The t_g was higher than the dewpoint temperature (DPT_a, 21.93°C) of the atmosphere. The atmosphere state point was within the BCDE area of the rewetting aeration window (Fig. 2), and the ventilators were turned on.

At 19:01 on October 20th, 2013, the moisture of the grain bulk was 13.8%, the automatic detection system showed average grain bulk temperature (t_g) of 16.5°C, atmosphere temperature of 18.0°C, and atmosphere RH of 91%. The t_g was equal to the dewpoint temperature (DPT_a, 16.5°C) of the atmosphere. The atmosphere state point was at the edge of the BCDE area of the rewetting aeration window (Fig. 3), thus the rewetting aeration condition was not satisfied and the ventilators were turned off.

At 22:00 on October 21th, 2013, the moisture of the grain bulk was 13.8%, the automatic detection system showed average grain bulk temperature (t_g) of 16.8°C, atmosphere temperature of 17.3°C, and atmosphere RH of 92%. The t_g was higher than the dewpoint temperature (DPT_a, 15.98°C) of the atmosphere. The atmosphere state point was within the BCDE area of the rewetting aeration window (Fig. 4), thus the rewetting aeration condition was sufficient and the ventilators were turned on.
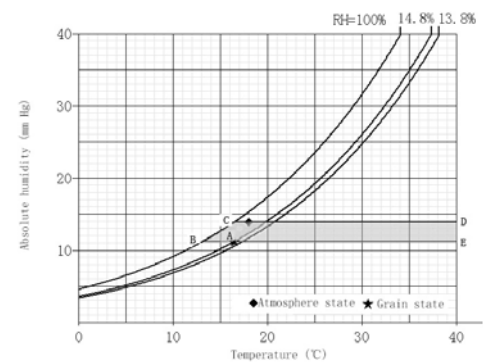
Fig. 3 The ventilators were turned off at 19:01 on October 20th, 2013.
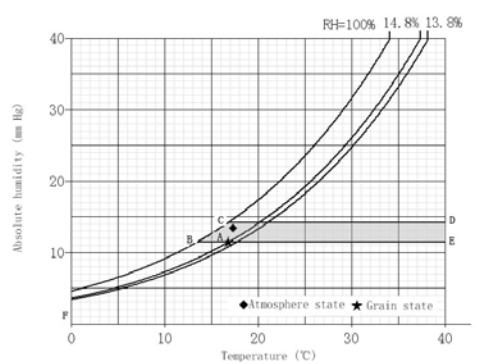
Fig. 4 The ventilators were running at 22:00 on October 21th, 2013.

At 11:14 on October 31th, 2013, the moisture of the grain bulk was 14.1%, the automatic detection system showed average grain bulk temperature (t_g) of 15.6°C, atmosphere temperature of 15.8°C, and atmosphere RH of 95%. The t_g was slightly higher than the dew temperature (DPT_a, 15.01°C) of the atmosphere. The atmosphere state point was outside of the BCDE area of the rewetting aeration window (Fig. 5), and the ventilators were turned off.

The rewetting aeration in No.12 warehouse was ended on November 2nd, 2013. Table 1 shows the data of automatic bulk detection. At 10:29, the grain bulk had a maximum temperature of 16.3°C, a minmum temperature of 15.8°C, and a mean temperature of 16.0°C. The temperature gradient in the grain bulk was ≤1°C m-1 grain layer. The air temperature and RH above the grain bulk surface was 16.0°C and 91%, respectively; the temperature and RH of the atmosphere were 16.3°C and 95%, respectively. The moisture of the grain bulk was 14.2%.

3.1.2. Energy consumption and profit anlysis for automatic paddy aeration

The rewetting manipulation was being carried out while the grain bulk was retrieved from storage. The moisture of the outbound grain was 14.1-14.2%. The moisture content in the remaining 612 t of paddy was 14.1% on November 2nd, and rewetting aeration was stopped. The total output grain was 1324 t until November 12th, and grain loss was 5.85 t. The aeration system ran 181.5 h, and power consumption was calculated to be $181.5 \times 2 \times 0.576 = 209.1$ kWh The annual mean grain loss in the depot was 0.8%, the grain loss for 1330 t paddy should be 10.64 t. Therefore, the increase in grain weight by rewetting was 4.79 t. The price for output paddy was 2.16 yuan kg-1, the sale of 4.79 t of paddy was 10346.4 yuan. The electricity charge per kW·h was 0.92 yuan, and electricity cost was 192.37 yuan, thus the net profit was 10154.03 yuan. The unit energy consumption [kW·h (1%moisture·t)-1] was $\frac{209.1}{10346.4 \times (14.1 - 13.5)\%} = 0.3336$.

Tab. 1 Detection data in No. 12 paddy warehouse before and after rewetting aeration.

Time	Grain layer	Mean grain layer temp. (°C)	Min. grain temp. (°C)	Max. grain temp. (°C)	Mean grain bulk temp. (°C)	Temp. above bulk surface (°C)	Atmosphere temp. (°C)	RH above bulk surface (%)	Atmosphere RH (%)
23:31 Oct. 8th, 2013	1	23.6	23.5	23.8	23.6	23.3	22.3	92.0	92.0
	2	23.6							
	3	23.7							
	4	23.8							
10:25 Nov. 2nd, 2013	1	16.0	16.3	15.8	16.0	16.0	16.3	91.0	95.0
	2	16.1							
	3	15.9							
	4	15.9							

Tab. 2 The running time of two ventilators.

Date	Running time	Date	Running time
Oct.8th-9th	9 h 43 min	Oct.22th-23th	12 h 20 min
Oct.9th-14th	Turned off	Oct.23th-24th	11 h 10 min
Oct.15th-16th	14 h 19 min	Oct.24th-25th	10 h 54 min
Oct.16th-17th	19 h 52 min	Oct.25th-26th	10 h 42 min
Oct.17th-18th	9 h 59 min	Oct.28th-29th	10 h 21 min
Oct.18th-19th	9 h 44 min	Oct.29th-30th	19 h 30 min
Oct.20th-21th	12 h 10 min	Oct.30th-31th	14 h 30 min
Oct.21th-22th	9 h 34 min	Oct.31th-Nov.1st	8 h 30 min
		Amount	181 h 38 min

3.2. The rewetting aeration in No. 13 warehouse in Qihe, Shandong province

3.2.1. Predicting paddy EMC from meteorological data
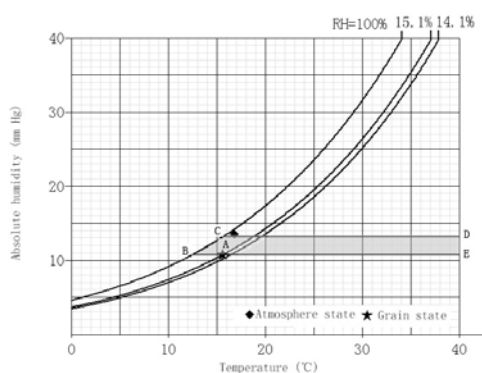
Fig. 5 The ventilators were turneded off at 11:14 on October 31th, 2013.
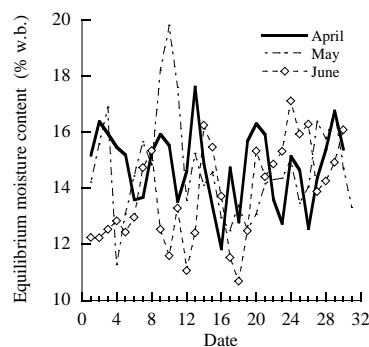
Fig. 6 The predicted EMC of paddy in Qihe, Shandong province.

The daily atmosphere RH and temperature during 17:00 to 8:00 from April to June in 2015 and 2016 was reviewed to predict the EMC of paddy. The number of days that the predicted desorption EMC is above 13.5% w.b. was 25, 22, and 16 in April, May, and June, respectively. The mean desorption EMC in April, May, and June was 14.81%, 14.71%, 13.83%, respectively, yielding an average of 14.45%. This indicated that the local atmosphere RH and temperature could be used to rewet paddy to 14% MC with automatic aeration.

3.2.2. Efficacy of automatic rewetting aeration
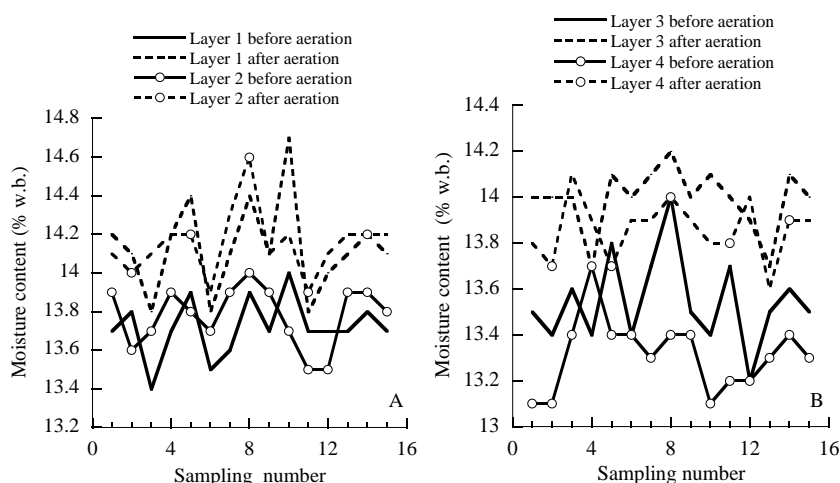
Fig. 7 Change in sample MC in paddy with automatic rewetting aeration.

Sampling number shows the average value of four bulk layers.

Tab. 3 Effect of controlled rewetting aeration on percentage of head rice yield in samples.

Sampling site	Before	aeration			After	aeration		
	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
			Head	rice	yield	(%)		
1	67.4	69.9	67.5	-	70.6	73	71.9	-
2	68.1	66.4	-	-	71.3	70.8	-	-
3	68.2	-	69.5	-	72.1	-	72.8	-
4	-	70.1	-	68.3	-	71.8	-	71.4
5	-	68.1	-	66.3	-	70.6	-	72.2
6	-	69.1	68.8	70.5	-	71.2	72	71.9
7	-	67.7	67.5	68.2	-	71.2	72.1	70.4
8	67.5	-	66.2	-	71.6	-	70.8	-
9	68.3	67.8	-	-	72.2	71.3	-	-
10	-	68.5	67.7	-	-	71.6	70.9	-
11	-	67.9		68	-	71.2	-	70.7
12	70.1	69.5	68.8	68.2	71	72.4	71.9	71.5
13	67.5	67.2		69.3	72.2	71.4	-	72.6
14	-	68.1	69.4	-	-	70.9	72.4	-
Average	68.2	68.4	68.2	68.4	71.6	71.8	71.9	71.5

The No. 13 warehouse in Qihe depot was chosen for rewetting on April 13th, and the treatment ended on June 16th, 2017. During automatic aeration over two months, three conditions were set: (i) when the atmosphere relative humidity (RH_a) is ≤80% and its temperature (t_a) is <28°C, the average grain temperature (t_g) is higher than the dew temperature (DPT_a) of the atmosphere, and the equilibrium absolute humidity (EAH_g) of grain moisture plus 1 percentage point is lower than the absolute humidity (AH_a) of the atmosphere, the two axial-flow fans were turned on; and (ii) when RH_a >80% and t_a <28°C, the two axial-flow fans were turned on. Whenever the t_a is above 28°C, the two fans were switched off.

This rewetting aeration increased grain moisture from an initial moisture content of 13.5% to 14.0% (Fig. 7) within the accumulated power consumption of 566.1 kWh using two 0.85 kW axial-flow fans. The unit energy consumption was 0.455 kW·h (1% moisture·t)$^{-1}$. The percent of average head rice yield in the whole depot after aeration was 71.7% (Tab. 3), significantly higher than that (68.2%) of

the non-rewetted paddy. The Chinese national standard of paddy (GB1350-2009, China) stipulates that the percentage of head rice yield of first grade japanic paddy should be higher than 61%. Tab. 4 shows the percent of damaged grains at each sampling location. It had some difference among different sampling sites in each bulk layer, but its mean values among four layers were not significantly different, indicating even moisture distribution in the whole paddy bulk. The percent of damaged grains in the whole warehouse was 7.7±1.8%.

Tab. 4 The percentage of damaged kernels in samples after controlled rewetting aeration.

Sampling site	1	2	3	4	5
Layer 1	10.01±2.89b	4.22±0.98ab	5.80±4.39ab	2.01±1.22b	10.41±4.64ab
Layer 2	5.77±5.34bc	4.13±1.41ab	6.54±0.44a	2.99±1.42b	14.50±1.97a
Layer 3	0.45±2.01c	6.22±2.06a	0.82±2.07b	10.92±4.61a	8.08±1.21b
Layer 4	13.34±0.42a	3.45±0.48b	10.47±3.68a	13.45±0.86a	7.38±2.34b

Sampling site	6	7	8	9	10
Layer 1	5.34±1.34a	3.20±1.58b	1.76±1.38c	3.62±2.01b	2.99±0.96b
Layer 2	7.94±3.78a	8.41±3.36a	16.82±0.84a	9.34±1.34b	9.27±2.81a
Layer 3	4.03±1.27a	5.76±2.11ab	10.08±4.65b	16.65±4.37a	0.50±2.23b
Layer 4	3.41±2.41a	10.04±4.34a	21.20±4.11a	7.73±4.47b	9.34±0.84a

Sampling site	11	12	13	14	Average
Layer 1	5.92±1.27b	10.75±4.81bc	5.83±0.53a	5.34±0.15b	5.52±2.98a
Layer 2	2.22±1.79c	7.52±1.98c	0.61±2.27b	6.47±1.81b	7.24±4.58a
Layer 3	11.13±1.64a	24.92±4.11a	5.83±1.11a	9.62±1.17a	8.22±6.68a
Layer 4	9.97±0.15a	11.20±0.91b	8.36±3.21a	8.15±6.91ab	9.83±4.43a

The damaged kernel was determined as described by Li et al. (2016). The different small letters in the same column show significant different ($p<0.05$) at LSD-test.

4. Discussion

Banaszek and Siebenmorgen (1990) reported that air conditions of 12.5°C/RH 50%, 15°C/RH 50% and 12.5°C /RH 90% were not obtainable with the RH and temperature control unit used for their adsorption EMC experiment with rough rice. The reason is not clear. We found that for "Longyang" variety japanic paddy samples from northeast China with 13.57% initial MC and under 65% ERH it had moisture adsorption at 10°C, but had moisture desorption at 20 to 35°C. Below 86% ERH condition, it had moisture adsorption at 10 to 35°C (Li, et al., 2015). These results suggest that paddy samples with 13.5% MC could be rewetted at 80% RH and 10-25°C of ambient condition.

Acknowledgements

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Drying Ginger and Preserving 6-Gingerol

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Abstract.

Ginger rhizome (*Zingiber officinale*) is widely used as a spice or as a medicinal plant. The major bioactive compound in fresh ginger rhizome is 6-gingerol and it is known for having a number of physiological effects. This compound is heat-sensitive and during cooking or drying will transform into 6-shogaol. Hence, the 6-gingerol content is used to evaluate the quality of dried ginger. The content of 6-gingerol during drying was measured using HPLC. Several factors that could affect the 6-gingerol content were considered and a predictive model for changes in 6-gingerol has been developed from the experimental data. The predictive model includes a single term drying model that predicts the changes of moisture content during drying. Drying time and relative humidity (ranging from 10% to 40%) impacted 6-gingerol content whereas drying air temperature (ranging from 30ºC to 60ºC) had a lesser effect. It was also found that the 6-gingerol content in fresh rhizomes was highly variable and thus required thorough testing prior to drying to be able to make the prediction more accurate.

Keywords: ginger, air drying, 6-gingerol, HPLC, predictive model.

1. Introduction

Background

Ginger, with a scientific name of *Zingiber officinale* Rosc, is a member of the tropical and sub-tropical family Zingiberaceae. It originates in tropical rainforests in southern Asia and spread to Mediterranean regions by the 1st century. In ancient Rome, ginger was a popular spice used to make delicacies. Throughout the history of global trade, ginger has been traded longer than most other spices. In the ancient world, it was regarded as a costly herb for its medicinal merits and nutritional value.

Over the long history of ginger trading around the world, ginger has been planted on most continents. Given different growing environment, ginger has developed into several cultivars. In commercial trading, ginger is often designated by the country where it originates from, such as Chinese ginger, Indian ginger, Australian ginger or Jamaican ginger. However, ginger has a large cultivar diversity, so that even in one country, there could be dozens of cultivars. Generally, a cultivar comes from a specific growing place, and hence many cultivars were named after their growing place.

Chemical composition of ginger

Ginger rhizomes contain a variety of compounds. Researchers have found more than one hundred compounds which can be classified into three groups: essential oils, gingerol and diarylheptanoids.

Essential oils are hydrophobic liquids, containing volatile chemical constituents. Distillation and extractions are the most common ways to isolate the essential oils. The major components of essential oils are the terpenoids, including monoterpenes and hemiterpenes. Most compounds from these two groups have a strong volatile aroma and biological activity, which are important ingredients in medicine, cosmetics and food production.

Gingerols are major pungent constituents of ginger which are made up of several different compounds. Gingerols have a 4-hydroxy-3-methoxyphenyl group in the chemical structure, varying according to different aliphatic chains attached to the main group. Gingerols can be classified as gingerol, shogaol, gingerdione and gingerdiol.

Gingerols are thermally labile due to the presence of a β-hydroxy keto group in the structure and produce corresponding shogaols via a dehydration reaction (Bhattarai, 2001). The dehydration process will be affected by drying air temperature and residence time. It is reported that raising the reaction temperature and extending time significantly increased the conversion of 6-gingerol to 6-shogaol (Kou et al., 2017). Among gingerols, 6-gingerol has been studied more thoroughly compared to other gingerols such as 8-gingerol and 10-gingerol. This is because the proportion of 6-gingerol in fresh ginger is the highest among all gingerols.

Effects of ginger on human health

Among all of the compounds in ginger, essential oils play an important role in improving consumers' mood. The benefits of ginger essential oils include offering a warm, spicy aroma which enhances feelings of vitality, promotes feeling of physical well-being, and helps improve body blood circulation. It is a frequent addition to blends for massage, arthritis and muscle aches and pains. Ginger oil is commonly used to soothe, comfort and balance digestive discomfort. Gingerols may make a contribution towards human health effects in medical applications of ginger. Studies have shown antitumor activity of 6-gingerol （Parket al., 1998), analgesic and anti-inflammatory effects (Young et al., 2005), and 10-gingerol and 12-gingerol have antibacterial activity against periodontal bacteria (Park et al., 2008). The content of gingerols varies significantly with ginger varieties and cultivating locations.

Use of ginger and derived products

Ginger is used as a main ingredient in many products throughout the world. Fresh ginger roots are juicy with a mild taste and can be used as spices for sweet or salty food such as soup, meat, vegetable, seafood, pickle, curries, drinks and cake. However, due to the strong pungent flavour of fresh ginger root, fresh ginger is normally dried, and used to produce ginger powder, which makes the spicy flavour weaker. Yet, the major use of ginger is in the pharmaceutical area. In many Asian countries, especially China, India and Japan, ginger is treated as one of the additives in traditional medicine, rather than as herb. Therefore, ginger is considered as herbal medicine with strong health benefits.

Dried ginger and dried ginger products account for the largest amount of ginger consumption around the globe, as fresh ginger is mainly produced in tropical and subtropical countries. Dried ginger significantly lowers the cost of transporting and storing. Generally, dried ginger is produced from fresh mature ginger rhizomes whereas immature ginger rhizomes are processed to make preserved ginger, as the mature rhizome has stronger flavour and aroma.

Aim of this study

The aim of this study was to investigate the ability of a two-layer drying model to predict the optimum conditions for drying fresh ginger rhizomes for the maximisation of the retention of 6-ginerol in the dried ginger.

2. Materials and Methods

Ginger samples

The samples of fresh ginger rhizomes were obtained from Buderim Ginger Pty Ltd in Yandina, Queensland, Australia. They were shipped to Sydney in a refrigerated container and then placed in

a freezer at -20°C until being used for experiments. The day before the experiments the samples were defrosted overnight in a fridge at +4°C.

Defrosted ginger rhizomes were peeled and sliced to 5 mm thickness using a slicer. A sample of about 10 g of slices was used for determination of initial moisture content. Duplicate samples were placed in a convection oven at 105°C for 24 h.

Drying

The drying experiments were conducted in a cabinet dryer constructed in the workshop of the School of Chemical Engineering of the University of New South Wales. The cabinet dryer (see Fig. 1) had an electric heater (15 kW) fitted with a PID controller and a fan (0.75 kW). The airflow was parallel to the tray on which the drying samples were placed in a thin layer. The temperature and relative humidity were monitored and recorded with a datalogger. The weight loss was recorded with an electronic balance placed under the sample holding tray.
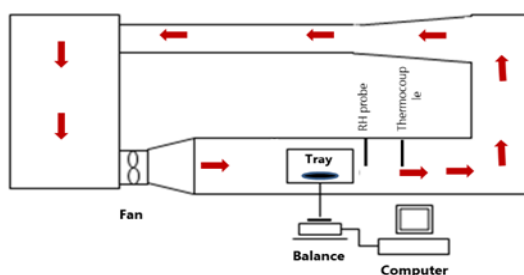
Fig. 1 Cabinet dryer.

The drying conditions in the experiments were either a constant temperature and relative humidity (RH), as in Runs 1-3, or a changing temperatue and RH, as in Runs 4-11 (see Tab. 1).

The reason for this experimental design was that drying temperature was expected to affect the drying process. Since the properties of the ginger samples were changing during the drying process, a change of the drying conditions in the dryer was allowing for investigation of the effects of the changed conditions on the drying behavior of the samples.

The wet sample of sliced rhizome was placed on the tray as quickly as possible in order to reduce the influence of ambient conditions on those in the drying chamber.

After the weight of the sample became steady, i.e., the sample reached the equilibrium moisture content corresponding to the drying conditions (temperature and RH), the drying run was finished. A duplicate dried sample (10 g each) was taken and used for the determination of the final moisture content.

The remainder of the dried sample was weighed and preserved in a vacuum-sealed bag. It was kept in a cool place for the determination of the 6-gingerol content.

Determination of 6-gingerol

The 6-gingerol standard ($\gg$ 98% purity) was obtained from Sigma-Aldrich. Methanol of HPLC grade was purchased from Burdick & Jackson. Water for HPLC analysis was purified with a Milli-Q water system. Agilent vials for HPLC with caps were used. Whatman filter paper had a pore size of 0.45 μm.

Dried ginger slices were pulverized using a grinder and passed through a 40 mesh (0.42 mm) sieve before extraction. In contrast, the fresh ginger was crushed with a mortar and pestle prior to extraction. The sample of ginger powder or paste (1 g) was dissolved in 25 mL HPLC-grade methanol and sonicated for 30 min. The mixtures were centrifuged at 10000 rpm for 10 min and supernatant

was filtered through Whatman filter paper. Then the supernatant was diluted with water to reach a final concentration of 10% methanol and 90% water. Extracts of ginger were transferred to the HPLC vials and capped. All the extracts were kept at 4°C until being used.

Tab. 1 Summary of drying conditions.

Run number	Temperature (°C)	RH (%)
1	40	30
2	50	20
3	60	10
4	60	10
	50	20
5	60	10
	50	30
6	60	10
	50	40
7	60	10
	40	30
8	60	10
	30	40
9	50	30
	40	30
10	50	20
	60	10
11	40	30
	50	20

From Run 4 to Run 11, the drying conditions were changed from those in the 1st rows to those in the 2nd rows when the sample had lost 50% of its weight.

For the calibration curve of 6-gingerol, a stock solution of 5.0 mg/mL of standard in HPLC-grade methanol was prepared. Serial standard dilutions were made from the stock solution to obtain concentrations of 5.0, 10.0, 20.0, 40.0, 60.0 and 80.0 µg/mL. All dilutions of 6-gingerol standard were capped and stored at 4°C.

The HPLC system used in this study was from Shimadzu, model Prominence LC-20AD. The separation of the compounds was conducted in a C18 column (XTerra), 3.5 µm, and 2.1×150 mm. Water (A) and methanol (B) constituted the mobile phase for the separation. The following linear gradient was used: 0-5 min, 50% B; 5-10 min, 50-60% B; 10-15min, 60% B; 15-25min, 60-80% B; 25-30 min, 80% B; 30-35min, 80-50% B; 35-50 min, 50% B. The injection volume was 20 µL and the flow rate was 0.2 mL/min. The detection wavelength of the UV detector (0~1000 nm) was set at 281 nm and the column temperature was maintained at 30°C. The tests were done in triplicate.

3. Results

Content of 6-gingerol in dried samples

Prior to the drying experiments, 6-gingerol content of fresh ginger rhizomes was determined and found to be on average 0.59 ± 0.06 µg/mg 6-gingerol on dry matter basis.

Tab. 2 shows the summary for three constant drying conditions and multiple comparisons while Tab. 3 shows the results of the statistical analysis of the samples (ANOVA). There was a considerable variability within the results of each treatment as indicated by the value of the coefficient of variation (CV). It appears that the highest 6-gingerol content in a dried sample (0.456 µg/mg) was obtained after drying at 50°C and 20% RH. This was far below the initial content in fresh sample (0.5 µg/mg). From the ANOVA test, see Tab. 3, the 6-gingerol content from the three runs was significantly different from each other. Run 2 showed a higher 6-gingerol content than Run 1. This was expected since the drying temperature in Run 2 was higher and the drying time shorter.

However, a considerably shorter drying time in Run 3 did not necessarily result in a higher 6-gingerol content.

Tab. 2 Characteristics of samples subjected to different drying treatments with constant temperature and relative humidity.

Run	Drying conditions	Drying time (min)	6-gingerol content* (µg/mg)	CV (%)
1	40°C 30% RH	314	0.337	9.52
2	50°C 20% RH	293	0.456	4.57
3	60°C 10% RH	154	0.444	3.43

*Average of three experiments

Tab. 3 ANOVA test between constant drying conditions runs

Run comparison	P value
1 vs 2	0.000
1 vs 3	0.000
2 vs 3	0.360

ANOVA, P=0.05

Tabs. 4 and 5 show the summary for eight changing drying conditions and multiple comparisons. In general, the following trend in 6-gingerol content was observed: higher drying temperature and longer drying time resulted in lower gingerol content. This corresponds to the conclusions from the study of Bhattarai et al. (2001) that higher temperature results in rapid and faster dehydration of 6-gingerol and forming of the degradation product, 6-shogaol.

It is clear that there was no significant difference between Runs 4 and 10, Runs 5 and 9, and Runs 7 and 8. Runs 4 and 10 were conducted under similar conditions but Run 10 was 72 min longer, which indicated drying time had less impact on 6-gingerol reduction. There was a significant difference between Runs 4, 5 and 6, in which samples were dried at the same temperature but at different RH and with a different drying time. This showed that RH could have played a role in the decrease of 6-gingerol.

There was no significant difference between Runs 7 and 8. However, Run 8 had a lower average temperature and longer drying time. The reason for this result could be due to the combined impact of drying temperature and drying time.

Tab. 4 Characteristics of samples subjected to different treatments with changing conditions

Run	Drying conditions	Drying time (min)	6-gingerol content* (µg/mg)	CV (%)
4	60°C 10% RH to 50°C 20% RH	207	0.349	3.53
5	60°C 10% RH to 50°C 30% RH	133	0.578	4.79
6	60°C 20% RH to 50°C 40% RH	283	0.448	2.79
7	60°C 10% RH to 40°C 30% RH	253	0.401	2.47
8	60°C 10% RH to 30°C 40% RH	275	0.403	4.56
9	50°C 30% RH to 40°C 30% RH	231	0.588	4.40
10	50°C 20% RH to 60°C 10% RH	279	0.356	9.08
11	40°C 30% RH to 50°C 20% RH	282	0.497	0.98

*Average of three experiments

Tab. 6 shows ANOVA test results between constant drying conditions and changing drying conditions. Four groups showed no significant difference, which were Runs 1 and 4, Runs 1 and 10, Runs 2 and 6, and Runs 3 and 6. Runs 1 and 4 both obtained a lower gingerol yield while Run 4 was conducted at a higher temperature and lower humidity. The reason for this could be the much longer drying time of Run 1 (107 min longer). Similar situation happened in Runs 1 and 10, while Run 1 lasted 35 min longer than Run 10. Runs 2 and 6 had a similar drying time. However, Run 6 was conducted at a higher temperature and RH. This result again suggested that RH could affect 6-gingerol content in drying process.

Tab. 5 ANOVA test between changing drying conditions runs

Run	P value	Run	P value
4 vs 5	0.000	6 vs 8	0.000
4 vs 6	0.000	6 vs 9	0.000
4 vs 7	0.000	6 vs 10	0.000
4 vs 8	0.000	6 vs 11	0.000
4 vs 9	0.000	7 vs 8	0.863
4 vs 10	0.519	7 vs 9	0.000
4 vs 11	0.000	7 vs 10	0.000
5 vs 6	0.000	7 vs 11	0.000
5 vs 7	0.000	8 vs 9	0.000
5 vs 8	0.000	8 vs 10	0.000
5 vs 9	0.713	8 vs 11	0.000
5 vs 10	0.000	9 vs 10	0.000
5 vs 11	0.000	9 vs 11	0.000
6 to 7	0.0 00	10 vs 11	0.000

ANOVA, P=0.05

No significant difference between Runs 3 and 6 showed that drying at a higher temperature, shorter time and lower relative humidity results in a similar gingerol content as a run at lower temperature, longer drying time and higher humidity, which indicates that interactions between drying temperature, time and RH are likely to have an impact on 6-gingerol content.

Tab. 6 ANOVA test between constant and changing conditions.

Run	P value	Run	P value
1 vs 4	0.483	2 vs 8	0.006
1 vs 5	0.000	2 vs 9	0.000
1 vs 6	0.000	2 vs 10	0.000
1 to 7	0.001	2 vs 11	0.027
1 vs 8	0.001	3 vs 4	0.000
1 vs 9	0.000	3 vs 5	0.000
1 vs 10	0.301	3 vs 6	0.790
1 vs 11	0.000	3 vs 7	0.023
2 vs 4	0.000	3 vs 8	0.028
2 vs 5	0.000	3 vs 9	0.000
2 vs 6	0.662	3 vs 10	0.000
2 vs 7	0.005	3 vs 11	0.005

ANOVA, P=0.05

Predictive model for 6-gingerol

Drying time and drying temperature are considered as the two main factors affecting gingerol content (two-factors model). The prediction model function of 6-gingerol in this case is expressed by equation (1):

$$G = G_0 \exp(-k_{G_0} t) \qquad (1)$$

where G is the final 6-gingerol content, G_0 is the initial 6-gingerol content, k_{G_0} is the 6-gingerol rate constant and t is the drying time.

Combining equation (1) with the single term drying model for ginger we obtain equation (2):

$$G = G_0 \exp\left(-k_{G_0} \exp\left(\frac{h}{RT}\right) t\right) \qquad (2)$$

where h is the activation energy (J), R is the gas constant and T is the product absolute temperature (K).

Tab. 7 shows the experimentally determined value vs the model calculated 6-gingerol content for each of the drying treatments.

Tab. 7 Summary of 6-gingerol content from different drying treatments: experimental and model calculated values.

Run	Stage	Drying time (min)	6-gingerol content (µg/mg)	
			Experiment	Model
1		282	0.336	0.402
2		293	0.454	0.413
3		154	0.444	0.479
4	1	35		
	2	172	0.348	0.463
5	1	32		
	2	101	0.578	0.510
6	1	53		
	2	231	0.448	0.419
7	1	33		
	2	220	0.401	0.436
8	1	54		
	2	219	0.403	0.423
9	1	60		
	2	170	0.587	0.448
10	1	49		
	2	230	0.356	0.421
11	1	77		
	2	204	0.494	0.420

"-" means constant drying conditions. 1 and 2 means before changed drying conditions and after changed drying conditions.

The quality of the fit between the experimentally obtained values and the model calculated values was evaluated by the coefficient of determination (R^2) and root mean square error (RMSE). For the results shown in Tab. 7, they were R^2=0.4994 and RMSE= 0.0713.

The reasons for this relatively poor fit may have been due to the fact that there was a considerable level of variability in the initial moisture content of ginger rhizomes. Furthermore, the model did not take into account the RH of the drying air. In order to study the effects of RH, anew model (see equation (3)) was developed including RH:

$$G = G_0 \exp\left(-\left(k_{G_0} + A * RH\right) exp\left(\frac{h}{RT}\right) t\right) \qquad (3)$$

where A is a constant, RH is the relative humidity (decimal). Equation (3) is based on equation (2), and is the simplest modification to this model for including the effect of relative humidity on 6-gingerol depletion.

The new models showed an improvement of the fit having a higher coefficient of determination (R^2=0.5923) and a lower RMSE (0.0675).

4. Discussion

Combining a single term drying model and a three factors model for predicting gingerol content allows for devising the most appropriate drying conditions to obtain a maximum 6-gingerol yield.

A shorter drying time, a lower drying temperature and a higher RH reduce the 6-gingerol depletion in dried ginger.

The results suggest that the optimum drying condition of ginger is: air temperature of less than 40°C; RH of 40% at the late stage of drying, drying time not exceeding 300 min.

Acknowledgement

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Numerical modeling of the horizontal flow and concentration distribution of nitrogen within a stored-paddy bulk in a large warehouse

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Abstract

The insect population in grain stores can be kept under control by maintaining a high concentration of N_2 gas throughout the grain bed. The development of controlled atmosphere storage technology for insect control requires an accurate prediction of the distribution of introduced gases in bulk grain. In this paper, based on the convective-diffusion model, the horizontal flow of N_2, which was introduced into the paddy bulk in a large warehouse by means of the horizontal ventilation system, are modeled as fluid flow in a porous medium. The experimental data for N_2 transfer and flow through ducts and bulk paddy were used to validate the model. The equations were solved using the finite difference method, and the predictions from the proposed model were in good agreement with the experimental results. The concentration distribution and flow uniformity of nitrogen in stored paddy were also analyzed during the nitrogen-filling procedure for CA storage. It was shown that it is feasible and practical to introduce nitrogen into stored bulk grain in a large warehouse by means of the horizontal ventilation system.

Keywords: numerical modeling, stored paddy, concentration distribution, nitrogen-filling procedure

1. Introduction

Chemical control methods such as fumigation with phosphine are effective against insect pests, but have disadvantages including residue problems and development of tolerance by insects (Banks et al.,1990). In the recent past, the use of controlled atmosphere (CA) as a safe residue-free alternative to chemical fumigants and protectants has gained popularity for controlling insects infesting stored grain. Controlled atmosphere with low oxygen (O_2) and high nitrogen (N_2) in storage by injecting nitrogen into the storage displacing the oxygen is just one of a number of methods that can be used for controlling pests in stored products.

Controlled atmosphere storage for insect control involves the alteration of the proportion of the normal atmospheric gases, i.e., N_2, carbon dioxide (CO_2) and O_2, to create an atmosphere that is lethal to the insects. The success of CA and fumigants in killing stored product pests depends on the movement of gas through and uniform distribution of the gas in the stored grain, and maintaining a lethal gas concentration level for the required exposure period.

Three-dimensional heat, mass and momentum transfer models with concentration species were developed by Singh et al. (1993), Lawrence et al. (2013a, 2013b) and Mat Isa et al. (2014) for predicting fumigant concentrations in a rectangular domain or cylindrical silo. These models need to be validated under a wider range of conditions, and can then be used to evaluate causes of fumigation failures and to develop best management practices to prevent the failures. Although

the Lawrence et al. model was not validated, it included gas sorption and insect extinction models which were empirically based.

A mathematical model of the three-dimensional movement of CO_2 in stored wheat was solved using the finite-element method (Alagusundaram et al., 1996a, b). The model simulates the initial bulk flow of CO_2 as it sublimates and expands from a dry ice source. The mean relative errors between the predicted and measured CO_2 concentrations in a bin with an open top surface were 24% at 3 h and 16% at 21 h. The model developed by Alagusundaram et al. (1996c) handles the loss of CO_2 by modifying boundary conditions. It can predict the CO_2 concentrations at any point in a grain bulk stored in any shape or size structure and can be used by engineering designers to determine the best location for adding CO_2, the approximate amount of CO_2 needed, and the expected level of insect mortality. The boundary conditions for gas transfer models in stored grain can be quite variable (Jayas, 1995). The CO_2 leaving the grain surface will diffuse rapidly into the head space air. If the head space is not well ventilated by wind through openings in the roof and wall, the CO_2 concentration may rise above the atmospheric level. The top surface could be covered with a gas impermeable sheet, resulting in zero flow across the boundary.

Uniform and fast application of N_2 to all parts of the grain storage is fundamental to effective pest management. However, relatively few studies have been reported on the movement of N_2 through the stored grain and uniform distribution of N_2 in the stored grain. This study investigated the flow and concentration distribution of nitrogen inside a grain storage structure during the nitrogen-filling procedure. The specific objective was to model the flow uniformity of nitrogen within a stored-paddy bulk in a large warehouse using computational fluid dynamics. In this paper, the horizontal flow, concentration distribution and uniformity of nitrogen were evaluated and verified within a stored-paddy bulk in a large warehouse during the nitrogen-filling procedure by means of a ventilation system.

2. Materials and Methods

2.1. Controlled atmosphere (CA) grain storage model

The simulation used a 3-dimensional model of a stored paddy warehouse with dimensions of 18 m wide, 50 m long and 5.0 m flat height. The flat top surface of the stored paddy was covered by a gas impermeable sheet of polythene (Fig. 1). The stored grain was represented by a porous zone. Nitrogen from a nitrogen generator was introduced into the stored paddy at the main horizontal and branch vertical perforated ducts on the north side, then cross-flowed the stored paddy to the main and branch perforated ducts on the south side using suction fans which exhausted the gas to the outside of the warehouse.
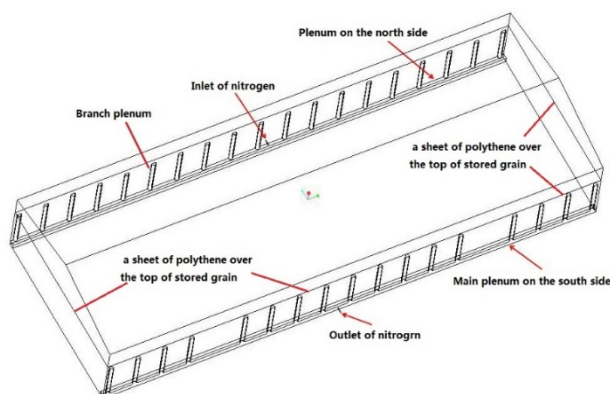
Fig. 1 Schematic diagram of the horizontal ventilation system.

2.2. Mathematical Model and CFD Model Parameters

2.2.1 Mathematical model of nitrogen flow and convection-diffusion in stored grain mass

The physical processes that occur in bulks of stored grain obey conservation laws. These phenomena were captured mathematically by a partial differential equation of the form:

$$\varphi \frac{\partial C}{\partial t} + u_j \frac{\partial C}{\partial x_j} = \frac{\partial}{\partial x_j}\left[D_{eff} \frac{\partial C}{\partial x_j} \right]$$

(1)

where φ is the grain bulk porosity (φ =0.55), u_j is the component of velocity in the jth direction (m/s), t is the time (s), C is the nitrogen concentration (species fraction) in stored grain, D_{eff} is the nitrogen effective diffusion coefficient in stored grain (D_{eff} =2.46 * 10^{-5} m²/s) (Thorpe, 2008).

2.2.2 CFD model parameters

ANSYS Fluent was used to solve the transient species transport model. For the porous zone, a porosity of 0.55 for paddy and a computed viscous loss coefficient of 2.037 x 10⁸ /m² were used as inputs; the inertial loss coefficient was 26767 (Pa•s²/kg) (Thorpe, 2008). Nitrogen inlet was given a fixed velocity of 5.0 m/s, i.e., the volume flow rate of nitrogen at the inlet was 60 m³/h. The concentration of nitrogen at the entrance was 0.998 (species fraction). The initial temperature was assumed 25°C. The walls were considered no-slip wall boundary conditions. The initial species mass fractions were assumed 0.78 for N_2 and 0.22 for O_2.

3. Results

3.1 Numerical simulation results and analysis

Fig. 2 shows the streamlines of nitrogen flow in the stored paddy. It can be seen that the nitrogen uniformly entered from the ducts on the north side, then cross-flowed through the stored paddy with a superficial velocity of 6.67×10⁻⁵ m/s to the ducts on the south side. Fig. 3 shows nitrogen continues to move forward horizontally in the grain mass covered by a sheet of polythene. The average concentration of nitrogen in the grain mass was above 0.99 (species fraction) when the time of the nitrogen-filling procedure was about 61 hours (above 2.5 days) (Fig. 4), while nitrogen concentration of the outlet reached 0.978 (species fraction). According to the standard of CA with nitrogen in China (more than 0.97), the nitrogen-filling process can be stopped at this time.
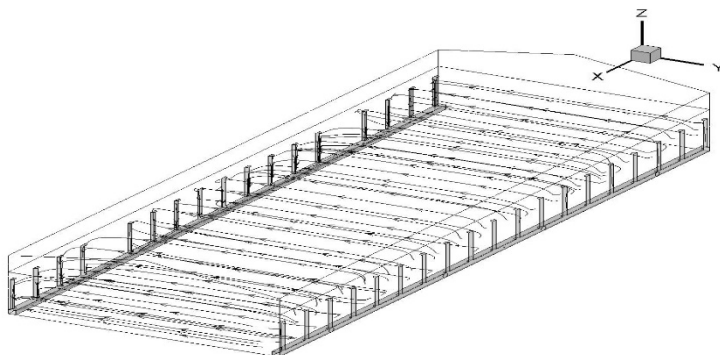
Fig. 2 The streamlines of nitrogen in the stored paddy inside the storage warehouse.
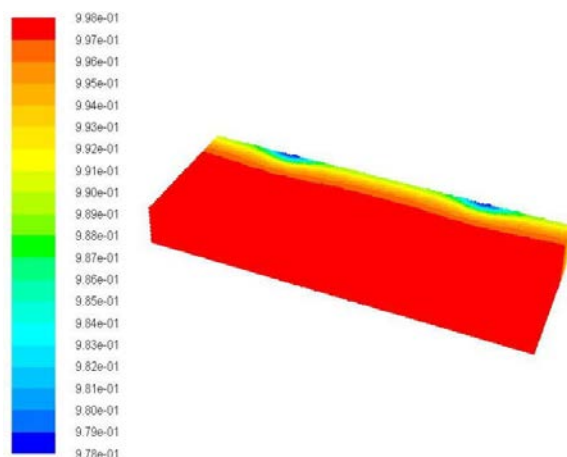
Fig. 3 Distribution of nitrogen concentration at 61 hours during the nitrogen-filling procedure in stored paddy.
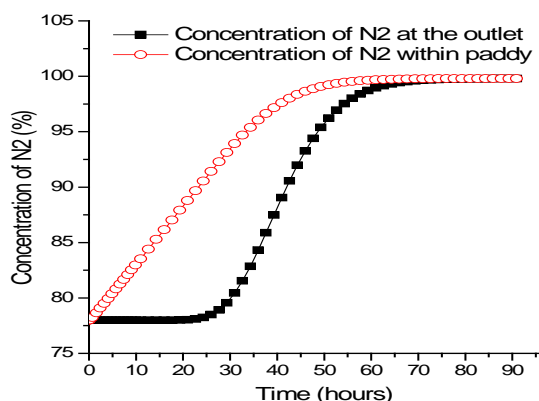
Fig. 4 Variation of nitrogen concentration in stored paddy with the time of nitrogen-filling.

3.2 Evaluation of uniformity of nitrogen concentration in the grain mass

The uniformity index was used to evaluate the uniformity of nitrogen concentration distribution in the stored paddy during nitrogen-filling. The formula of uniformity presented by Weltens (1993) was used to calculate the value of uniformity of nitrogen concentration inside stored paddy. Fig. 5 is a layout of the monitoring points of nitrogen concentration at each level in the stored paddy. For monitoring of the nitrogen concentration, 27 monitoring points at three levels were placed inside the stored paddy, and each layer had nine points. The heights of the monitoring points for the upper, middle and bottom layers were 1, 2.5 and 4 m, respectively, away from the warehouse floor. Each monitoring point was 1 m away from the inner wall of the warehouse. The evaluation of observed and simulated uniformity values of nitrogen concentration distribution inside the stored paddy is summarized in Tab. 1. It was found that the nitrogen gas was distributed uniformly in the stored paddy after 61 hours of nitrogen-filling. A good agreement between the observed and simulated nitrogen concentrations indicated that the model and the parameter values used in the model are applicable for predicting nitrogen gas concentration in stored grains.
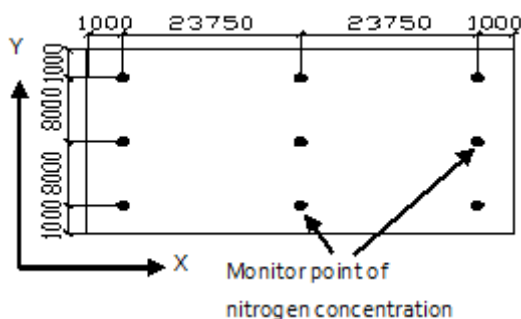
Fig. 5 Layout of monitoring points of nitrogen concentration at each layer in stored paddy.

Tab. 1 Comparison of uniformity values of nitrogen concentration distribution from a field observation and simulation.

	Uniformity of nitrogen concentration in X direction			Uniformity of nitrogen concentration in Y direction			Uniformity of nitrogen concentration in Z direction		
Location (m)	X=1.00	X=24.75	X=48.50	Y=1.00	Y=9.00	Y=17.00	Z=1.00	Z=2.50	Z=4.00
Value of monitoring (species fraction)	0.9911	0.9897	0.9916	0.9890	0.9997	0.9882	0.9948	0.9936	0.9987
Value of simulation (species fraction)	0.9997	0.9997	0.9997	0.9999	0.9999	10.000	0.9997	0.9997	0.9996

4. Conclusions

Application of controlled atmosphere technology in grain storage using nitrogen is highly efficient, environmentally friendly and safe. It is essential to investigate the movement of gas through the grain mass and the uniformity of gas distribution in the stored grain in order to maintain a lethal concentration level for the required exposure period. In this paper, numerical simulation of the horizontal flow and concentration distribution of nitrogen within a stored-paddy bulk in a large warehouse was conducted. The concentration distribution and flow uniformity of nitrogen were also analyzed during the nitrogen-filling process for CA storage. The following specific conclusions were drawn from this study:

(1) Numerical modeling can accurately predict the flow and concentration of nitrogen inside a stored grain mass during the nitrogen-filling process.

(2) It is feasible and practical to introduce and distribute nitrogen gas into a stored grain bulk in a large warehouse by means of the horizontal ventilation system of the warehouse.

Acknowledgement

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Study on Rapid Detection of Degree of Freshness of Paddy Rice in China

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Abstract

This paper describes research results and progress of rapid detection of the degree of freshness of paddy. We studied the changes of degree of freshness, fat acidity value and taste evaluated value of paddy under different storage conditions in the laboratory. The correlations between the degree of freshness, fat acidity value and taste evaluated value were analyzed. The results showed that there was a significant negative correlation ($p < 0.01$) between the degree of freshnessand fat acidity value. The correlation coefficient was -0.845. The degree of freshness was significantly positively correlated with the taste evaluated value, and most of the correlation coefficients were above 0.9. The nationwide investigation result of paddy's degree of freshness showed that there was an obvious distinction in the degree of freshness between newly harvested rice and rice harvested in previous years. The degree of distinction of indica rice achieved 85%. Due to its special reasons, japonica rice had a lower degree of distinction, but it also reached 75%.

Keywords: paddy, degree of freshness, fat acidity value, taste evaluated value.

1. Introduction

Rice is a staple food for more than 60% of the world's population, especially in China (Wei et al., 2007). As a primary dietary source of carbohydrates, rice plays an important role in meeting caloric requirements and nutrient intakes (Yang et al., 2006). Aging during storage results in numerous changes in the chemical and physical properties of rice (Patindol et al., 2005; Singh et al., 2006; Sodhi et al., 2003). These changes in pasting properties, color, flavor, and composition affect rice cooking and eating quality (Srikaeo K et al., 2013; Park C E et al., 2012). The fresh rice is prefered in the market in China. So it is particularly important to detect the degree of freshness rapidly during acquisitions, and during daily or long-term storage of paddy rice.

Since 2013, we have developed an instrument which could detect the paddy freshness rapidly for the degree of freshness. The higher the degree of freshness of paddy is, the fresher it is. The detection principle of the degree of freshness of paddy is that milled rice is mixed with special reagent, according to the different contents of ketones and aldehydes, the solution reveals different color. Analysing the spectrum of these colors, we can quantify the degree of freshness of paddy. The instrument is easy to use, and the results are objective and accurate.

In nearly two years, the research on rapid detection of degree of freshness has made new progress. We studied the changes of paddy freshness qualities during different conditions of storage in a

laboratory and the correlations between the degree of freshness, taste evaluated value, and fat acidity value. The result proved that the degree of freshness is a sensitive index which can reflect its freshness quality in the aging process. We have detected the degree of freshness of paddy rice of different producing areas, varieties and production years for three years across the country. A total of 9381 samples were statistically analyzed, yielding the standard of determination and evaluation of degree of freshness of paddy.

2. Materials

2.1 Materials of Storage Experiment

Fourteen samples of fresh japonica rice and indica rice were selected from 6 provinces which included Jiangsu, Heilongjiang, Jilin, Jiangxi, Zhejiang and Anhui. The japonica rice samples were numbered from 1 to 9, and the indica rice were numbered 10 to 14.

2.2 Nationwide investigation of paddy degree of freshness

The producing area of indica rice samples covered 14 provinces and the harvest years were from 2012 to 2016. The number of samples of indica rice was 3106. There were 2097 new havest samples in 2015 and 2016, and 1032 samples harvested in previous years from 2011 to 2014.

The producing area of japonica rice covered 6 provinces and the harvest years were from 2011 to 2016. The number of samples of japonica rice was 1612. There were 1177 new havest samples in 2015 and 2016, and 357 samples harvested in previous years from 2011 to 2014.

3. Instruments and Equipment

Degree of freshness tester of paddy rice: JCXD10, Beijing Dongfu Jiuheng Instrument Technology Co., Ltd.

Rice hulling machine: JDMZ, Beijing Dongfu Jiuheng Instrument Technology Co., Ltd.

Rice mill: JNM - Ⅲ, Chengdu Shitewei Technical and Development Company.

Hammer Cyclone mill: JXFM110, Shanghai Jiading Grain and Oil Instrument Co., Ltd.

4. Experimental Method

4.1 Grain storage and sampling method

After packing and sealing, experimental paddy samples were stored at indoor constant temperatures of 25℃ and 35℃, respectively. Three hundred and fifty grams of paddy were sampled periodically and the specific sampling times are shown in Tab. 1.

Tab. 1 Sampling Time.

Sampling Frequency	Sampling Time	
	25℃/(W)	35℃/(W)
start	0	0
first	8	2
second	20	4
third	60	7
fourth	92	12
fifth		24
sixth		32
seventh		64

W: means per week

4.2 Degree of freshness determination

In accordance with LS/T 6118-2017 Inspection of grain and oils-Determination and evaluation of degree of freshness of paddy.

4.3 Fat acidity value determination

According to the appendix A of *GB/T 20569-2006 The determination rules of rice quality in storage*.

4.4 Taste evaluated value determination

According to the appendix B of *GB/T 20569-2006 The determination rules of rice quality in storage*.

5. Results and Analysis

5.1 Results of storage experiment

5.1.1 Change of each quality indicators

The change in degree of freshness, fat acidity value and taste evaluated value in fourteen paddy samples stored respectively under 25℃ and 35℃ constant temperature conditionsare as shown in Figs. 1, 2 and 3 over lngth of storage time.

a 25℃　　　　　　　　　　　　　　b 35℃
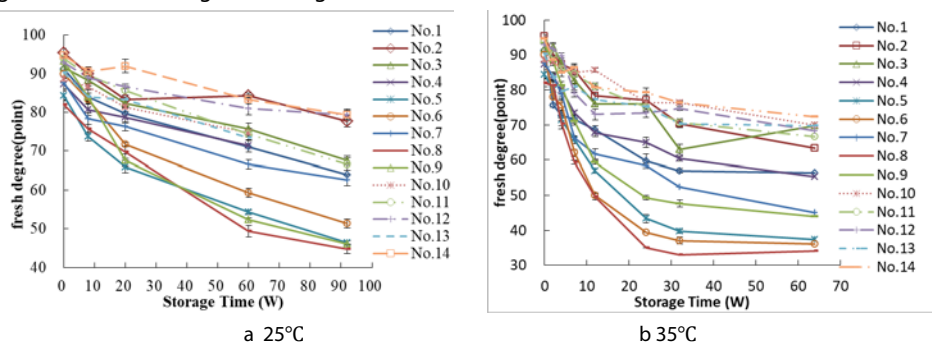
Fig. 1 Change in degree of freshness during storage time.

a 25℃　　　　　　　　　　　　　　b 35℃
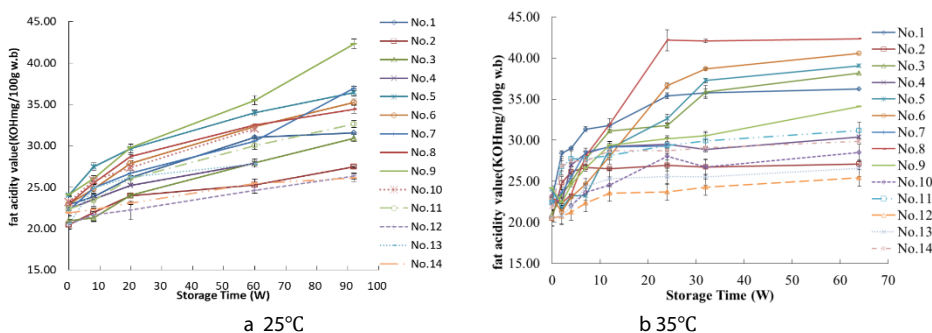
Fig. 2 Changein fat acidity value during storage time.

a 25℃   b 35℃
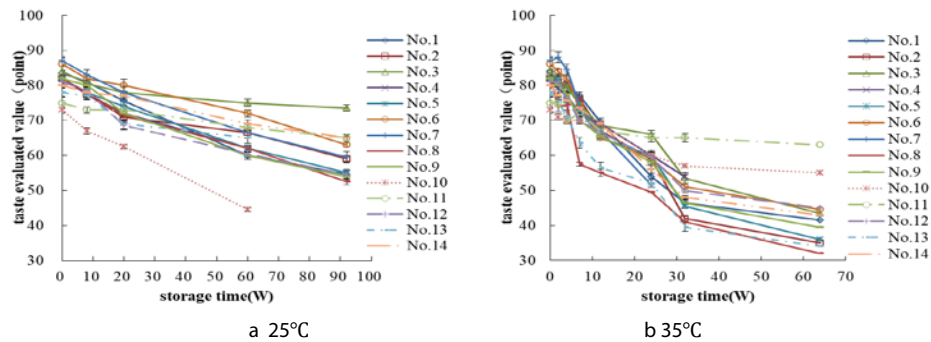
Fig. 3 Change in taste evaluated value during storage time.

These figures show that paddy degree of freshness and taste evaluated value decreased over storage time, while fat acidity value increased. Thus, these three indicators can accurately reflect the degree of deterioration of paddy's quality during time in storage.

5.1.2 Correlation between degree of freshness and fat acidity value

The density ellipse of degree of freshness and fat acidity value of storage samples at a confidence level of p = 0.95 is shown in Fig. 4.
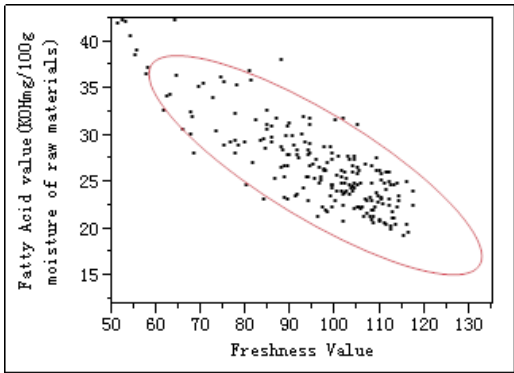
Fig. 4 Correlation of the degree of freshness and fat acidity value of storage samples at a confidence level of p=0.95.

The correlation and significance test of degree of freshness and fat acidity value of storage samples are shown in Tab. 2.

Tab. 2 Correlation and significance test of the degree of freshness and fat acidity value of storage samples.

variable	mean value	standard deviation	correlation r	significance probability p	quantity
Degree of freshness	91.22	14.78	-0.845	＜.0001*	162
Fatty acids value	27.68	4.96			

The correlation coefficient of degree of freshness and fat acidity value was -0.845, and the significant probability was smaller than 0.0001. These values suggest that the degree of freshness of paddy is significantly negatively correlated with its fat acidity value.

5.1.3 Correlation between degree of freshness and taste evaluated value

Due to the great influence of paddy varieties on the taste evaluated value, the correlation analysis between the degree of freshness and the taste evaluated value was carried out separately for each sample. The results are shown in Tab. 3 and Tab. 4.

Tab. 3 Relationship between degree of freshness and taste evaluated value of paddy stored at 25 °C.

Number	Indicator	Storage time (w)					Correlation coefficient
		0	8	20	60	92	
1	TAV	84	81	76	60	55	0.98
	FD	91	84	80	71	64	
2	TAV	82	78	71	67	59	0.97
	FD	95	90	83	84	78	
3	TAV	84	81	78	75	74	0.97
	FD	92	88	82	76	68	
4	TAV	82	78	72	62		0.98
	FD	87	81	79	71		
5	TAV	82	78	74	62	55	0.98
	FD	84	74	66	54	46	
6	TAV	86	82	80	72	63	0.96
	FD	90	83	72	59	51	
7	TAV	87	83	78	67	60	0.97
	FD	88	78	76	67	63	
8	TAV	82	78	72	62	53	0.98
	FD	82	76	70	49	45	
9	TAV	82	80	72	60	54	0.98
	FD	93	84	68	52	46	
10	TAV	73	67	63	45		0.98
	FD	89	87	81	75		
11	TAV	75	73	73	68	65	0.99
	FD	94	90	86	74	67	
12	TAV	81	79	69	61	55	0.98
	FD	93	89	87	81	79	
13	TAV	78	77	69	65		0.87
	FD	90	84	83	73		
14	TAV	80	78	77	69	65	0.99
	FD	95	91	92	83	79	

Tab. 4 Relationship between degree of freshness and taste evaluated value of paddy stored at 35 °C.

Number	Indicator	Storage time (w)								Correlation coefficient
		0	2	4	7	12	24	32	64	
1	TAV	84	80	80	77	70	54	47	42	0.90
	FD	91	76	73	72	69	60	57	56	
2	TAV	82	81	80	76	67	59	42	35	0.97
	FD	95	90	87	86	78	77	70	63	
3	TAV	84	82	80	75	69	66	54	44	0.92
	FD	92	92	88	83	76	76	63	70	
4	TAV	82	79	77	72	69	60	54		0.97
	FD	87	84	80	73	68	65	60		
5	TAV	82	82	76	73	65	60	46	36	0.94
	FD	84	80	71	66	57	43	40	37	
6	TAV	86	84	82	75	65	59	51	45	0.96
	FD	90	78	76	62	50	39	37	36	
7	TAV	87	88	85	74	67	52			0.93
	FD	88	84	79	66	62	58			
8	TAV	82	74	75	58	55	50	41	32	0.95
	FD	82	81	70	59	50	35	33	34	
9	TAV	82	80	78	70	66	58	47	40	0.95
	FD	93	88	81	71	59	49	48	44	
10	TAV	73	71	70	70	69	60	57	55	0.98
	FD	89	88	86	85	86	76	76	70	
11	TAV	75	75	70	72	67	65	65	63	0.96
	FD	94	90	86	85	81	75	71	67	
12	TAV	81	81	76	70	67	60	50	45	0.90
	FD	93	92	90	79	73	73	75	68	
13	TAV	78	76	71	64	57	52	40	34	0.95
	FD	90	84	80	81	77	75	70	70	
14	TAV	80	77	76	74	69	56	48	43	0.93
	FD	95	89	85	86	81	79	76	72	

Note: TAV means taste evaluated value. FD means degree of freshness

These experiments were terminated once insects and mould appeared in individual samples during the later period of storage.

The data in Tabs. 3 and 4 show that the degree of freshness of paddy was positively correlated with the taste evaluated value, and most of the correlation coefficients were above 0.9

5.2 Results of nationwide investigation of paddy degree of freshness

5.2.1 Analysis and results of indica rice

The results of degree of freshness of 3106 indica rice samples were analysed, and the distribution of degree of freshnesss of the samples is shown in Fig. 5.
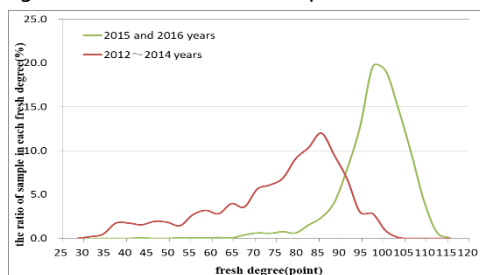
Fig. 5 Distribution of the degree of freshness of indica rice samples.
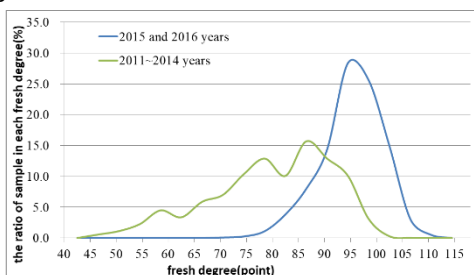
Fig. 6 Distribution of degree of freshness of japonica rice samples.

As shown above, there was an obvious distinction of degree of freshness between new harvest rice and rice harvested in previous years. With data analysis, the discrimination of new harvest samples in 2015 and 2016 and the rice harvested in previous years was close to 85%, ranging from 89 to 90 points.

5.2.2 Analysis and results of japonica rice

The distribution of the degree of freshnesss of 1612 japonica rice samples is shown in Fig. 6.

The results of statistical analysis showed that the discrimination of the degree of freshness of new harvest samples in 2015 and 2016 years and the samples harvested in previous years was close to 76%.

The discrimination of japonica rice was lower than that of indica rice, mainly because of good storage conditions of low temperature and humidity in its production area. Meanwhile the samples of japonica rice with good quality was larger than indica rice during the previous year's production.

Discussion

Above all, the degree of freshness is a rapid detection indicator that can accurately reflect changes of quality of paddy (fresh or not) and has significant correlations with fat acidity value and taste evaluated value. There was a significant negative correlation ($p < 0.01$) between degree of freshness and fat acidity value with a correlation coefficient of -0.845. However, degree of freshness was significantly positively correlated with taste evaluated value. The correlation coefficient was above 0.9.

According to the results of **a** nationwide investigation, there was an obvious distinction between degree of freshness of new harvest indica rice and indica rice harvested in previous years. The degree of distinction achieved 85%. For pecial reasons, japonica rice had a lower degree of distinction, but it also reached over 75%, which conforms to preserving quality according to China's legale storage requirements.

The above results show that the rapid detection technology of paddy's degree of freshness has great applicability to distinguish fresh and non-fresh paddy in China.

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Fumigation with Ph3 using automatic generation - Presentation of results of recent trials

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Abstract

Fumigation is essential part of preservation of grains, other edible commodity and perishables. Phosphine is most commonly used fumigant since more than 65 years. It is now practically the only fumigant and most commonly used. While fumigating with conventional metal phosphide formulations most common problems or concerns are operator safety, laborious to apply, gas retention in structure, uniformity of gas concentration in the structure, solid residues left in the commodity, limitations in ambient conditions to apply the fumigant and others. Bad fumigation practices lead to failed fumigations. These are blamed on insect resistance. Scientists have noted higher tolerance levels, but not resistance to phosphine. To address all the concerns referred, and limitations of conventional fumigants, we have developed a Phosphine generator and a suitable formulation for use with the same. This is a fully automatic machine. The formulation is granular and dust free. Those using our generators have stopped using conventional formulations of phosphine. The paper presents merits of technology, results of trials in various locations and on different commodity. This is the only system, which ensures uniform distribution of gas in entire structure to give 100 % guaranteed fumigation results.

Browning Mechanism and Process Optimization during MaizeMaize KX7349 Drying

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Abstract

Browning of KX7349 maize during drying occurred mainly in the pericarp layer. Browning was caused by oxidation of water soluble matter in the pericarp layer. Moisture content had no significant influence on browning rate. Drying temperature, drying time and drying method (vacuum drying or hot-air drying) had significant influence on the browning rate. Through lab research, a prediction model for the relationship between browning rate and drying air temperature was developed. Total drying time is $y=-13.086+0.289X_1+1.045X_2$, where y is the browning rate （%）, X_1 is drying temperature （℃）, X_2 is total drying time （h）, the value range of X_1 was 30～80, the value range of X_2 was 2～10. The concurrent and counter current dryer was applied in Nenjiang to optimize the drying process. The hot air temperature in each drying stage was reduced. When the hot air temperature of the 1st, 2nd, 3rd drying stage was reduced to 95℃,75℃,60℃

respectively, the browning rate was reduced to 15%～16%. Keeping the hot air temperature constant at each drying stage, by drying twice, the browning rate was reduced to 4%～6%.

Keywords: KX7349, drying, browning mechanism

1. Introduction

Maize is one of the most important cereal grains in the world. It is cultivated worldwide. America and China are the main producers of maize, which yielded 57.5% of maize production in 2015 in the world. In China, maize is cultivated widely. According to geographical and climatic features, there are six maize planting areas. Among them, the northern spring maize region accounts for 30% of maize production in China. This area includes Heilongjiang, Jilin, Liaoning and Inner Mongolia. When the maize is harvested in this area, the temperature decreases rapidly, maize cannot be sundried sufficiently. The moisture content is high. Especially in Heilongjiang province, on some occasions, the MC is up to 30% wet-basis. Rapid moisture-removal technology is needed to achieve a safe stoage moisture level and to inhibit the growth of microorganisms. Hot-air drying is an appropriate approach.

Maize KX7349 is a variety bred by KWS, a German seed company. It is planted widely in Inner Mongolia, Heilongjia and Jilin. After drying, many maize kernels undergo browning, which results in a rapid drop in price. It is rejected by the food industry if premium quality is needed.

This phenomenon motivates us to find the reasons and explore methods of improving the process of drying. Studies were conducted to determine the factors affecting browning and the separation and extraction of browning materials.

2. Materials and Methods

Materials: KX7349 maize, harvested in 2014, provided by Heilongjiang province.

Main instruments: DHG-9146A electric constant temperature drying oven (Shanghai Yiheng Science Instruments Co., Ltd.); DZF-6090 vacuum drying oven (Shanghai Yiheng Science Instrument Co., Ltd.); AL204 electronic scales (Shanghai Mettler-Toledo Instruments Co., Ltd.); HSNT25 concurrent and counter current dryer (COFCO engineering Co., Ltd.); HPLC-ELSD analyzer with a sugar analysis column (Agilent Technologies Co., Ltd.).

Main methods:

- Browning rate

Browning rate (%) = Weight of browning shelled maize×100/ Total weight of shelled maize

- Main factors affecting browning rate

The KX7349 shelled maize at 30% MC was dried by the electric constant temperature drying oven at 30℃, 40℃, 50℃, 60℃, 70℃ and 80℃, respectively. The browning rate was tested every two hours.

The KX7349 shelled maize with original MCs of 14%, 20%, 25% and 30% was dried at 80℃. The browning rate was tested every half an hour to analyze the effect of MC on the browning rate.

KX7349 maize growing in different areas was tested to evaluate the effect of growing area on browning rate.

Also, two different drying methods, namely hot-air drying and vacuum drying, were compared.

- Separation and extraction of browning materials

The maize pericarp was peeled off the kernel. The maize pericarp and the kernel were dried at 100℃ for 30 min to test the colour changes. The pericarp was also treated in distilled water for 2 hours and then dried for 30 min at 100℃. Browning and non-browning shelled maize were analyzed by the HPLC-ELSD analyzer equipped with a sugar analysis column.

- Drying process optimization

The KX7349 shelled maize at 30% MC was dried by the electric constant temperature drying oven at 30℃, 40℃, 50℃, 60℃, 70℃ and 80℃, respectively. The browning rate was tested every two

hours.Maize

The concurrent and counter current dryers were applied in Nenjiang to optimize the drying process. The initial MC of maize was 27% ~ 31%. The drying process included two concurrent flow stages, one counter current flow stage and one cooling stage.

3. Results

- Main factors affecting browning rate

Table 1 shows the results of browning rate of KX7349 maize being dried at different temperatures and drying times. The browning rate increased as both drying time and temperature increased. At 80℃, after 10 hours the browning rate was highest at 19.99%. At 80℃, the browning rate increased much more rapidly as compared to drying at the other temperatures. The analysis showed that the drying time and temperature significantly affected the browning rate ($p < 0.01$).

Table 1. Browning rate (%) at different drying temperatures and times.

	2 h	4 h	6 h	8 h	10 h
30℃	1.13±0.12	1.47±0.05	2.01±0.02	3.00±0.02	5.01±0.01
40℃	1.51±0.09	2.00±0.09	3.05±0.15	4.13±0.05	5.81±0.09
50℃	3.96±0.13	6.96±0.14	7.84±0.05	8.94±0.14	11.93±0.07
60℃	6.07±0.14	7.98±0.03	9.99±0.09	13.87±0.06	17.87±0.12
70℃	7.96±0.22	10.02±0.07	12.97±0.06	16.96±0.06	19.98±0.10
80℃	9.93±0.14	13.05±0.09	16.82±0.06	19.87±0.12	19.99±0.02

Table 2 shows changes in the browning rate of different original MC maize being dried at 80℃. Regardless of the original maize MC, the browning rate increased with drying time. maizeAlso, the analysis showed that the moisture content of maize had no effect on the browning rate (p=0.647).

Table 2. Browning rate (%) of maize with different MCsmaize.

MC (%)	0.5 h	1 h	1.5 h	2 h
14	2.56±0.09	5.40±0.01	7.24±0.05	9.23±0.06
20	2.44±0.04	5.33±0.12	7.29±0.09	9.29±0.07
25	2.56±0.15	5.26±0.11	7.31±0.05	9.23±0.09
30	2.59±0.12	5.33±0.09	7.28±0.03	9.30±0.04

Table 3 shows the browning rates of maize from two different areas being dried at 90℃. At the same drying conditions, the browning rate of the maize from Nenjiang area was much higher than the maize from Qitaihe area. The analysis showed growing area of maize had a significant effect ($p < 0.01$). Because the annual accumulated temperature was different in these two places, the maturity level was different for maize from these two places.

Table 3. Browning rate (%) of maize from different areas.

Drying method	Nenjiang	Qitaihe
Hot-air drying 90℃，30 min	43.73±0.42a	6.70±0.13b
Hot-air drying 90℃，1 h	72.50±0.19a	25.12±0.15b

Table 4 shows the browning rates from the different drying methods. After 30 min of drying, the browning rates by vacuum and hot-air drying were 6.24% and 43.72%, respectively. After 1 h, the browning rate by vacuum drying was 6.03%, while the browning rate by hot-air drying was 72.50%. Compared with hot-air drying, vacuum drying could obviously inhibit maize browning. This indicated the maize underwent browning due to the participation of oxygen.

Table 4. Browning rate （%） for maize dried by two different methods.

Drying method	Browning rate (%)
Vacuum drying 90℃, 30 min	6.24±0.24
Vacuum drying 90℃, 1 h	6.03±0.12
Hot-air drying 90℃, 30 min	43.73±0.42
Hot-air drying 90℃, 1 h	72.50±0.19

- Separation and extraction of browning materials

Fig. 1. Before drying.   Fig. 2. After drying.
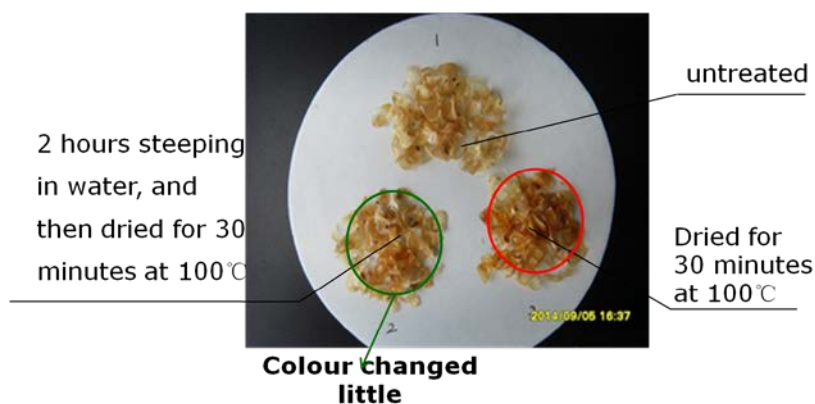
Fig. 3. Dissolution characteristics of browning materials.

The maize pericarp was peeled off the kernel. After drying, the maize pericarp underwent browning, however, the endosperm and embryo changed only slightly (Fig. 1 and Fig. 2). This indicated that most of the browning materials were in the pericarp. The pericarp was treated in distilled water for 2 hours, and then dried for 30 minutes at 100℃. Compared with the untreated pericarp after being dried at the same condition, the colour changed slightly, indicating that the browning materials were soluble in the water (Fig. 3).

From the HPLC fingerprint spectrums (Fig. 4), Peak 1 was found. The retention time of Peak 1 corresponded to that of glucose. The results showed that the glucose content of the browning in maize was twice as much of the non-browning maize. From this point, we deduced that browning might be caused by Mailard reaction. In addition, we concluded the following new findings: 1. browning materials were mainly in maize pericarp and soluble in water, 2. Browning was induced by oxidation of the water-soluble materials and 3. glucose content was higher in browning maize than that in non-browning maize without drying.
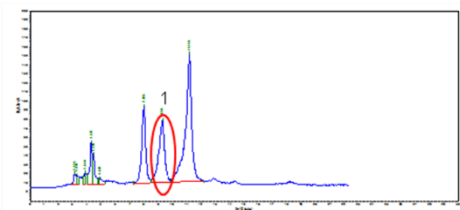
Fig. 4. Sample of browning   Fig. 5. Sample of non-browning
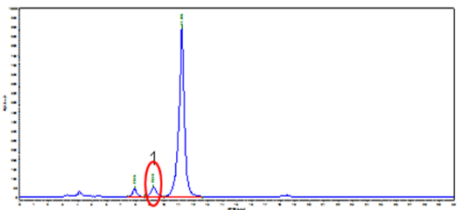
- Drying process optimization

From the results of the above analysis, we found that main influence factors of browning rate were drying temperature, drying time and drying method. Because vacuum drying has not been applied commercially in practice, drying temperature and drying time became the most important factors determining the maize browning rate.
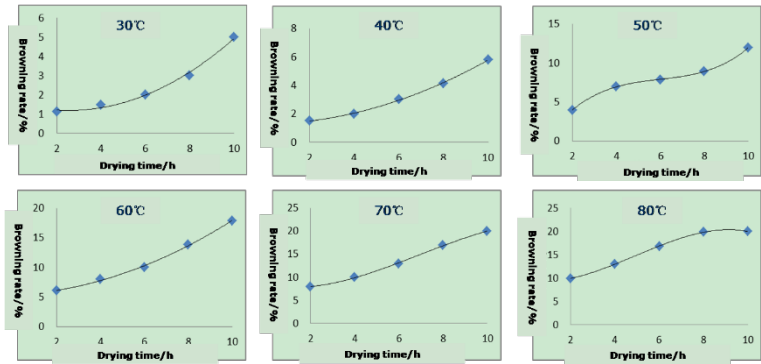
Fig.6. Changes in browning rate with drying time and temperature.

Fig. 6 shows changes in browning rate with drying time and drying temperature. The browning rate increased with drying time for every drying temperature. During the early drying period, the slope of the curve was very steep for the higher temperature, compared with the lower temperature, suggesting that drying temperature had more obvious effect on browning rate than drying time.

Fig. 7. Concurrent and counter current dryer.
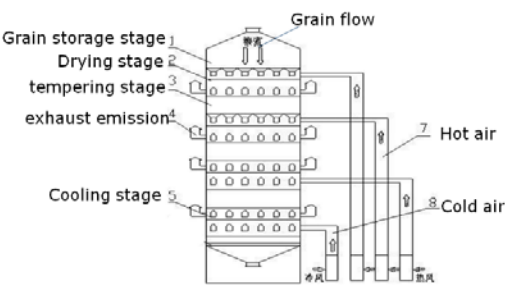
Fig.8. Schematic drawing of concurrent and counter current dryer.

The concurrent and counter current dryer (Fig.7) was applied in Nenjiang to optimize the drying process. In the process, the hot air temperature in each drying stage was gradually reduced. When the hot air temperatures of the 1st, 2nd and 3rd drying stages were reduced to 95°C, 75°C and 60°C, respectively, the browning rate was reduced to 15% ～ 16% (Table 5). Keeping the hot air

temperature constant at each drying stage, by drying twice, the browning rate was reduced to 4%～6%.

Table 5. Browning rate (%) of MaizeKX7349 maize.

1st drying stage		2nd drying stage		3rd drying stage		Draining maize (Hz)	Browning rate (%)
Hot air (℃)	Grain (℃)	Hot air (℃)	Grain (℃)	Hot air (℃)	Grain (℃)		
120	40	100	52	70	45	10	25 ~ 30
110	30	95	50	70	43	9	22 ~ 28
100	30	80	40	70	41	8	17 ~ 20
95	26	75	37	60	45	7	15 ~ 16
95	24 25	75	35 40	60	42 43	15 16	(drying twice) 4 ~ 6

This can be explained by the fact that between the first and second drying stages, there was a sufficiently long tempering stage. During the long tempering stage, moisture transported from the interior of a kernel to the surface, and consequently, the water near the surface could be removed easily when subjected to drying conditions again. This not only reduced the drying time but also the energy consumption.

4. Discussion

Drying temperature, drying time and drying method were the main influence factors of the browning rate. Initial MC of maize hardly influenced browning. Increasing drying temperature and drying time led to an increase in the browning rate. The analysis showed drying temperature and time significantly affected the browning rate. This finding had important implications in optimization of the drying process.

Regarding the browning mechanism, the following new findings were concluded: 1. browning materials were mainly in maize pericarp and soluble in water, 2. the browning was induced by oxidation of the water-soluble materials, and 3. glucose content was higher in browning maize than in non-browning maize.maizemaizemaize

The concurrent and counter current dryer was applied in Nenjiang to optimize the drying process. The hot air temperature in each drying stage was gradually reduced. When the hot air temperatures of the 1st, 2nd and 3rd drying stages were reduced to 95℃, 75℃ and 60℃, respectively, the browning rate was reduced to 15% ~ 16%. Keeping the hot air temperature constant at each drying stage, by drying twice, the browning rate was reduced to 4% ~ 6%.

Acknowledgement

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Session 5
Physical and Biological Control

Temperature: Implications for Biology and Control of Stored-Product Insects

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Extended Abstract

Insects are affected by temperature in all aspects of their biology, ecology, reproduction, behaviour, physiology and biochemistry (Fields, 1992; Beckett et al., 2007). Stored-product insects reproduce between 15 and 35°C, with maximum reproduction occurring at approximately 33°C. Above and below these temperatures insects can move, but cannot complete their development. Temperatures below 5°C and above 40°C insects cannot walk, and will eventually die. Between -15 and -25°C insects freeze and die instantaneously. There are significant changes to these general patterns depending upon species, life stage and acclimation. For example, insects can become 10 times more resistant to cold if acclimated at cool temperatures (5-15°C) before being exposed to sub-zero temperatures.

Temperature also effects trapping (Fargo et al, 1989). The speed and direction of movement is affected by temperature (Flinn and Hagstrum, 1998). Insects move faster at higher temperatures, so that if the populations are the same, more insects will be trapped at higher temperatures. Insects will move towards warm temperatures, and avoid temperatures that are too hot.

In general insecticides work better at higher temperatures (Kenaga, 1961; Iordanou and Watters, 1969; Fig. 1), but some insecticides have only a small increase in efficacy (methyl bromide), others have a large increase in efficacy (carbon tetracholoride), whereas others have a decrease in efficacy (pyrethrins) with higher temperatures. Contact insecticides degrade faster at higher temperatures (Desmarchelier, 1977).

Given the many effects of temperature on every aspect of stored-product insects, researchers should carefully design experiments to avoid unseen effects by temperature, understand its effect on their area of study. Grain managers need also to be aware of the many ways, sometimes not obvious, that temperature can affect storage.

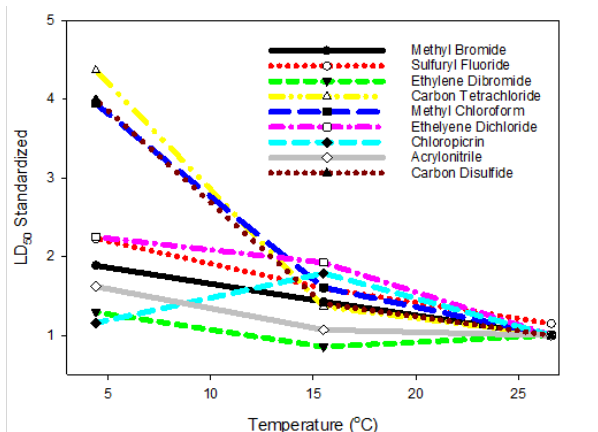**Keywords:** insecticide, degradation, behaviour, heat, cold

Fig. 1 Effect of temperature on efficacy of fumigants on *T. confusum*. LD_{50} are expressed as a proportion of LD_{50} at 26.7°C, data from Kenaga, 1961.

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Evaluation of insecticidal efficacy and persistence of Nigerian raw diatomaceous earth against *Callosobruchus maculatus* (F.) on stored cowpea

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DOI 10.5073/jka.2018.463.092

Abstract

The insecticidal efficacy and persistence of Nigerian raw diatomaceous earth (DE) were evaluated in the laboratory on cowpea against *Callosbruchus maculatus* (F.) (Coleoptera: Chrysomelidae). The raw DE was applied to 1.5 kg lots of cowpea seeds at 0 (untreated control), 250, 500, 750, 1000 and 1500 mg/kg, and a commercial DE formulation (Protect-It®) applied at 1000 mg/kg was included in the test as positive (treated) control. The treated cowpea seeds were kept under ambient laboratory conditions (26 - 34°C and 24 - 93% RH. Bioassays were conducted on samples taken from each treatment at the day of storage and every 30 d for 6 consecutive months. Adult *C. maculatus* were exposed for 3 and 5 d to the samples and adult mortality was assessed over this exposure interval and progeny production and seed damage were assessed after additional 30 d. On freshly treated cowpea, both the raw DE and Protect-It® were highly effective against *C. maculatus* causing 100% adult mortality following 5 d of exposure. In general, the raw DE was less persistent on cowpea providing complete adult mortality only for two months. Protect-It® on the other hand was stable over the 6-month period of storage causing 95.8 to 100% adult mortality. None of the treatments completely inhibited progeny production after 2-3-moths storage period. The results of this study indicated that Protect-It® may provide suitable protection for 6 months against *C. maculatus*, but the raw DE in its present state is not suitable for long-term protection against this insect pest.

Keywords: *Callosobruchus maculatus*, raw diatomaceous earth, cowpea, residual activity

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most economically and nutritionally important indigenous African grain legumes produced throughout the tropical and subtropical areas of the world (Abate et al., 2011). It is a source of relatively low cost, high quality protein, and for many West and Central African farmers a major cash crop (Langyintuo et al., 2003). As production and consumption do not occur simultaneously, producers and traders need efficient storage systems to ensure year round cowpea availability for consumers. Consumers, on the other hand, want to buy cowpeas at the cheapest cost without compromising quality characteristics (Ndong et al., 2012).

Nigeria is the largest producer and consumer of cowpea, accounting for about 45 percent of world's production (Lowenberg-DeBoer and Ibro, 2008) and a per capita consumption of 25 - 30 kg per year (Nurudeen and Rasaki, 2011). The major storage pest of cowpea in Nigeria is *C. maculatus* (Adedire, 2001). As a field to-store pest, the attack, which starts before harvest and intensifies during storage, may cause total losses (Faroni and Sousa, 2006). The damage by *C. maclatus* is caused by oviposition on the surface of grains or pod and subsequent larval penetration into the grains. The attack results in weight loss, nutritional value, reduced level of product hygiene (presence of droppings, eggs, and insects), reduced seed germination resulting in decreased retail value (Almeida et al., 2005). According to Singh et al. (2002), a 5% annual production loss to this bruchid in Nigeria alone would cost about $100 million USD, or a loss of over 40,000 tonnes of cowpea. Fumigants, chiefly phosphine and dichlorvos are the major synthetic insecticides used in controlling *C. maculatus* in Nigeria. The storage conditions available to most farmers enable re-infestation, increasing the frequency of insecticide use. These chemicals may result in deleterios effects ranging from cowpea poisoning, environmental contamination, residues in grain, development of genetic resistance due to improper usage, and hazards to workerIn addition, the high costs of chemicals may also make it difficult for small-scale farmers to access (Lowenberg-DeBoer and Ibro, 2008), accompanied by increased infestation and losses.

The search for alternatives to synthetic insecticides in stored-products for insect pest management has been intensifing. One alternative is the use of diatomaceous earth which has received considerable attention, and are considered among the most promising alternatives to synthetic residual insecticides in stored-grain protection (Athanassiou et al., 2003).. During the last 20 years, DE has been the subject of several review papers with the numerous references cited within each of review. Also DE is now registered as a grain protectant or for structural treatment in several countries (Korunic, 2016). The mode of action of DE is different from the synthetic insecticides. DE absorbs the insect's cuticular waxes, and insects die from desiccation (Korunic, 2013). The advantages of using DE are its low mammalian toxicity, its stability, leaving no toxic residues on grains, control of the synthetic insecticide resistant pests and applied using the same technology for conventional grain protectants (Vayias et al., 2006).

Regional deposits of DE have been shown to be effective against local populations of stored-product insect species. For example varying deposits exist in Croatia (Korunic et al., 2009; Liska et al., 2015), Greece and Romania (Athanassiou et al., 2016) and Iran (Ziaee et al., 2013; 2016). There are also sevaral deposits of DE in Nigeria, however, their insecticidal efficacy has not been widely investigated. Kabir et al. (2011) first reported the insecticidal efficacy of Bularafa DE against *Tribolium castanem* (Herst) then against *Rhyzopertha dominica* (F.) (Kabir et al., 2013). Later, Nwaubani et al. (2014) reported the efficacy of Bularafa and Abakire diatomites agaisnt *R. dominica* and *Sitophilus oryzae* (L.). Information on insecticidal efficacy is important for commercial development of Nigerian DE deposits for use as grain protectant. The objective of this research was to evaluate the insecticidal efficacy and residual activity of Bularafa raw DE to control *C. maculatus* in stored cowpea.

Materials and Methods

Test Insect

Callosobruchus maculatus were obtained from laboratory culture, wich were maintained on cowpea for about a year. Adult insects were used to establish new insect cultures for the experiments. Two (1 litre capacity) glass jars were filled with 400 g of cowpea grains and 100 mixed-sex adults of the test insects were introduced into culture medium to oviposit. Each jar was covered with nylon mesh and secured with rubber bands. Parent insects were removed five days after introduction and the resulting F_1 progeny aged 0-2 days were used for the bioassay. New cultures were set up monthly to ensure availability of adult insects throughout the experiments.

Cowpea seeds

Insecticide free cowpea grains (Var. Borno Brown), were obtained from Borno State Agricultural Development Programme (BOSADP) Maiduguri, Borno State. The grains were cleaned and disinfested according to Kabir (2013), then equilibrated with laboratory condition for 10 days.

Diatomaceous earths

The raw diatomaceous earth (RDE) in the form of soft chalky rock was obtained from mines located 6 km North of Bularaffa village (Latitude: 11^0 8' 48" and Longitude: 11^0 49' 17" E) in the Gujba Local Government Area of Yobe State, Nigeria. The DE was oven dried, ground and put through a 63 µm sieve. Its pH and tapped density were analyzed in accordance with methods described by Korunic (1997) while its mineral composition was analyzed in the Geology Laboratory, Ahmadu Bello University, Zaria, Nigeria. It has the following properties: tapped density- 312.5 g/L, pH-9.2; mineral composition: SiO_2 - 80.43%, Al_2O_3 -5.02%, CaO – 0.48%, Na_2O – 0.07%, K_2O -0.14%, Fe_2O_3 -0.17%, ZnO - 0.01%, and MnO - 0.01. The commercial formulation of DE (Protect-It®) was obtained from Diatom Research and Consulting Inc., Toronto, Canada. It is an enhanced DE that contains approximately 83.7% amorphous SiO_2, 5.6% Al_2O_3, 2.3% Fe_2O_3, 0.9% CaO, 0.3% MgO and 1.9% other oxides e.g. TiO_2 and P_2O_3), and 3-5% moisture content (m.c.). The median particle size is between 5 and 6 µm with 10% silica aerogel (Athanassiou et al., 2009).

Bioassay Procedure

Adult *C. maculatus* adults were bioassayed at RDE doses of 0 (untreated control), 250, 500, 1,000, 1500 mg/kg RDE and Protect-It at 1000 mg/kg . De's were applied to cowpea grains under ambient conditions (31-34°C and 24 - 30% R.H.). For the acute toxicity test, the appropriate amounts of DE were applied to 50 g of cowpea and placed in 150 ml glass bottles that were tumbled manually for 5 min to achieve an even distribution of the DE on the grains. Then, 30 mixed-sex adult insects were introduced into each bottle, capped with perforated plastic lids and kept on a laboratory shelf. Each treatment combination was replicated four times. Adult mortality was recorded on 3 and 5 d after exposure, while progeny production and grain damage were assessed 40 days after infestation (DAI). The residual toxicity was assessed on 1500 g lots of cowpea grains treated with above mentioned doses and stored in plastic containers for 180 days (from April to October) under laboratory conditions (26-32°C and 33-93% RH). Similar bioassay procedures and observations as described above were conducted at 30 days intervals

Data Analysis

Where necessary, mortality data obtained were first corrected for control using Abbott's (Abbott, 1925) formula and together with data on grain damage were arcsine transformed. Data relating to number of F1 progeny were square root $\sqrt{(x + 1)}$ transformed. All were then subjected to Analysis of Variance (ANOVA Statistix 8.0). Differences between treatment means were separated using Tukey-Kramer Honestly Significant Difference (HSD) test at ($P \leq 0.05$).

Results

There are significant ($P<0.05$) variations in mortality levels of *C. maculatus* adults caused by different doses of RDE, when exposed for 3 days (Fig. 1). Irrespective of storage period, adult mortality increased with increase in raw DE dose. Protect-It was the most effective DE causing 100% adult mortality following 3 days of exposure to freshly treated cowpea grains and on those treated and stored for upto 60 days. With the RDE, similar effects were achieved, however, only on freshly treated seeds. Within each month there were significant declines ($P<0.05$) in mortality levels among raw DE doses.

Adult mortality increased with extended exposure period (Fig. 2). After 5 days of exposure mortality levels recorded for all RDE and Protect-It dosages significantly ($P<0.05$) increased irrespective of

post-treatment storage period. The RDE applied at 1500 mg/kg caused 100% adult mortality only on freshly treated cowpea and after 30 days of storage, whereas with Protect-It caused complete adult mortality for upto 90 post-treatment days of storage. Efficacy of both RDE and Protect-It declined with an increase in post-treatment storage period. In the case of RDE applied at 1500 mg/kg, adult mortality level decreased from 85% after 90 days 58.3% after 180 days post-treatment; and a similar trend was observed for other doses. With Protect-It, however the minimum mortality level caused (92.5% adult mortality) was recorded at 120 days post treatment and did not significantly change thereafter (Fig. 2)

Both DEs had significant impact on progeny production of *C. maculatus*. Effect on progeny production was significantly ($P<0.05$) influenced by DE dose and storage interval. Throughout the post treatment period, the untreated control supported significantly ($P<0.05$) higher number of progeny than the treated grains, except on those treated at 250 mg/kg after 60 days post-treatment. Furthermore increase in raw DE dose resulted in increased progeny suppression. Even the highest dose of RDE could not prevent progeny development, although in allcases the number of progeny was less <10. Protect-It was more effective in progeny inhibition inducing complete suppression on grain freshly treated or treated and stored for 30 days. The progeny that emerged thereafter was <3 per bottle (Fig. 3).

Progeny development in all treatments were drastically reduced after 90 days post-treatment. The percent of damaged seeds followed the same trend with number of progeny produced. Significant ($P>0.05$) differences in grain damage were noted among RDE doses and storage periods (Fig. 4). Higher grain damage was record in the untreated control and grains treated at 250 mg/kg of RDE, where differences were not significant except on freshly treated grains and after 30 post-treatment.
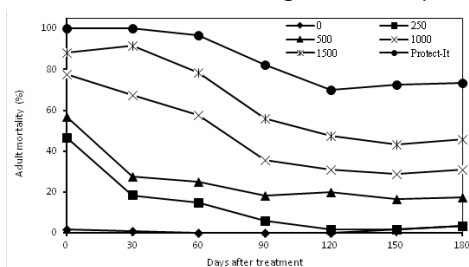
Fig. 1. Mean mortality of *C. maculatus* adults after three days of exposure to cowpea treated with different doses of DE and stored for various periods
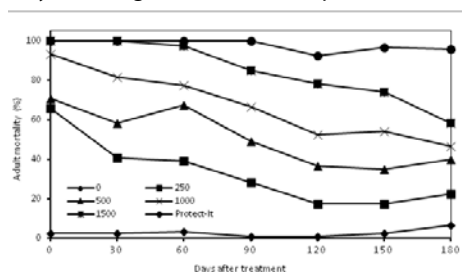
Fig. 2. Mortality of *C. maculatus* adults after five days of exposure to cowpea treated with different doese of DE and stored for various periods
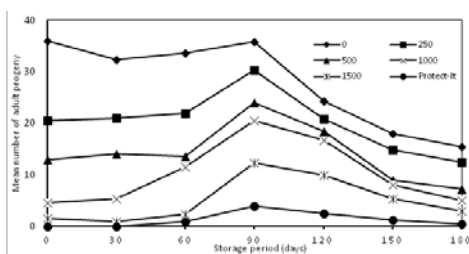
Fig. 3. Mean number of *C. maculatus* F1 progeny after 40 DAI on cowpea treated with different doses of DE and stored for different periods
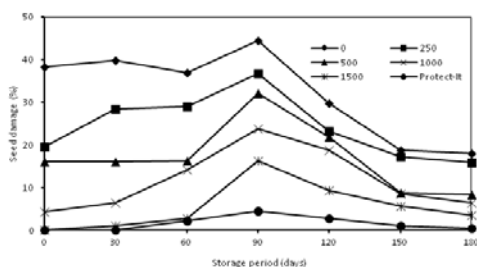
Fig. 4. Seed damage caused by *C. maculatus* 40 DAI on cowpea treated with different doses of DE and stored for different periods

Even on grain treated at 1500 mg/kg grain, damage could not be contained. After about 90 days post treatment, at all DE doses and the untreated control, there was a slight but significant increase in grain damage. Protect–It prevented grain damage on freshly treated seeds and after 30 days post-

treatment; and even where grain damage was recorded they were less than 3% and differences between storage periods were not significant (P>0.05).

Discussion

Adult progeny emerged in all DE treatments except in Protect-It treated (freshly treated and 30 days post treatment) grains, possibly because oviposition occurred before the adults died before exposure to the DE (Subramanyam and Roesli, 2000). However, in all treatments and the untreated control, progeny production significantly increased at 90 days post-treatment. This being the period coinciding with, middle of the rainy season in Maiduguri. This period is characterized by lower ambient indoor temperature (26 - 29°C) and higher relative humidity (>80%) as compared to the first three months of the experimentation (May-July, when the r.h. was bet ween 24 and 58%). DE efficacy is related to relative humidity, temperature and changes in physical proprieties of treated grain (Athanassiou et al., 2005). During this period, it is likely the DE absorbed moisture from the atmosphere (Stathers et al., 2004). Other Studies have also shown that an increase in relative humidity reduces DE efficacy (Fields and Korunic, 2000; Rojht et al., 2010; Beris et al., 2011). Given that DE efficacy is reduced by higher moisture, there are direct consequences of DE effectiveness for grains stored in ventilated structures, especially in humid areas. On the other hand, the relatively higher efficacy of the RDE and Protect-It during the first 60 days of storage (May and June) which coincided with a period of high temperature (32) could be attributed to the fact that at higher temperatures insects are more mobile (Arthur, 2000) increasing contact with the DE particles, thus resulting in greater damage of the insect cuticle and water loss (Athanassiou et al., 2005; Wakil et al., 2010; Athanassiou et al. 2016). These results suggest that raw DE could be more effective in the Sudan and Sahel savannah regions, characterized by long dry season, high temperature and low relative humidity than in the humid areas. Another interesting finding of this study is the general reduction in progeny production including the untreated control after 90 days of storage (Fig. 3). The reason could not be explained. Perhaps cowpea grains became unsupportive of the pest's reproduction. This hypothesis needs to be verified by experiments.

One of the major drawbacks limiting the widespread use of DE is its reduction in efficacy under high moisture storage conditions (Korunic et al., 2016). This limitation could be overcomed in humid areas by thorough grain drying before storage and limiting moistuture equillibration with the sorrounding by using hermetic storage sructures.

In conclusion, this study indicated that the Nigerian RDE may not be suitable for long-term storage of cowpea grains against *C. maculatus* when applied at a dose rates of 1500 mg/kg; perhaps 2000-2500 mg/kg may be effective. Given that DE efficacy decreased during the months with high relative humidity, it is necessary to store DE treated grains in airtight structures or modify storage structures to limit moisture absorption from the surrounding environment in order to increase the benefits of DE treatments. Further studies on different particle sizes, higher dose rates and enhancement of Nigerian RDE are recommended.

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Thermal disinfestation of stored grains by solar energy

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Abstract

Chemical control especially fumigants is the most commonly used method to control stored-grain pests. A safer alternative for disinfestation is by heating up grains to a temperature of 50-60 °C. However, this alternative consumes high thermal energy due to the relatively high temperature required to achieve the required goal. Using solar energy as heat source for low temperature applications has become a viable mean for heating applications. Heating of grains using solar energy requires special design of grain storage system as well as development of efficient heat transfer mechanism to increase grain temperature over a limited period of time. The main objective of the current study is to use thermal disinfestation as a non-chemical, safe control method for grain management. A heating system based on solar energy has been developed as heat generator to control stored-grain insects. The target temperature range is 50-60 °C, which is enough to kill most of stored-grain insects. The grain hopper heating system relies on hot water supplied from a solar collector. The temperature of grains can be controlled based on the amount of grains contained in the hopper and the amount of energy transmitted to grains inside the hopper. The effectiveness of the system will be measured by reaching the best temperature and time combination for each insect species without affecting the seeds quality. The best temperature and time combination for cowpea beetles will be discussed in more details.

Retrospect, insights and foresights:
Biological control of *Anobium punctatum* with *Spathius exarator*

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Abstract

Biological control using beneficial organisms is getting more and more important in Integrated Pest Management. An effective strategy in the fight against the most common timber pest species, the furniture beetle *Anobium punctatum*, is based on the parasitoid wasp species *Spathius exarator*. This braconid wasp parasitizes its host species by piercing its ovipositor directly through the wood surface followed by oviposition onto the beetle larva. After feeding on the larva and pupation, the adult wasp emerges through a tiny 0.5 mm wide wood hole, which can be clearly distinguished from the 2 mm wide hole of *A. punctatum*. This enables us to observe easily the treatment success as each new *S. exarator* exit hole is equivalent to one killed beetle larva.

Between 2012 and 2017, the braconid wasps were introduced into about 80 *A. punctatum* infested buildings. At least twelve treatments over a period of up to three years were performed. On exactly defined areas, the newly emerged exit holes of *A. punctatum* and *S. exarator* were counted and the parasitisation rate was calculated. Here we present pooled data of 29 *A. punctatum* infested churches, successfully treated and monitored over a period of one to five years. Furthermore, as a representative sample, we show the results of one church over a period of six years.

We demonstrate the biological control of the common furniture beetle with this braconid wasp as an efficient, sustainable alternative to conventional residual methods. However, after a period of up to three years intensive treatment, a continuous monitoring-program with necessary additional single treatments should follow.

Key words: biological control, wood pest, cultural heritage, common furniture beetle, parasitic wasps

1. Introduction

Many chemical products for wood preservation are currently in a review process and possibly will be restricted from the European Biocidal Product Regulation. Thus, the expansion of alternative methods for pest control will be required like physical treatments or biological control. Physical methods like heat treatment or anoxia for controlling wood boring insects are well established and have a long tradition in practical experience. Biological control using natural enemies, on the other hand, had not been applied so far, although many antagonists against common wood boring species are known (Haustein, 2010; Schmidt 1952) and some reports of laboratory research or practical experience had been published (Lygnes, 1956; Haustein, 2010). Advantages in using parasitoids for biological control are the exclusive feeding on their host individual and the pinpointing of their hosts in hidden places, even at low densities (Schöller & Prozell, 2011). Described enemies of the common furniture beetle *Anobium punctatum* are amongst others the checkered beetles (Cleridae) *Opilo domesticus* and *Korynetes caeruleus* and the parasitoids *Spathius exarator*, *Sclerodermus domesticus* (Schmidt, 1952) and *Cerocephala cornigera* (Becker, 1942). So far, laboratory breeding experiments with predators in the family Cleridae revealed less success in mass rearing, thus making them currently unsuitable candidates for biological control (Haustein, 2010). Further practical monitoring (Ott, 2005; Paul et al., 2008; Schöller et al., 2008) confessed, that the braconid wasp *S. exarator* is one of the most common natural enemies of the furniture beetle in historic buildings in Germany. With the scientific knowledge of Becker (1942) and Lygnes (1956), the innovative pest control company APC AG from Nuremberg bred *S. exarator* as a commercial biological control method against *A. punctatum* (Auer and Kassel, 2014). After successful mass rearing, first results of laboratory as well as several praxis tests were published (Kassel and Auer, 2015; Biebl and Auer, 2017).

This publication shows the practical use of the parasitoid *S. exarator,* presenting pooled data from 29 infested objects, as well as one representative church, selected from currently more than 80 *S. exarator* treated objects.

2. Material and Methods

Biology of Spathius exarator

The hymenopteran wasp *S. exarator* has a body size of up to 9 mm, with females possessing an ovipositor about their body length. A female wasp localizes its host feeding within timber by its movements and gnawing. After drilling the ovipositor through the wood, it paralyzes the larva and lays a single flexible egg onto it. At a temperature of 20°C and humidity of 60%, the *S. exarator* larva hatches after 3 to 5 days and feeds from its host larva. After pupation, the adult wasp hatches 28 to 30 days after oviposition from the wood through a self-gnawed exit hole (diameter up to 0.5 mm), which can easily be distinguished from the exit holes of *A. punctatum* (diameter 1-2 mm).

General treatment and monitoring procedure

Depending on intensity of infestation, at least twelve treatments over a period of up to three years at a building temperature of >15°C were performed by the company APC AG. About 100 bred *S. exarator* were assembled for each defined infested area inside the building. Usually, a total of 500-800 wasps were released per object and treatment. After a period of up to three years of intensive treatment, monitoring was continued and, if necessary, further single treatments were conducted.

On exactly defined areas, the new exit holes of wasps and furniture beetles were counted and documented after each treatment. From these data, the reduction of newly hatched *A. punctatum* beetles per year was calculated. As data of hatching beetles in the year before the treatment are lacking, a real blank value is missing. Thus, additionally the parasitisation rate using the following formula was calculated:

$$\frac{\text{no. of } S.\ exarator \text{ exit holes}}{(\text{no. of } S.\ exarator \text{ exit holes}) + (\text{no. of } A.\ punctatum \text{ exit holes})}$$

Pooled Data

Presented data compare the basic parasitisation rate at the day of the first treatment with the parasitisation rate after the last monitoring of 29 buildings treated with *S. exarator* up to five years, using Mann-Whitney U-test. Furthermore, we show the data of the decline in newly appeared *A. punctatum* exit holes and the parallel mean cumulative increase of *S. exarator* exit holes in these objects. From 53 treated objects up to 2016, 24 could not be included in the analyses since monitoring modalities have changed (n=5), basic parasitisation rates could not be calculated because of missing data (n=6) or monitoring data were not collected (n=13).

Chapel P. (Bavaria)

In the Chapel P. in Bavaria, eight, six and eight treatments were performed in the years 2012 to 2014. In 2015 and 2016 two treatments per year and in 2017, one treatment was done. At each treatment, about 500 braconid wasps were released at infested foot stools and the altars of the chapel.

Statistics

Statistical analyses were conducted using the software PAST: Paleontological Statistics software package for education and data analysis (version 2.12; Hammer et al., 2001). Figures were made with Microsoft Excel or the Microsoft Excel add in SSC-Stat (Statistical service center, University of Reading, UK).

3. Results

Results from five years of practical application in 29 *S. exarator* treated objects showed promising results. The mean number of treatments per year were 5.8, 5.4, 3.7, 0.7 and 1.7 for treatment years one to five, respectively. Parasitisation rates in the monitored areas increased after treatment with *S. exarator*: Before the onset of applications, parasitisation rates ranged from 0 to 0.276 (0.085±0.088; mean ± standard deviation; n=29). Parasitisation rates in objects treated for one to five years were significantly higher, ranging from 0.017 to 0.565 (0.206±0.114, mean ± standard deviation; n=29; Mann-Whitney U-test, p<0,001; Fig. 1).
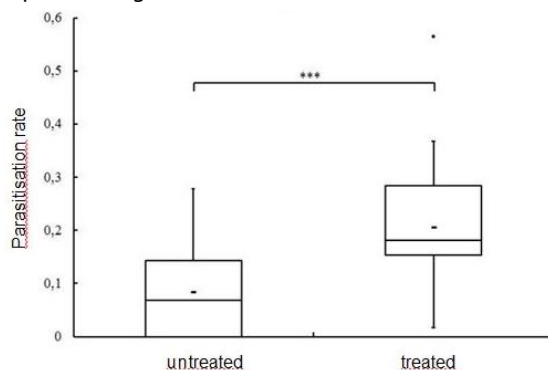
Figure 1. Mean parasitisation rates ± SD of 29 churches. Untreated: before first treatment; treated: during last monitoring.

Asterisks indicate significant differences (Mann-Whitney U-test, p≤0.001). Rhombus: outlier.

In table 1, an overview is given on the number of treated objects, the mean number of treatments per year as well as the mean number of newly hatching adult furniture beetles and wasps over treatment years one to five.

Number of newly hatched adults of *A. punctatum* continuously decreased over the first three years of treatment, as indicated by the declining number of newly appeared exit holes of *A. punctatum*. In the second year, an overall reduction by 61.22% was reached, and in the third year, a significant

reduction of even 92.61% was achieved (Mann-Whitney U-test, p=0.016). After three years of treatment, the number of adult *A. punctatum* slightly increased. Compared to the first year, the overall decline of newly hatched *A. punctatum* still was 74.14% in the fourth and 67.68% in the fifth year. However, only few objects were treated over a period longer than three years (n=3, year four and five, respectively) and the number of applications (0-2) in these objects was rather small.

Simultaneously to the decreasing number of newly appeared *A. punctatum* exit holes, the number of *S. exarator* exit holes continuously increased over the treatment years (table 1).

Table 1. Number of annually new eclosion holes of *A. punctatum* and the cumulative number of eclosion holes of *S. exarator* in treatment years 1 to 5; SD: standard deviation

Year of treatment (n=number of objects)	Mean number of treatments per year	Mean number of *A. punctatum* eclosion holes (±SD)	Mean number of *S. exarator* eclosion holes (cumulative) (±SD)
1 (n=29)	5.8	15.47 (±17.74)	37.10 (±30.35)
2 (n=17)	5.4	6.00 (±7.13)	59.12 (±51.53)
3 (n=7)	3.7	1.44 (±0.83)	61.43 (±47.69)
4 (n=3)	0.7	4.00 (±4.33)	116.33 (±34.08)
5 (n=3)	1.7	5.00 (±4.08)	123.67 (±40.27)

In Figure 2A, the number of newly hatched furniture beetles in the Chapel P. in Bavaria is shown, as indicated by the number of their eclosion holes. From 2012 to 2014, after repeated treatments each year, a strong decline was measured (34, 7 and 1 new exit holes, respectively). However, a slight increase after the fourth treatment year was found (10 new exit holes). In the following treatment years, a decline in newly hatched furniture beetles could be observed to an overall reduction of 91% compared to the first year (2016: 5 and 2017: 3 new exit holes).
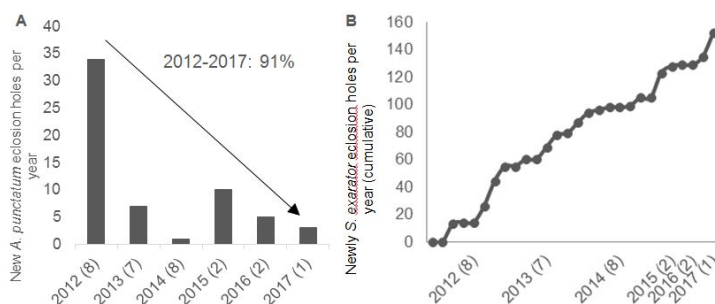
Figures 2 A–B: Eclosion holes by (A) *A. punctatum* and (B) *S. exarator* in foot stoles in Chapel P.

A. Number of newly found *A. punctatum* eclosion holes per year; B. Cumulative number of newly found *S. exarator* eclosion holes; Numbers in brackets indicate numbers of treatments per year

Figure 2B shows the amount of newly hatching *S. exarator*, represented by the number of their eclosion holes. In 2012 before first treatment, no *S. exarator* exit holes were found. At the end of 2012, after eight treatments, we found 55 new exit holes. This amount continuously increased over the treatment years up to a cumulative amount of 152 newly occurring adult wasps until now.

4. Discussion

The monitoring of the treated objects, presented in this publication, showed promising results. In all treated churches, the decline in newly hatched adult furniture beetles can be attributed to parasitisation by the released wasp *S. exarator*, as indicated by the increased number of their

eclosion holes. Thus, *S. exarator* appears to be an efficient and sustainable biological control method against the common furniture beetle. Furthermore, the success of a treatment can be estimated easily, as the eclosion holes of *S. exarator* and *A. punctatum* can be distinguished just by their size.

Larvae of the parasitic wasp *S. exarator* need larvae of *A. punctatum* for their development and, in consequence, each exit hole of *S. exarator* represents a parasitized and killed larva of *A. punctatum*. Over a period of five years of treatment, a mean number of 123.67 new *S. exarator* wasps hatched in the narrow-monitored areas of each treated church, representing as much killed furniture beetle larvae in this area. Corresponding to that, the number of annually newly appeared *A. punctatum* exit holes continually decreased with an overall reduction of 92.61% between the first and the third treatment year. However, in the fourth and fifth year of treatment, the number of newly eclosed furniture beetles slightly increased. However, only few objects were treated over a period longer than three years (n=3, year four and five, respectively) and additionally, the number of application (0–2) in these objects was rather small. Thus, monitoring objects over a longer period will reveal further insights in the population dynamics of *A. punctatum* and *S. exarator*.

It has to be noted that the amount of new *A. punctatum* exit holes in the first treatment year is already a reduced value. We started the first treatment about two months before the yearly one-time eclosion of *A. punctatum*. Thus, the wasps had been able to parasitize the beetle larvae for about two months until we documented the number of hatching *A. punctatum* beetles for the first treatment year. For a better estimation of the infestation level, it might be useful to monitor the eclosion of *A. punctatum* for one year before the onset of the treatment period.

As shown in literature (Ott, 2005; Paul et al., 2008; Schöller et al., 2008; Haustein, 2010), *S. exarator* appears to be a naturally occurring parasitoid of *A. punctatum*. Our own observations confirm that, since we found eclosion holes of *S. exarator* in two third of the 29 *S. exarator* treated churches before first treatment. After collecting data of exit holes of *A. punctatum* and *S. exarator* in untreated objects for two years, Haustein (2010) calculated a prey-predator relationship of 26.5 yearly eclosed *A. punctatum* per *S. exarator* (1:26.5). Even despite the knowledge of different data sets, our data show clearly reduced relationships of 1:1.6 after one year of treatment and 1:0.2 after the second treatment year (Auer and Kassel, 2017). Due to natural predator-prey relationships, the use of a natural antagonist in controlling pest organisms will not result in a 100% elimination of the pest (Graf, 1992; Querner, 2017) but in a decline of their population under a predefined minimum infestation level (Haustein, 2010). This is approved by our data, as the number of newly found eclosion holes of *A. punctatum* in treated objects slightly increased after a short period with a reduced number of treatments or without releasing *S. exarator*. Consequently, a continuous monitoring program with a well-adjusted treatment protocol is strongly recommended. This is even more important in the light of the relatively long development period of *A. punctatum* which can take up to 5 years (Pinniger, 1996).

Additionally, the parasitisation success of *S. exarator* also depends on various factors like paintings on infested objects (Biebl and Auer, 2017), previous insecticide treatments, type of wood and infestation level and should be investigated in further laboratory experiments as well as practical experience. By revealing the effects of these parameters, an elaborated application program adapted to the respective conditions in the treated objects can be developed and enhances the efficiency of the biological control of *A. punctatum*.

Acknowledgement

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Prospects of Entomopathogens in Post-Harvest Integrated Pest Management

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Abstract

In these exploratory experiments, entomopathogenic nematodes and fungi were investigated for the management of the populations of postharvest insect pests. Nematodes were screened for pathogenicity to *Plodia interpunctella* (Hübner), while nematodes and fungi were investigated for virulence to the maize weevil, *Sitophilus zeamais* (Motschulsky). Adults and larvae of *P. interpunctellea* were screened for susceptibility to the following six nematodes: *Heterorhabitis bacteriophora* Poinar (HP88, Lewiston and Oswego strains); *H. indica* Poinar, Karunakar and David (Homl strain); *H. marelatus* Liu and Berry (Point Reyes strain); *H. megidis* Poinar, Jackson, and Klein (UK211 strain); and *H. zealandica* Poinar (NZH3 strain). The nematodes that had the highest virulence to larvae and adults of *P. interpunctellea* were *H. indica*, *H. megidis*, and *H. marelatus*. Six strains of nematodes were studied, namely *H. bacteriophora*, *H. indica*, *H. georgiana* (K22), *Steinernema feltiae* SN and *S. carpocapsae.* All strains of fungi, *Beauveria bassiana* (GHA) and *Metarhizium brunneum* (F52) were evaluated for infectivity to adults of *S. zeamais*. The two strains of Steinernematidae nematodes and a strain of fungus, *B. bassiana* were found to cause significant mortality of the weevils compared to the rest of the entomopathogens and the control. To demonstrate the practical application of entomopathogens, wettable dust of *B. bassiana* were dispensed on jute bags after which weevils were exposed to the treated surfaces for 30 min. The exposed weevils recorded between 90 to 100% mortality 14-d after exposure. Additional study demonstrated that the parasitoid, *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) could be integrated with entomopathogenic nematodes. These experiments demonstrate the potential usefulness of entomopathogens in the management of stored product Lepidopteran and Coleopteran pests.

Keywords: entomopathogens, nematodes, fungi, parasitoid, virulence.

1. Introduction

Stored product Lepidopteran and Coleopteran pests are cosmopolitan pests that cause severe postharvest losses of grains and processed commodities. In the past, chemical pesticides have been used to disinfest commodities of storage pests. Integrated pest management (IPM) strategies in postharvest systems based on chemical pesticides pose health, legal and financial risks due to pesticide residues that may occur in foods (Monaco et al., 2002). Furthermore, there are dramatic restrictions in the use of synthetic pesticides to control pest populations in storage facilities. Natural enemies present alternative methods to overcome the potentially harmful effects of chemical pesticides especially in the management of postharvest pests since natural enemies are mostly environmentally safe and do not pose any dangers to humans or the environment. Natural enemies employed in postharvest IPM have been mostly parasitoids. Other forms of biological control such as use of entomopathogenic nematodes and fungi have recently started to attract attentions. This study investigated the potential of entomopathogens in the management of stored product pests. *Plodia interpunctella (Hübner)* (Lepidoptera: Pyralidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Dryophthoridae) were the test insects in this study.

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae spp.) have the potential to control a broad range of arthropod pests including stored product insects. Nematodes kill their insect hosts with their mutualistic relationship with bacteria *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae (Gram-negative Enterobacteriaceae) that inhabit the intestinal lumen of nematodes as symbionts (Boemare, 2002). Free living and infective juveniles (IJs) or third stage juveniles of nematodes enter the hemocoel of the host insects through the natural openings such as mouth, anus and spiracles or respiratory system, and release their pathogenic bacteria that propagate rapidly and kill insects within 48 hours (Poinar, 1990). The nematodes develop and complete 2 to 3 generations before leaving the host insect. Mbata and Shapiro-Ilan (2010) investigated the pathogenicity of different spp. of entomopathogenic nematodes to *P. interpunctella* larvae and reported *Heterorhabditis indica* (HOM1) strain to be most virulent of all the strains tested. In a laboratory experiment, strains of Steinernematidae and Heterorhabditidae showed higher virulence for the larvae of *Ephestia kuehniella* Zeller, *Tenebrio molitor* (Linnaeus) and adults stages of *Acanthoscelides obtectus* (Say) compared to *Sitophilus zeamais* and *S. oryzae* (Barbosa-Negrisoli et al., 2013). In another study, *H. indica* was reported to be pathogenic to several stored product pests including *S. zeamais* (Maketon et al., 2011). Ramos-Rodriguez et al. (2006) reported that *T. molitor*, *Oryzaephilus surinamensis* (Linnaeus) and *Tribolium castaneum* (Herbst) were found to be susceptible to *Steinernema riobrave* Cabanillas, Poinar and Raulston.

Entomopathogenic fungi have also been demonstrated to be a promising alternative to chemical pesticides for biological control of arthropod pests. Pathogenicity of two strains of *Purpureocillium lilacinum* to *T. confusum, R. dominica* and *S. zeamais* has been reported (Barra et al., 2013). The pathogenic effect of ten strains of *Beauveria bassiana* and two of *Metarhizium brunneum (Metschnikoff)* treated with 1×10^9 conidia/ ml against *S. zeamais* resulted in weevil mortality in the range of 53 to 93% (Ruelas-Ayala et al., 2013). In contrast, Trevisoli et al. (2015) reported that *S. zeamais* is less susceptible to the fungal strains of *B. bassiana*, *M. brunneum* and *Isaria fumosorosea*. Thus, susceptibility of the maize weevil to *B. bassiana* and *M. brunneum* requires further elucidation.

The studies reported here comprised of results from exploratory investigation into the potentials of entomopathogenic nematodes and fungi for biocontrol of two stored product pests, P. interpunctella and S. zeamais. In addition, combined application of Braconid wasp, H. hebetor, with entomopathogenic nematodes were compared with applications of nematodes or wasp alone to determine if the two biocontrol agents could be integrated for the management of P. interpunctella populations.

2. Materials and Methods

Rearing of insects and natural enemies

Plodia interpunctella stock culture was originally obtained from USDA-ARS, Grain Marketing and Research Laboratory, Manhattan, KS in 2001 and had since been maintained on the artificial moth diet at 28 ± 1.5 °C, 70 ± 5% RH and a 16h: 8h photoperiod in Biology department of Fort Valley State University (FVSU), Fort Valley, GA.

Foundation culture of *S. zeamais* was obtained in August, 2006, from the University of Georgia's center for Invasive species and Ecosystem Health, Department of Entomology, Tifton, GA. Populations of *S. zeamais* have been maintained in the insectary of FVSU.

Wasp culture was originally collected from the Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK. Wasps were reared on 50 late instars of *P. interpunctella* in 1000 mL glass rearing jars kept in experimental chamber maintained at 28 ± 1.5 °C, 70 ± 5% RH and a 16: 8 (L:D) photoperiod.

Nematodes were reared at ~ 25ºC in last instar of greater wax moth, *Galleria mellonella* (Linnaeus) following a procedure described by Woodring and Kaya (1988). The larvae of *G. mellonella* were obtained from Webster's Waxie Ranch (Webster, WI). Nematodes were stored at 13 °C for 15 d or less before being used for experiments.

Cultures of *H. bacteriophora* (HP88) and *H. megidis* were obtained from the MicroBio Group of Becker Under Wood (West Sussex, UK), *H. bacteriophora* (Lewiston) and *H. indica* from Integrated BioControl Systems (Lawenceburg, IN), *H. bacteriophora* (Oswego) from Dr. Elson Shields (Cornell University, Ithaca, NY) and *H. marelatus* and *H. zealandica* from P. stock (University of Arizona, Tucson, AZ). *H. indica* Poinar, Karunakar, and David (Homl strain), *H. georgiana* (K22), *Steinernema feltiae* (SN), and *S. carpocapsae* (All) were obtained from USDA-ARS culture collection in Byron, GA.

Entomopathogenic fungi, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (GHA strain) and *Metarhizum brunneum* Petch (F52 strain) were originally obtained from Stefan Jaronski (USDA-ARS) and cultured on Sabouraud dextrose agar supplemented with 0.2% yeast extract according to a procedure described by Goettel and Inglis (1997). Established cultures of fungi were stored at 4 ºC for one week before experimentation begins.

Pathogenicity of entomopathogenic nematodes to third and fifth instars of Indian meal moths

Virulence of the nematodes was tested at 150 and 480 dose rate of IJs per larva in plastic cups (3-4 cm internal diameter, 3 cm deep; Bioserv Frenchtown, NJ) at ~28 ± 1.5 °C and 70 ± 5% RH against 10-d third and 18-d-old fifth instars of *P. interpunctella*. One larva with 3.5 mL moth rearing medium was placed in each cup that was covered with plastic lid. Inoculum of IJs in 0.5 mL water was added to each cup 1-d after the introduction of larva and incubated until the emergence of adults and assessment of mortality. Moisture content of the medium was ~14% after adding 0.5 mL water. Four replicates of 10 cups per treatment of nematode strain and untreated control (water) of third and fifth instar of *P. interpunctella* were set up and two trials were conducted for both larval stage. Mortality of third and fifth instars was recorded after 10-d and 21-d respectively in treated and control cups.

Influence of combined application of nematodes and parasitoid versus single of either nematodes or parasitoid

This laboratory experiment assessed the virulence to *P. interpunctella* and interaction among biological control agents was conducted in 1 l rearing jars (7.4 cm diameter and16.8 cm height). A set of four jars were set up with two host larval densities, 20 and 40. Treatments consisted of *H. indica* and *H. hebetor*; 8000 IJs with 2 mL water (200 and 400 IJs/insect) were applied to larvae in one treatment, three pair (three males and three females) of adult parasitoids exposed to larvae in

second treatment, combination of exposure of 2 mL of water consisting of 8000 IJs and three pairs (2-d old males and females) of adult *H. hebetor* in treatment three and in treatment four, control was set up with 2 mL water. All jars were covered with filter paper and maintained at 28 ± 1.5 °C, 70 ± 5% RH and a 16: 8 (L:D) photoperiod. After 3-d period, mortality of host larvae and parasitoid was observed and afterwards all jars containing larvae with parasitoids, IJs or to both were transferred to incubator until the complete development of F_1 parasitoids. Each treatment was replicated four times and three runs were conducted.

Pathogenicity of entomopathogenic nematodes to *Sitophilus zeamais*

The protocol for inoculating the weevils with entomopathogenic nematodes followed a method used in screening *P. interpunctella* for susceptibility to entomopathogenic nematodes (Mbata and Shapiro-Ilan, 2005). Dose-response evaluation of the nematodes was carried out with infective juveniles (IJs) of *H. bacteriophora* Poinar (VS) and *S. carpocapsae* (All). Infective juveniles of nematodes in aqueous solutions were inoculated onto 7 cm filter papers (Whatman grade 40) placed in petri dishes (6 cm diameter) with 0.35 µL of nematode suspensions at the rates of 100 IJs/cm^2 (2400 IJs/0.350 uL), 200 IJs/cm^2 (4800 IJs/0.350 µL) or 400 IJs/cm^2 (27458 IJs/0.350 µL) to determine the rate of application that was infective to *S. zeamais*. Based on dose-response experiment, the application rate of 400 IJs/cm^2 was selected as the effective rate for screening of the six nematodes for virulence to *S. zeamais*. The controls were set up as described above but consisted of 0.35 µL of tap water sprayed onto 6 cm filter papers in petri dishes. Ten *S. zeamais* adults (1-3 d old) and a kernel of maize were transferred to each of the petri dishes. The experiment was organized in a completely randomized design with nine replicates of 10 weevils each per treatment and control that were grouped into three sets for examination of weevil mortality 3, 7 and 14 dpi (days post inoculation), consecutively. The experiment was conducted over four consecutive trials with new generations of weevils. The petri dishes were kept in a controlled chamber maintained at 25 °C and uncontrolled but high relative humidity due to the moist filter paper.

Pathogenicity of entomopathogenic fungi to *Sitophilus zeamais*

Beauveria bassiana (GHA strain) and *M. brunneum* (F52 starin) were investigated at three different concentrations designated as low (1 x 10^7 conidia), medium (1 x 10^8 conidia) and high (1 x 10^9 conidia) doses. The experimental design involved 7 treatments consisting of three different doses of each of the fungi and the control. Nine petri dishes for each of the strains, three for each dose, were prepared with filter paper and each petri dish was added with a different concentration of the fungal suspension from the cultures at the rates specified above that corresponded to 6857 (low), 13714 (medium), and 27482 (high) conidia/mL. One maize kernel, and 10 adult weevils were added to each petri dish and the dishes were sealed with parafilm. Observations of the weevils for mortality were carried out 7 and 14 ds post inoculation. The experiment was repeated over two consecutive trials with new generations of weevils.

Infectivity of fungal spores applied to jute bags against *Sitophilus zeamais*

The more virulent entomopathogenic fungus to *S. zeamais* determined in the test described above, *B. bassiana*, was investigated further for the protection of bagged maize grain by applying the wettable powder to jute bags used in the postharvest storage of maize. At this stage, it is reasonable to investigate the fungi further since they do not require moist surface for survival and dispersal[29]. Wettable powder of *B. bassiana* (GHA strain) 4.4 x 10^{10} conidia/g (Botanigard 22wp® WPO) was obtained from BioWorks (Victor, NY).

Fungal application during the experiment was carried out under a biosafety cabinet to contain the fungal powder and prevent contaminating the control weevils. The jute bags (surface area = 2.06 x 10^5 mm^2) were each treated with one of three rates of the wettable powder comprising of 2.13 x 10^7 conidia/mm^2, 1.07 x 10^7 conidia/mm^2, and 0.5 x 10^7 conidia/ mm^2. Control bags were not treated with any powder. Appropriate quantities of the wettable *B. bassiana* powder were weighed out for

each of the treatments and transferred into containers or bins (L 50.8 x W 47.8 x D 15.2 cm). Jute bags were placed in each of the containers dispensed with the wettable *B. bassiana* powder. Following the replacement of the lids of the containers, the containers were shaken vigorously to ensure even distribution of the powder through the surface of the jute bags. Each treatment and the control were replicated three times. Twenty weevils per bag were released to crawl on bags for a 30 min period and were transferred thereafter to 60 mm petri dishes lined with filter papers moistened with 0.35 mL of tap water. A kernel of maize that served as food for the weevils was placed in each of the petri dishes. The lids on dishes were taped down on to trays to prevent insects from escaping. Survival of insects was determined 7 and 14 d post-exposure. The entire experiment was repeated two times.

3. Results

Mortality of third and fifth instars of *P. interpunctella* exposed to entomopathogenic nematodes

Mortality differences in the 3rd and 5th instars of *P. interpunctella* exposed to nematodes were observed between all treatments and the control (Tab. 1; $F = 6.61$; $df = 12, 67$; $P < 0.0001$). Third instar larvae were found to be more susceptible at the rate of 480 IJs/insect than 150 IJs/insect. At the higher exposure rate of 480 IJs/larva, *H. marelatus* (Point Reyes), *H. megidis* (UK211), *H. indica* (HOM1) and *H. marelatus* strains caused higher larval mortality than the control whereas at the rate of 150 IJ/larva *H. zealandica* and *H. indica* caused greater mortality than the control (Tab. 2).

Influence of combined versus separate application of nematodes and parasitoid

Moth larvae exposed to nematodes at the rate of 200 (4000 IJs/2 mL) and 400 (8000 IJs/2 mL) IJs per larva had significantly different larval mortality (at 200 IJ/larva: F = 276.23; df = 3, 36; P = 0.0001; Fig. 1 and at 400 IJ/insect: F = 110.02; df = 3, 36; P = 0.0001; Fig. 1). Combined treatment of nematodes at the dose rate of 200 and 400 IJs per larva with *H. hebetor* (3 pairs of males and females) caused higher *P. interpunctella* larvae mortality.

Chi-square value ($χ^2$) indicated that combined effect of nematodes and parasitoids on the larval mortality were not antagonistic but could possibly be additive or synergistic because at both exposure rates of nematodes with *H. hebetor*, high mortality of host larvae was achieved (for 200 IJ/larva: $χ^2 = 1.81$; M = 0.87; $P < 0.05$ and for 400 IJ/larvae: $χ^2 = 2.26$; Me = 0.84; $P < 0.05$).

Pathogenicity of nematodes to *Sitophilus zeamais*

No significant differences were observed on the survival (%) of maize weevils following 3 and 5 dpi with nematodes compared to the control but survival of adult S. *zeamais* at 7 dpi was significantly lower than the control. (Tab. 3: F = 2.78; *df* = 6, 41; *P* < 0.0001). Maize weevils treated with *S. carpocapsae* (All) exhibited the highest susceptibility with survival rate at 28.3%. Pathogenicity of *S. carpocapsae* against weevils was significantly higher than the rest of the nematode strains except *S. feltiae*.

Pathogenicity of entomopathogenic fungi to *Sitophilus zeamais*

Survival of maize weevils was significantly reduced at 14 dpi (F= 6.05; df = 6, 41; *P* ≤ 0.0004; Tab. 4) following exposure to fungi but at 7 dpi survival of weevils exposed to fungi was not significantly different from the control. Survival of weevils exposed to low dose of *M. brunneum* (6857 conidia/mL) was significantly different from the survival of weevils exposed to medium fungi doses (13714 conidia/mL) at 14 dpi. Significant reduction in the survival of maize weevils was obtained at low, medium and high rates of *B. bassiana* compared to *M. brunneum* and control.

Fungal strains infectivity to jute bags against *Sitophilus zeamais*

Wettable *B. bassiana* powder applied to jute bags at 25, 50 and 100 g highly affected the survival of weevils at 14 dpi (Tab. 5: F= 76.16; df = 3, 15; P < 0.0001) compared to 7 dpi (Tab. 5 : F= 40.75; df = 3, 15; P < 0.0001) and the control. No significant differences were observed on the percentage survival of maize weevils exposed to low dose (25 g) of *B. bassiana* and the control weevils. Higher rate (100 g) of *B. bassiana* generated 100% mortality of treated weevils at 14 dpi.

Tab. 1. Mortality of third instar of *Plodia interpunctella* after 10-d exposure to nematodes strains at rates of 480 and 150 IJs/larva.

	Mean ± S.E.	
Nematode strain	**480 nematodes/larva**	**150 nematodes/larva**
H. bacteriophora **(HP 88)**	4.11 ± 0.68bc	2.78 ± 0.43cd
H. bacteriophora **(Lewiston)**	4.22 ± 1.23bc	2.44 ± 0.88cd
H. bacteriophora **(Oswego)**	4.00 ± 1.00bc	2.67 ± 0.60cd
H. indica **(Homl)**	5.56 ± 1.12ab	3.78 ± 0.92c
H. megidis **(UK 211)**	5.44 ± 1.20ab	2.11 ± 0.75d
H.marelatus **(Point Reyes)**	6.22 ± 1.04a	2.00 ± 0.71d
H. zealandica **(NzH3)**	3.22 ± 0.88cd	4.22 ± 0.92cd
Control	1.33 ± 0.44d	1.50 ± 0.46d

Mean ± SE (out of 10) within a column followed by same letter are not significantly different (Tukey's test, P < 0.05).

Tab. 2. Effect of strains of entomopathogenic nematodes on the mortality of fifth instars of *Plodia interpunctella*.

Nematode strain	**Mean ± S.E.**
H. bacteriophora **(HP 88)**	3.6 ± 0.33a
H. bacteriophora **(Lewiston)**	4.1 ± 0.43a
H. bacteriophora **(Oswego)**	4.2 ± 0.36a
H. indica **(Homl)**	4.1 ± 0.41a
H. megidis **(UK 211)**	3.9 ± 0.31a
H.marelatus **(Point Reyes)**	4.1 ± 0.46a
H. zealandica **(NzH3)**	4.3 ± 0.42a
Control	1.4 ± 0.22b

Means within a column followed by same letter are not significantly different (Tukey's test, P < 0.05)

Tab. 3. Percentage survival of adult *Sitophilus zeamais* after exposure to entomopathogenic nematodes for 7-d. Control consisting of water only.

Nematode strain	**Mean ± S.E.**
Control	100 ± 0.00a
H. georgiana	78.33 ± 3.07b
H. indica	60.00 ± 10.95bc
H. bacteriophora **(Lew)**	56.67 ± 2.10bc
H. bacteriophora **(Osw)**	50.00 ± 7.30c
S. carpocapsae	28.33 ± 7.40d
S. feltiae	41.67 ± 4.01cd

Different letters within a column are significantly different (Student-Newman-Keul's test, P < 0.05%).

Tab. 4. Survival (%) of maize weevils exposed to entomopathogenic fungi for 14-d. Control is with water.

Fungal strain	**Mean ± S.E.**
Control	90.00 ± 6.32a
Bb-lo	65.00 ± 12.58bc
Bb-med	61.67 ± 9.45bc
Bb-hi	38.33 ± 10.13c
Met-lo	75.00 ± 12.58ab
Met-med	78.33 ± 7.03ab

Met-hi	48.33 ± 6.00bc

Different letter (within column) is significantly different at 5% significant level (Student-Newman-Keul's test).

Tab. 5. Seven and 14 dpi survival (%) of maize weevils exposed for 30 min. to jute bags treated with different rates of wettable *B. bassiana* powder. Control was water only.

Dose of Fungi	7 dpi	14 dpi
Untreated control	98.33 ± 1.66a	76.66 ± 3.80a
Bb-25 g (low)	71.67 ± 2.10b	8.33 ± 4.01b
Bb-50 g (medium)	64.17 ± 5.83b	0.83 ± 0.83c
Bb-100 g (high)	72.50 ± 2.14b	0.00 ± 0.00d

Different letter (within column) is significantly different at 5% significant level (Student-Newman-Keuls test).
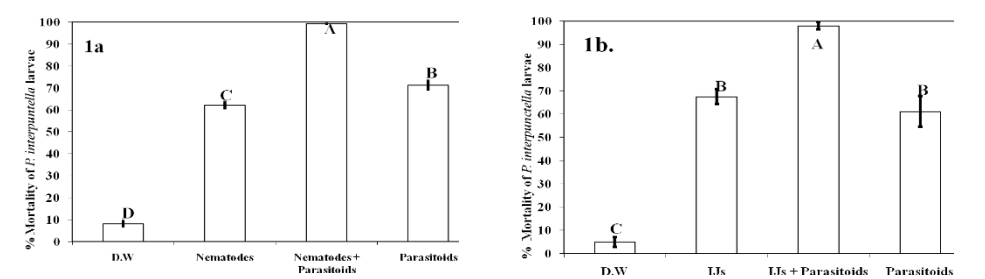
Fig. 1. Mortality (%) of Indian meal moth larvae at the exposure rate of 200 (1a) and 400 (1b) IJs of *Heterorhabitis indica/moth larva*, parasitoids (*Habrobracon hebetor*) or combination of *H. indica* and parasitoids. D.W. = Distilled Water for control, IJs= Infective juveniles nematodes. Different letter above bars indicate significant difference (Student-Newman-Keuls test, *P* < 0.05%).

4. Discussion

Heterorhabditis marelatus (Point Reyes), *H. megidis* (UK211), and *H. indica* (HOM1) showed more pathogenicity and caused higher mortality in Indian meal moth larvae. These nematodes have unique characteristics that recommend them for consideration for year round management of *P. interpunctella*. *H. marelatus* and *H. megidis* are considered to be cold tolerant (Grewal et al., 1994, Berry et al., 1997) while *H. indica* is heat tolerant (Shapiro and McCoy, 2000). This implies that these three nematodes could be used at different times of the year to regulate populations of *P. interpunctella*.

Combination of nematodes and parasitoids enhanced the mortality of *P. interpunctella*. Dillon et al. (2008) observed that the interaction between the nematodes *H. downesi* or *S. carpocapsae* and the parasitoid *Bracon hylobii* enhanced the mortality of the pest host, *Hylobius abietis*. Interaction between entomopathogenic nematodes and the parasitoid could possibly be additive or synergistic.

Maize weevils were more susceptible to Steinernematid strains particularly *S. carpocapsae* (All) and *S. feltiae* (SN) compared to the Heterorhabditidae. Entomopathogenic fungi, *B bassiana*, exhibited strong virulence against adult maize weevils compared to *M. brunneum* (Vega and Hofstetter, 2014). High concentration of wettable powder of *B. bassiana* applied to jute bag surface caused 100% mortality in maize weevils 14 days after inoculation. This implies that a path exists by which entomopathogens could be integrated in the IPM of postharvest arthropods.

Acknowledgment

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Chilled Aeration to Control Pests and Maintain Grain Quality During the Summer Storage of Wheat in North Central Region of Kansas

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Abstract

Chilled aeration allows to cool grain, independent of ambient conditions, to "safe" temperatures where insect, fungi, and spoilage is reduced to the minimum. The objective of this research was to evaluate the advantages of using grain chilling to preserve the quality of grain and reduce post-harvest losses, compared to conventional

aeration and storage strategies used during the summer storage of wheat in Central Kansas, U.S.A. The research trials were developed in two 1,350 metric ton (t) steel silos in a Farmer's Cooperative during the summer and fall of 2015 and 2016. One of the silos was chilled and the other was used as a control managed by the Cooperative. Variables evaluated were: grain temperature, moisture content (MC), grain quality, insect development and reproduction rate. The chilling treatment reduced the grain temperature from 28°C- 39°C to a minimum of 17°C-17.6°C in less than 250 hours. Grain temperatures below 25°C were not possible during the summer using ambient aeration. Minimum variation of MC was observed in the Chilled silo while ambient aeration reduced the MC by 0.5%. Reproduction rates of RFB and LGB were significantly reduced by chilled temperatures lower than 17°C. Lower temperatures also reduced insects discovered in probe traps and insect damaged kernels (IDK). The energy cost of the grain chiller was between 0.26 US $/t- 0.32 US $/t higher than ambient aeration.

Keywords: Ambient aeration, Grain chilling, Summer storage, Post-harvest losses, Wheat.

Introduction

Grain that is harvested during the summer season of the Northern Hemisphere presents the inconvenience that it is collected when the ambient temperature is high (26°C to 40°C). In these conditions, the grain goes into storage at a high temperature, which makes it prone to immediate insect infestation and mold growth that can affect its quality. Therefore it is imperative that the grain temperature is decreased as soon as possible (Reed and Arthur, 2000). Nevertheless, cool ambient conditions may be limited during this part of the season, thus the use of chilled air could be considered as a solution. Chilled air refers to aeration air that is cooled before it comes contact with the grain by passing through an evaporator coil of a grain chilling unit (Maier and Navarro, 2002). When the chilled air comes in contact with the grain, it lowers the temperature of the grain, independent of ambient conditions (Maier and Navarro, 2002). This technology makes it possible to cool down grain temperature between 20°C and 15°C immediately after summer harvest, which reduces insect populations and consequently the need for chemical control (Navarro et al., 2002).

Based on field tests using chilled aeration on low-moisture wheat stored in Michigan, Maier (1992) simulated chilling in the Midwestern region of the U. S. The computer simulation showed that chilled aeration was capable of lowering the temperature of 579 metric tons (t) of wheat from 30°C to 15°C in just one week. Continuous ambient aeration took 1.5 times longer to cool the grain down to 10°C, which caused higher dry matter losses (DML). Other grain chilling field trials developed in 2,500 t wheat silos in Central Kansas determined that the cost of chilling the grain from 32°C- 35°C to 15°C– 17°C in six days was less than 0.16 US $/t, while the cost of fumigating and turning the non-aerated silo was 0.67 US $/t, plus the additional shrink loss cost of approximately 7.5 t from the bulk (Hellemar, 1993).

Maier et al. (1996) compared eight combinations of ambient aeration, fumigation and chilled aeration strategies in three different locations of the U.S. through computer simulations. Chilling the grain below 17°C in a short period of time proved to be the best strategy to avoid DML and reduce the populations of maize weevil (MW) *Sitophilus zeamais*.

While the strategy of chilling grain is the effective control of insects, there are other benefits that come from the grain chilling technology, such as the possibility of storing damp grain for a limited time, predictable drying capability and better preservation of end-use quality (Hellemar, 1993; Maier and Navarro, 2002).

The objective of this research was to evaluate the advantages of using grain chilling technology to preserve the quality of grain and reduce post-harvest losses caused by insects and fungi, compared to the conventional aeration and storage strategies used during the summer storage in North Central Kansas.

Materials and Methods

This research was conducted at Farmer's Cooperative in the North Central region of Kansas, U.S.A., from August to November 2015 and from June to September 2016. The research trials were conducted in two 1,350 t steel silos of 11.3 m in diameter and 16.8 m in height from the bottom to the eave, filled almost completely with hard red winter wheat (HRW) harvested in the summer of

2015 and 2016. Before each harvest, the silo walls were cleaned up to 6 m from the bottom and the remaining grain on the floor of the silo was vacuumed out. Attached to these silos there were two centrifugal fans in parallel, each with a 10 HP (7.5 kWh) motor (Baldor Electric Co., Fort Smith, AR). One of the silos was chilled (Chilled silo) and the other one was used as a treatment control (Control silo) managed by the Cooperative using their regular grain quality management strategies.

Grain chiller setup and monitoring of air and grain conditions

The grain chiller GCH-20 used in this project was facilitated by the Brazilian company Coolseed (Santa Tereza do Oeste, Brazil). This equipment has the rated capacity to chill 100 to 170 t per 24-hour continuous operation in silos of up to 1,800 t, according to specifications of the manufacturer.

The grain chiller was connected to the grain silo through thermally insulated ducts that were connected into the two inlets of the aeration fans that where removed from the (Figure 1).

Figure 7. Grain chiller GCH-20 setup: (a) Insulated duct connected to the chiller's outlet at one end and to a "T" connector at the other, (b) Two ducts attached to the fan transition parts of the aeration fans that were removed.

The conditions inside the Chilled and Control silo were monitored through three temperature cables (TSGC Inc., Spirit Lake, IA) in each silo, that were attached to the roof and the floor of the silo. Additionally, temperature and relative humidity (RH) sensors were placed in the fan transitions, outside of the silos to record ambient conditions. In the 2016 trials, additional sensors were placed in the fan outlet of the grain chiller and inside the insulated ducts.

The wheat moisture content (MC) was measured using a GAC 2500-UGMA (Dickey John, Auburn, IL) every 30 days. Grain samples were taken at four different depths and three depths in each of the silos. The samples collected per location were put together and homogenized to make up a composite sample per location in each of the silos. The composite sample from each location was considered a replication for the calculation of significant differences between sampling dates. Statistical analysis was performed using the SAS statistical software (SAS Institute Inc., NC). Statistically significant differences were analyzed with Tuckey's test ($p < 0.05$).

Insect pest population monitoring and quantification

Insect bioassays

The effect of chilled aeration on the survival rates of insects was quantified using insect bioassays with the species Lesser Grain Borer (LGB) *Rhyzopertha dominica* and Red Flour Beetle (RFB) *Tribolium castaneum*. The bioassays consisted of plastic jars of 0.2 L with holes on the bottom and top, covered with wire mesh and filled with an exact number of adults of each species, together with a mix of flour, yeast and broken kernels for insect feeding.

In each of the silos, a bioassay of each species was located in the center of the silo and next to each temperature cable, and buried 0.3 m below grain surface. A fifth bioassay per species was located

in one of the fan transition parts. In 2016, three jars per location were put inside the grain mass and transition parts. One jar from each location was taken out every 28 days.

When the jars were taken out of the silos in each sampling date, the number of dead and live adults were quantified and then discarded, and the larvae, pupae and eggs (if any) were kept in a growth chamber and counted 28 days after as adults. The total progeny number was calculated by the total insect count (initial dead and live insects when jar was pulled out of the silo plus the progeny number after 28 days in the growth chamber) minus the original number of insects put into the jar. Statistical analysis was performed using the SAS statistical software. Statistically significant differences were analyzed with Tuckey's test ($p < 0.05$).

Endemic insect population sampling

Insect populations inside the silos were quantified by placing five perforated insect probe traps model Storgard W.B. Probe II (Trece Inc., Adair, OK) of approximately 0.6 m in length in the North, South, East, West, and Center sections of the silos, approximately 1.5 m from the walls. Insects inside the probe traps were checked every 28 days and identified (up to the genus level). Adults of the main insect pests of stored-products were counted.

Grain quality analysis

Grain samples for these analysis were collected using the same procedure described to collect the MC grain samples. In 2015, the samples were only collected in the first two months of the trial, while in 2016, the sampling period was expanded for one more month.

For the grain quality analysis, only one composite 2,500 g composite sample was taken per silo each sampling date. This composite sample was sent to the Kansas Grain Inspection Service (KGIS) in Topeka, Kansas, for grading.

Electrical cost of chilled and ambient aeration strategies

The energy consumption during the chilling treatment was measured using a kWh counter that was installed at the of the power inlet of the grain chiller. The energy consumed by the aeration fans in the Control silo were calculated according to the hours of operation reported by the Cooperative. The costs of the ambient and chilled aeration process were calculated based on the energy consumption, using an average cost of 0.084 $/kWh (obtained from the local electrical service provider), and considering additional charges for basic service and consumption fees.

Results and Discussion

Ambient and chilling aeration trials

Trial of 2015

The grain chilling treatment started on August 22nd, and the cool air front reached the top of the grain mass after 175 hours of active chilling at an airflow rate of 0.07 m³/min/t and at a temperature of approximately 17°C (initial grain temperature: 28°C). Due to technical difficulties with the grain chiller during certain periods, the equipment was left running longer to tests its capacity, until September 14th, 2015, for a total of 314 hours (Figure 2).

The ambient aeration strategy applied in the the Control silo by the Farmer's Cooperative was based on turning on the fans when the ambient temperature was below 27°C in the summer, and below 18°C during the fall. The total active aeration time was 308 hours, at an average airflow of 0.11 m³/min/t. Temperatures inside the Control silo remained over 17°C until mid-November, which was about two months after this temperature was reached in the Chilled silo. According to Hagstrum and Subramanyam (2006), for every month that cooling is delayed, populations of insects can grow 5- to 25-fold their original frequency.

During the trial, the ambient air fluctuated between 8°C and 37°C, with an average of 23°C. The average ambient RH was 63.5% with a minimum of 27.4% and a maximum of 93.1%.
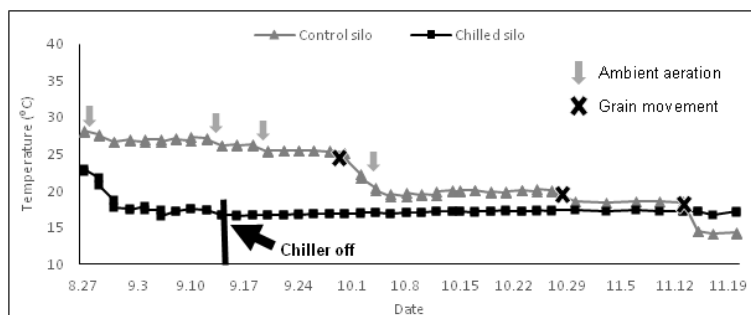

Figure 8. Grain temperature profile (°C) of the grain mass inside the Control and Chilled silo from Aug. 27th to Nov. 20th, 2015 in Farmer's Cooperative, Kansas, U.S.A.

In 2015, the average MC inside the Chilled silo was 11.4% and did not change significantly, while in the Control silo the average MC decreased significantly from 11.1% to 10.5% in the last two months (October and November) of evaluation.

Trial of 2016

In 2016, the initial grain temperature in the Chilled silo was higher than the previous year (39°C). The grain chilling trial started on June 21st, and reached the top of the grain mass after 245 hours of active chilling at aproximately the same airflow rate and temperature as the previous year. Once again, there were some issues with the grain chiller so the equipment was left running longer until July 12th, 2016, for a total of 384 hours (Figure 3).
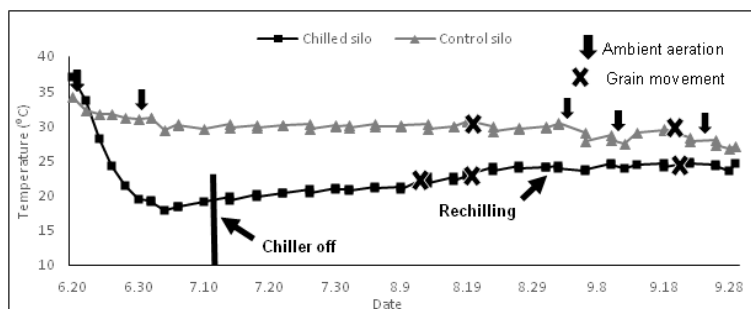
Figure 9. Grain temperature profile (°C) of the grain mass inside the Control and Chilled silo from June 20th to Sep. 29th, 2016 in Farmer's Cooperative, Kansas, U.S.A.

The additional temperature/RH sensors placed in the chiller outlet and insulated ducts in 2016 showed that the temperature of the chilled air coming out of the grain chiller was 12.5°C and increased by an average of 3°C in the transition parts. As well, the RH coming out of the grain chiller was 85% and decreased by an average of 13% in the transition parts.

During the chilling period, the average ambient temperature was 26°C, with 16.5°C and 38°C as minimum and maximum, respectively. The average relative humidity was 63.8%, with 22.7% and 91.2% as minimum and maximum, respectively.

Due to the high ambient temperature during most of July (over 32°C), the issues with the grain chiller, and the constant movement of grain, it was difficult to maintain the temperature of the grain below the optimum insect development threshold (25°C to 33°C) (Fields, 1992), so a rechilling

treatment was proposed by early-September, but the issues of the grain chiller persisted and the rechilling cycle did not have much of an effect on the grain temperature.

In the Control silo the initial grain temperature was 34°C. The aeration fans were activated using the same criteria as the previous year. The total fan run hours were 371 and the lowest temperature achieved was 25°C by late September.

In 2016, the average MC inside the Chilled silo was 10.2% and did not change significantly, while in the Control silo the average MC decreased significantly from 10.6% to 10.0% in the last sampling date (September). This was about the same tendency observed in 2015.

During the night of September 29th, one of the eaves from the Chilled silo cracked and a side of the silo split open. Given the incident it was decided to terminate the trial on this date.

Effect of grain chilling on insect reproduction and survival

During the 28 days the bioassays were inside the silos in 2015, the average temperature in the grain surface and fan transition part of the Chilled silo was 19°C and 17°C, respectively, while in the Control silo it was 27°C and 25°C, respectively. The cooler temperatures inside the Chilled silos significantly slowed down the total progeny development of LGB and RFB compared to the progeny observed in the Control silo (Table 1).

The temperatures of 17°C and 19°C in the top of the grain mass and transition part of the Chilled silo, respectively, are considered "safe" since population growth is almost insignificant at these temperatures (Navarro et al., 2002).

Table 2. Total progeny number (mean±SE) of adults of LGB and RFB for 2015 bioassays located 0.3 m below grain surface and in fan transition parts of the Chilled and Control silo for 28 days.

Year	Insect species	Location in the silo	Silo	
			Chilled	Control
2015	LGB	0.3 m below grain surface	2.3 ±0.7[B]	974.3 ±33.7[A]
		Transition	1.0	768.0
	RFB	0.3 m below grain surface	5.3 ±1.4[B]	21.3 ±5.6[A]
		Transition	0.0	7.0

[A, B] Mean values with the same letter within the same line are not significantly different by Tuckey's test (p>0.05).

The difficulty to maintain temperatures below 20°C in the Chilled silo during 2016 due to issues with the grain chilling unit, higher ambient temperatures and constant loading of warm wheat from the field during the trial, did not allow a significant difference to be observed between the insect progeny of the Chilled and Control silo. The average temperatures inside the Chilled and Control silo during the 68 days the bioassays were inside the silos were 23°C and 31°C, respectively. Although the average temperature inside the Chilled silo was lower, the fast temperature rise due to several issues previously mentioned caused an acclimation effect that basically eliminated the cooling effect on the development rate. According to Burks et al. (2000), if the temperature increases after the insect has been exposed to non-lethal cold temperatures, it may recover from the mild cold-injury effect.

Insect populations in the Chilled and Control silos

The main insect pests found in the probe traps of both silos were: flat grain beetle (FGB) *Cryptolestes* spp., flour beetle (FB) *Tribolium* spp. The populations of these genera increased faster in the Control silo than in the Chilled silo in both years (Table 2), even though the temperature difference between the silos was narrower in 2016.

Table 3. Total number of insects of main stored-product pests found in probe traps of Chilled and Control silo on Aug. 15th, Sep. 22nd and Nov. 20th, 2015, and Aug. 2nd, Sep. 20th and Sep. 30th, 2016.

Silo	Insect species	2015			2016		
		08/15	09/22	11/20	08/02	09/20	9/30[x]

	FGB	27	84	131	9	171	ʸ
Chilled	FB	1	80	74	5	78	ʸ
	WEV	0	10	270	1	29	ʸ
	FGB	33	3280	1236	44	719	328
Control	FB	4	1350	142	13	722	1241
	WEV	1	0	1	0	8	12

ʸProbe traps lost when the Chilled silo cracked.

ˣTrial terminated earlier due to the accident in Chilled silo.

The main internal insect pest found in the probe traps were weevils (WEV) of the genus *Sitophilus* spp. More individuals of this genus were found in the Chilled silo than in the Control silo and the reason could have been that this genus was in competitive disadvantage with the high populations of FB and FGB.

Grain quality evaluation

The grain quality results indicate that there was no change in grade throughout the trials in either of the silos (Table 3). This means that there was no noticeable quality deterioration of the wheat during the duration of the trials.

In 2015, one IDK was identified in the Chilled silo in each sampling date which indicates that, although this damage was present before the grain chiller was turned on, it did not increase, probably due to the chilled temperatures (Table 2).

Table 4. Grain quality analysis of wheat stored in Chilled and Control silos from samples taken on Aug. 15th to Sep. 22nd, 2015, and July 1st to Sep. 27th, 2016.

Year	Sampling date	Chilled silo			Control silo		
		July	August	September	July	August	September
2015	Insect Damaged Kernels (#/100 g)	-	1.0	1.0	-	0.0	0.0
	Grade	-	1	1	-	1	1
2016	Insect Damaged Kernels (#/100 g)	0.0	0.0	0.0	0.0	0.0	1.0
	Grade	1	1	1	1	1	1

In 2016, IDK increased in the Control silo after three months of storage, while there was no detection in the Chilled silo. This means that, although internal-feeding insects were detected in both silos according to the results of the probe traps, it seems like the slightly lower grain temperature in the Chilled silo discouraged the insect damage.

Power consumption and cost analysis

The total power consumption and cost per ton of the ambient aeration and grain chilling trials are shown in Table 4.

In both years, the cost of grain chilling nearly doubled that of ambient aeration. These results agree with those reported by Quirino et al. (2013). Nevertheless, it has to be taken into consideration that the temperature of the Chilled silo was taken down to levels considerably lower (approximately 17°C) in only 175 hours in 2015 and 245 in 2016, with basically no considerable shrinkage loss.   It also has to considered that the cost analysis did not include fumigation cost as these were not required during the trials, but previous research trials have demonstrated that grain chilling is economicallly feasible compared to the use of ambient aeration plus fumigation. Maier et al. (1997) determined that the annual operating cost for chilling wheat from 25°C- 27°C to 15°C- 17°C in 182-240 hours would lower the costs by 1.48 $/t compared to in-house fumigation combined with ambient aeration.

Table 5. Power consumption (kWh) and metric ton ($/t) for running chilling and ambient aeration in 2015 and 2016.

Year	Silo	Average Load (kWh)	Hours of Operation	Total Energy Consumption (kW)	$/t[3]
2015	Chilled	28[1]	314	8,794	0.54
	Control	15[2]	308	4,620	0.28
2016	Chilled	28[1]	384	10,752	0.66
	Control	15[2]	371	5,565	0.34

[1] Average load of system: 1 centrifugal fan of 7.5 kW+ 2 axial fans of 950 W/ea+ 2 compressors of 9.325 kW/ea.
[2] Two centrifugal fans of 7.5 kWh/ea. connected to the Control silo.
[3] Based on an average cost of 0.084 $/kWh

Conclusions

The grain chiller GCH-20 was capable of lowering the temperature of 1,350 t of wheat from 28°C-39°C to approximately 17°C in less than 250 hours. The shrinkage loss with the grain chilling treatment did not significantly increase in either of the trials. Using ambient aeration, the average grain temperature inside the Control silo remained over 25°C all summer during both years and there was a significant shrink loss of approximately 0.5%.

The stable low grain temperatures of 17°C in the Chilled silo in 2015 significantly slowed down the development rate of RFB and LGB, but in 2016, the increasing trend of the grain temperature in the Chilled silo from 17.6°C to more than 25°C avoided this effect to be observed.

The lower grain temperatures in the Chilled silo decreased drastically the progeny development of FGB and FB in both years. The most common internal-feeder found in the probe traps was the WEV, although proof of increasing levels of IDK were only found in the Control silo in 2016.

The cost analysis of the trials, based only on the power consumption of both aeration strategies, showed that the cost of grain chilling is between 0.26 $/t- 0.32 $/t higher than ambient aeration.

Acknowledgements

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Does it really work? 25 years biological control in Germany

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Stored-product protection, museum environments as well as protection of materials are growing fields of application of macro-organisms for biological control in Central Europe during the last 25 years.

Material destroying pests

Stored-product pests may destroy materials as well, either on their way to pupation sites or because the materials contain ingredients suitable for development. This initiated the interest in biological control of these pests in museums and other environments with cultural heritage items, as well as research in specific natural enemies of museum pests.

Spider beetles are mainly scavengers feeding equally on plant or animal materials. Beside their natural habitats, a number of species infest historic houses feeding on organic insulation materials and become a nuisance in residences (Howe, 1959). Moreover, spider beetles were found to infest historic books and herbaria (Gamalie, 2006). A number of spider beetle species were found to be suitable hosts for the larval parasitoid *Lariophagus distinguendus*, such as *Ptinus* spp. (Kaschef, 1955), *Gibbium psylloides* (Czenpinski, 1778) (Kaschef, 1961) and *Niptus hololeucus* (Faldermann, 1835) (Schöller and Prozell, 2011). Spider beetles are difficult to control in houses because the larvae develop hidden within walls and in dead floors, and no monitoring devices are available. In recent years, *L. distinguendus* was released against the hump beetle *G. psylloides* and the golden spider beetle *N. hololeucus* in Germany by pest control companies and became a regularly applied control technique (Kassel, 2008).

Larder beetles (Dermestidae) are among the cultural heritage pests most difficult to control by chemical means. Two approaches for biological control were tested so far, the control by a parasitoid naturally occurring in houses, and the control by a generalist predator transferred from the stored-product environment. The parasitoid *Laelius pedatus* (Say, 1836) (Hymenoptera: Bethylidae) is a gregarious ectoparasitoid of several larder beetle species including *A. verbasci* and *T. angustum*. The shiny black wasps measure 2 to 3 mm in length. During its life span a female wasp paralysed 74 ± 20 larvae of *A. verbasci* (Al-Kirshi, 1998). The average number of eggs per female wasp and day was 1.42 ± 0.2 if larvae of *T. angustum* were used as host. Most egg-laying activity was observed at temperatures between 25° and 28°C, while no oviposition occurs at 15°C. A mated female lives 6 to 8 weeks at room temperature (Al-Kirshi 1998). This parasitoid is occuring spontaneously in Central Europe in buildings, but there are not studies on the biological control potential of laboratory-reared wasps in field trials.

Stored product pests

Biological control in stored products is commercialized since 1998. Most applications were against stored-product moths in bakeries, food processing industries, retail trade and private households, and against weevils in grain on farms. Fifty percent of the types of application are control of pyralid

stored-product moths. The reasons for this might be the fact that biological control of pyralids was the first commercialized application and is best known in the public, and/or the fact that *Trichogramma* spp. are hardly visible under practical conditions due to their small size. The adults of these egg-parasitoids are 0.3 mm long. They lay their eggs into lepidopteran eggs, preferring freshly-laid eggs. Upon hatching, the wasp larva consumes the content of the egg. It pupates inside the egg and emerges as an adult wasp. Adult wasps mate shortly after emergence. A female wasp will parasitize approximately 50 eggs in her life-span of 3 to 14 days. While foraging for moth eggs, the females are usually walking. Typically parasitized eggs fixed to a card are applied (Prozell & Schöller, 2003). These cards are placed on shelves and palettes. The cards can be stored at 8 to 12°C in the dark for seven days.

Habrobracon hebetor is a cosmopolitan idiobiont gregarious ectoparasitoid. It develops on larvae of many Lepidoptera, mainly members of the family Pyralidae (Schöller, 1998). Actually the number of hosts even increased, but this is probably due to the presence of different strains in fields and warehouses (Heimpel et al., 1997). Today, *H. hebetor* is recommended for biological control and it has been studied from the biological and demographical point of view (Eliopoulos and Stathas, 2008; Akinkurolere et al., 2009).

Anisopteromalus calandrae is one of the most frequently found parasitoids in stored grain, and it is widely distributed. It has been reported as natural enemy of the following pests: *S. granarius*, *S. zeamais*, *Rhyzopertha dominica* (F.), *Stegobium paniceum* (L.), *L. serricorne*, *A. obtectus* (Say), and *Callosobruchus maculatus* (F.) (Williams and Floyd, 1971; Arbogast and Mullen, 1990; Ngamo et al., 2007). *A. calandrae* is a primary, idiobiont ectoparasitoid attacking the late larval stages and early pupae of beetles inside seeds and cocoons (Shin et al., 1994).

Lariophagus distinguendus has been reported as potential agent for biological control for a wide number of beetles that infest stored agricultural products (Steidle and Schöller, 1997): *S. oryzae* (Lucas and Riudavest, 2002), *S. granarius* (Steidle and Schöller, 2002), (Wen and Brower, 1994), *R. dominica* (Menon et al., 2002), *L. serricorne*, *S. paniceum* and *A. obtectus*. It is a solitary ectoparasitoid of larvae and prepupae.

In the meantime biological control was adopted by the conventional sector after its start in the organic market. Moreover, many pest control operators are using natural enemies. On the one hand, customers are demanding pesticide-free solutions and products, on the other hand the evaluation of non-chemical alternatives prior to the application of synthetic insecticides is regulated by law.

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Storage of Mungbean in Hermetic PVC Tank

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Abstract

This research was carried out to evaluate the effect of hermetic storage on quality of mungbean. About 260 kg of mungbean samples were stored in an especially design 350 L capacity hermetic PVC tanks (hermetic tank) and non-hermetic PVC tanks (control tank). Hermetic PVC tanks were closed air-tightly. All tanks were randomly placed in a warehouse. Each hermetic and control PVC tanks were artificially infested by 50 unsexed *Callosobruchus chinensis* kept in 4 glass jars containing 100 g of mungbean and jars were dipped in four different depths. The gas concentrations in the tanks were monitored up to 6 months intervals. Percentages of germination, moisture content, and grain damage were evaluated at the end of the storage. The oxygen content of hermetic samples was dropped to 11±1.2% and carbon dioxide content was increased up to 7±0.7% within 6 months of storage. Live insects of *C. chinensis* were not found in hermetic samples after 6 months but abundant population of *C. chinensis* was found in the control PVC tank just after one month. After 6 months, germination percentage of the mungbean samples stored in hermetic tanks had decreased from 95±3% to 82±4%, whereas it was decreased from 95±3% to 47±7% in control tanks due to grain damage. Percent grain damage of the hermetic sample was only 4.5±1% compared to the heavy insect damage of the control samples. Moisture content of hermetic samples remained unchanged compare to the control.

Keywords: Hermetic storgae, PVC tank, Mungbean, *Callosobruchus chinensis*

Introduction

Nearly 30-40% of cereals and grain legumes harvested in Sri Lanka are stored by farmers for consumption, seeds and future sale for a period of three to nine months (Adhikarinayake, 2006). Mungbean, cowpea, black gram, and soybean are major legume grain grown in Sri Lanka. These grains are mainly stored in polybags causing insignificant postharvest loss about 15% within 3-4 months (Sartaj and Ekanayake, 1991), but grain damage can be high as 68% after 4 months of stored in polysack bags (Prasantha et al., 2014a). Mungbean (*Vigna radiate* (L.) is cultivated around 9760 ha mainly by dry-zone farmers in Sri Lanka are yielding around 14000 MT per annum. Legumes are an inexpensive source of dietary protein supplement for more than 67% of Sri Lankans that consume them as an alternative to animal protein. However, local production is insufficient for local consumption and more or less 7000 MT is imported to Sri Lanka every year. Munbean and cowpea are highly susceptible to bruchids damage from pests such as *Callosobruchus chinensis* (L.) and *Callosobruchus maculates* (Fabricus) which are commonly known as southern cowpea weevil and cowpea weevil respectively. *C. chinensis* is the most common bruchid species that infests stored grain legumes in Sri Lanka. Mostly under poor storage conditions, *C. chinensis* attacks on stored grain legumes cause substantial losses to both quality and quantity. Although the infestation of grain legumes by bruchids begins in the field before seed maturation (Huignard et al., 1985), they reproduce rapidly in poor storage condition. New generation of weevil immerges in every 28 days (Prasantha et al., 2002) and may cause losses up to 12-15% in 2 months of storage. If infestation of weevil is not controlled, complete grain damage (100%) of mungbean could occur within 6 months where mungbean stored in common storage (in polybag) condition (Prasantha et al., 2014a). As a result, farmers try to sell their grains at low prices or apply hazardous insecticides to protect their stored grains soon after harvesting. Phosphine fumigation is not recommended at the farm level due to risk and safety issues in the application. Quality deterioration of mungbean is unavoidable under common storage in polybag. Hard-to-cook (HTC) defect is well-known quality deteriorating problem of mungbean which is related to the increase time of cooking due to poor storage (Prasantha et al., 2014a). The other problem is the loss of stored grain viability or percentage of seed germination due to insect infestation and development of HTC characteristics. Therefore, an effective storage method is necessary to prevent the insect infestation and avoid the development of HTC.

Hermetic storage is an airtight grain storage technique for controlling stored-product pests and avoid the development of HTC (Sanon, et al., 2011; Prasantha et al., 2014a). The respiration of insects, microorganisms and grains hinder the growth of insects as a result of creating high carbon dioxide (CO_2) and low oxygen (O_2) in the storage environment (Murdock, 2012). This indicates the importance of hermetic storage where early infestation can be avoided without substantial damage to the stored grains. According to previous studies hermetic storage of grain legumes in PET bottles containers and plastic bags can successfully control the damage of legume grains by bruchids (Murdock, 2012; Guenha et al., 2014; Prasantha et al., 2014b) more than 6 months. Although it is a relatively simple method of storage of mungbean, farmers are reluctant to adopt the method due to lack of appropriate plastic bags and handling problems of the bags. However, the farmers are preferring to use type of larger storage tanks where they can store larger quantities of grain with minimum space and low cost of larger numbers of bag handling.

The other major problem is the lack of information on final quality of stored mungbean such as germination and cooking quality. Therefore, it is important to study the applicability and effectiveness of hermetic storage on preservation of mungbean. This research was carried out to evaluate the suitability of bin type PVC hermetic tank for storage of mungbean to minimize postharvest losses and thereby to improve the seed germination and minimizing the HTC characteristics of mungbean.

Materials and Methods

The research was carried out at the "Palvehre" seed farm, Department of Agriculture, Sri Lanka. Mungbean samples (*Vigna radiate* (L.) Wilczek) were obtained directly from the field 2-3 weeks after harvesting and sun dried to moisture content about 12±1% (w.b) before storage. Approximately 260 kg of mungbean sample stored in an especially design 350 L capacity hermetic PVC tank (hermetic tank) and non-hermetic PVC tanks (control tank). Hermetic tank was air-tightly closed using thread seal with airtight PVC lid and covered by high vacuum silicon grease. Control tank was closed without hermetic sealing by PVC lid (Fig. 1). The control tank was also allowed to infest naturally similar to the common aerated storage.
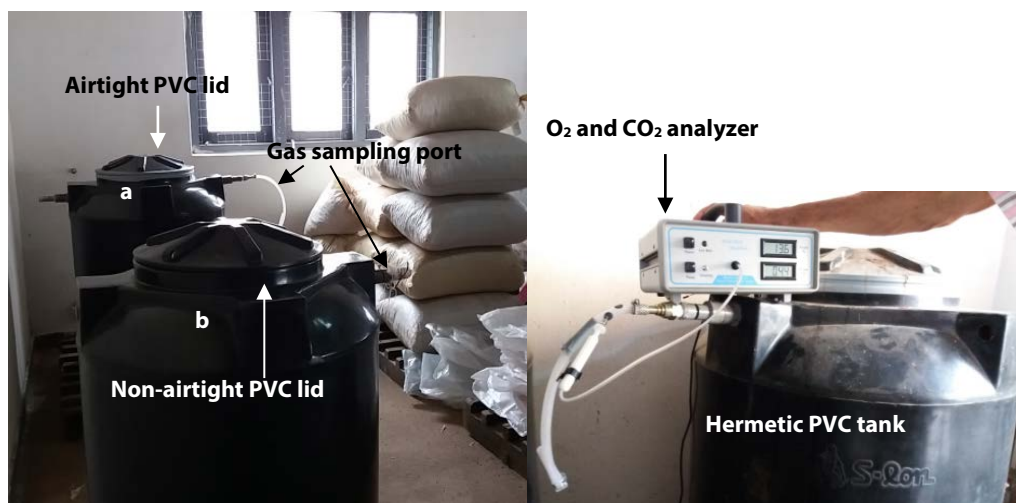

Fig 1. Storage of mungbean in PVC tanks (a) hermetic tank (b) control tank and (c) method of measuring of internal gas content

Biological tests

Prior to the experiment, 5 kg of mungbean sample was stored in a freezer (-18 °C) for about 2 weeks to destroy any hidden infestations of insects. Adults of *C. chinensis* were obtained from the same store and cultured on mungbean at the Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka, for 3 months. About 500 g of mungbean samples was obtained and stored in a 750 ml glass jar. The sample was artificially infested with 50 unsexed freshly emerged (1-3 days) adults of *C. chinensis* and the jar was covered with fine wire mesh. The samples were kept one week at ambient condition (26±1 °C and r.h. 78±3%) for egg laying and then weevils were removed from the samples through sieving. During grain loading, 3 artificially infested jars were buried inside each PVC tank at 3 different depths (i.e. bottom, middle and top) of each PVC tank. Number of adults emerged from the samples were determined by sifting three sample jars after 196 days of storage in hermetic and control tanks.

Hermetic storage

A rubber septum was glued onto the gas sampling rubber tube (10 mm internal diameter) as gas sampling port immerging from the centre of the PVC tank (Fig 1). Gas sampling port of the PVC tank pierced with a needle connected to the gas analyser (Quantek modle-902D, USA) to determine the percentage of O_2 and CO_2 contents. The initial O_2 and CO_2 content (atmospheric) were adjusted as 20.7% and 0.2% respectively. All experiments were conducted for 196 days in grain storage warehouse conditions. The temperature and relative humidity (r.h.) in these storages were 28±2 °C and 73±5%, respectively. Gas samples were measured almost at every 30-40 days intervals over the

period of 196 days. About 3-4 gas samples were withdrawn from each PVC tank in every test. This study was repeated 3 consecutive times during 2015-2017 at the same period (November-May) of each year. Altogether six PVC tanks were used in equal numbers for the control and hermetic study.

Storage grain quality

The m.c, of the initial, control and hermetic samples was determined (% w.b) by forced-air oven drying at 105 °C for 24 h. Grain germination of the initial, control and hermetic mungbean samples was tested after 196 days (ISTA, 2006). Samples of 100 mungbeans from each storage method were germinated on wet paper towels. Percent germination was calculated as the number of grains showing plumule and radicle emergence after 24 h of incubation at room temperature of 28±2°C. The HTC characteristics was evaluated using minimum cooking time (Singh et al., 1991). Two grams of mungbean samples were taken into a boiling tube and cooked by adding 20 ml of distilled water in a boiling water bath. The cooking time was determined by removing few grains at different time intervals during the cooking. The gains were pressed in between two glass slides until uncooked core was disappeared. This experiment was repeated for 4 times.

Storage losses

Percent grain damage was estimated using 50 g samples (Boxall, 2002) at the end of storage method using the following equation. Altogether 30 replicates were used to estimate the storage loss of this study.

$$Grain\ damage\ \% = \frac{N_d}{N_d + N_u} \times 100$$

Where;
Nd = Number of damaged grains in the sample
Nu = Number of undamaged grains in the sample

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SAS (1990) statistical package using PROC GLM procedure. Duncan's multiple range tests was used to separate means when ANOVA showed significance at $P < 0.05$. Other descriptive statistics and graphical methods were used to present the data with time and storage when appropriate.

Results and Discussion

Biological tests

After one month of storage, the large number of insects emerged from the control tank were identified as *C. chinensis*. It is important to note that there was no other species of bruchids were found in the samples. Artificially infested mungbean samples stored in the control tank were found with high number of insect emergence holes and an abundant number of *C. chinensis* progeny. However, 0.5±0.25% grain damaged was observed in the initial samples obtained from the field (Table 1). This indicates that mungbean samples obtained directly from the field had already been infested by *C. chinensis*. Generally, mungbeans are highly susceptible to damage by bruchids. According to Mutungi et al. (2014) ineffective methods of storage cause a substantial loss in quality and quantity of grains.

Storage losses

Grain damage had increased up to 98.5±1.5% in the control samples after 196 days of storage. It was remained significantly low at 4.5±1% (P<0.05) in the hermetic samples, which was a 96% reduction of grain damage compared to that in the control samples. Mortality of *C. chinensis* was

recorded at 100% in mungbean sample stored in hermetic conditions for 196 days. The number of progeny emergence of weevils was significantly lower (11±3.0) in the artificially infested samples stored in a hermetic tank. Prasantha et al. (2014b) noted more or less similar results of hermetic storage of mungbean.

Storage grain quality

Initial moisture content of mungbean was 12.2±0.1% (w.b.). Control samples showed comparatively higher moisture content than hermetic samples but no significant difference (P>0.05) was noted in the moisture content between hermetic and control mungbean samples. Comparatively high moisture content detected in the control samples may be related to the accumulation of metabolic moisture (both weevils and grains) and the absorption of atmospheric moisture.

Germination of the initial mungbean sample was 95±3% and it was significantly reduced ($P < 0.05$) to 46.8±7% in the control samples compared to the 82±3.8% germination remained in the hermetic samples after 196 days (Table 1). However, there was a 14% reduction of germination observed in mungbean stored in the hermetic tank compared to initial samples. Adikarinayake et al. (2006) reported that paddy stored in a hermetically sealed bin has completely lost its germination percentage after six months. Similar to this study, Prasantha et al. (2014a) also showed that germination of mungbean stored in the hermetic condition decreased slightly compared to initial sample after 6-12 months. Hamel, (1989) reported that high CO_2 storage can reduce the seeds viabilities of wheat, rape seed, soybean, and onion. The possible reason for this reduction might be the lowering of physiological and biochemical activities in mungbean due to development of HTC characteristics with ageing.

Table 1. Number of adults emerged, moisture content, percent germination, percent grain damage and minimum cooking time of initial, control and hermetically stored mungbean samples in PVC tank under ambient conditions.

Test parameters	Initial	PVC tanks	
		Control	Hermetic
Number of progeny	0	TNC[†]	11±3.0
Moisture (w.b %)	12.2±1.0[a*]	13.4±1.2[a]	12.7±0.7[a]
Germination (%)	95±3.0[c]	46.8±7.0[a]	82±3.8[b]
Grain damage (%)	0.5±0.25	98.5±1.5[b]	4.5±1.0[a]
Cooking time (min.)	25±1.2[a]	33±2.0[b]	26±1.0[a]

All data represent the mean ± SD of three-five replicates
*Values followed by the different small letters in each raw significantly different at $P < 0.05$
[†]TNC = Too numerous to count

Cooking time of hermetically stored mungbean samples did not show any significant change (P > 0.05) compared to the initial samples (Table 1). Cooking time of control samples significantly increased (P < 0.05) from 25±1.2 min to 33±2.0 min which was about 35% increase compared to the initial cooking time. Gradual development of high cooking time with storage is indication of grain hardness development and it is commonly known as HTC characteristics. Kon and Sanslulck (1981) reported that cooking time of common beans increased by about 5-fold when bean sample was stored at high r.h. and high temperature conditions. In contrast to finding of this study, Nasar-Abbas et al. (2008) reported HTC characteristics of faba bean increased significantly when beans stored in airtight bags. However, this study has revealed that hermetic storage can successfully delay the development of HTC in mungbean at least by 6 months.

Hermetic storage

A significant reduction of ($P < 0.05$) of O_2 and increase of CO_2 was observed in the hermetically stored mungbean samples in PVC tanks (Fig 2). Initially, the O_2 and CO_2 contents approximately dropped below 11% and increased more than 6% respectively, within the first 38 days after storage in

hermetic tank. Throughout the storage period, the O_2 content dropped to an average of 11.2±1.2% % and CO_2 increased to 6.8±0.7% in the hermetic PVC tank. There were no weevils found in the hermetic samples at the end of the storage period. The drop of atmospheric O_2 content in the hermetic PVC tank was approximately 48% compared to control tank sample, and there was no change in the gas composition detected in the control PVC tank. Similar results were observed by Murdock et al. (2012) and our previous studies of hermetic storage (Prasantha et al. 2014a and 2014b). Although the respiration of mungbean is low, but high metabolic activity of weevils and their developing immature stage (larval/ pupal) inside the mungbean were the reasons for lowering the O_2 and raising the CO_2 contents of inter-granular atmosphere of hermetic stored grains (Murdock et al., 2012; Navarro, 2012). The death of weevils may have occurred due to the low O_2 content and reduction of O_2 partial pressure within the inter-granular space during the storage period (Mbata et al., 2005). According to the data, successful developments of hermetic condition in the PVC tank without changing the gas composition during 196 days indicate that the suitability and sustainability of the hermetic PVC tank as a storage method for direct field application.
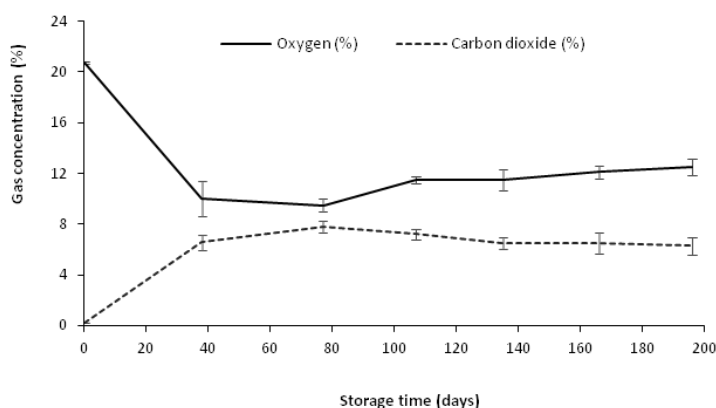

Fig 2: Changes in gas concentration (%) of hermetically stored mungbean samples in PVC tank. Data used are means ± SD of three tanks.

Conclusions

Storage damage of mungbean was mainly caused by *C. chinensis*. Hermetically storage of mungbean in PVC tack was successfully reduced the weevil development and grain damage. Although a slight change in percentage of germination was observed in the hermetically stored samples, moisture content and cooking time of beans did not change with the storage in hermetic tank. The increase of CO_2 and drop of O_2 contents in hermetic samples indicated the successful development of the hermetic condition within the stored mungbean in hermetic PVC tank. We conclude that hermetic storage can prevent the development of HTC characteristics and postharvest loss of mungbean.

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Combination of Mating Disruption and parasitoid Habrobracon hebetor against Plodia interpunctella in a chocolate factory

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Abstract

A field experiment of 4 years' duration was carried out to evaluate the efficacy of combining the mating disruption (MD) formulation Dismate ZETA (9Z,12E-tetradecadienyl acetate), with the parasitoid *Habrobracon hebetor* against the Indianmeal moth *Plodia interpunctella* in a chocolate factory. The experimental period began early in 2011 and ended in late 2014. Begane Dismate dispensers were placed in the facility from 2011 to 2014 and *H. hebetor* was released in 2014. Pheromone-baited traps were used to monitor the flight activity of the male

moths and oviposition Petri dish cups were placed to assess the progeny production of *P. interpunctella* females. Following the start of MD, a decrease in the number of *P. interpunctella* males caught in monitoring traps was observed from 2011 to 2013. A further decline in the moth population was noted in 2014, when MD was combined with the release of parasitoids. The presence of larvae in the oviposition cups was occasionally observed throughout the monitoring period, from 2011 to 2014. This study demonstrates that the combined system of MD and parasitoids is an effective and reliable technique that can be used to successfully control *P. interpunctella*.

Keywords: food processing, Integrated Pest Management, *Plodia interpunctella*, mating-disruption, *Habrobracon hebetor*

Introduction

The use of pheromones for suppressing pest populations through mating-disruption (MD) has been widely studied for stored-product Lepidoptera. For stored-product moths such as *Ephestia* spp. and *Plodia interpunctella* (Hübner), a single pheromone compound, known as TDA [(9Z,12E)-tetradecadienyl-acetate] can act as a male attractant (Trematerra, 2012; Trematerra et al., 2013). The enclosed environment of storage facilities provides an ideal area for the application of MD, given that the sources of external infestation in this environment are limited (e.g., the introduction of infested raw materials or the immigration of mated females from untreated areas) (Trematerra and Fleurat-Lessard, 2015). For instance, in a recent study, Trematerra et al. (2011) illustrated the reduction in the population of various Pyralidae following the use of MD dispensers in several areas and facilities in Europe, which clearly indicated that this technique can be of widespread use.

MD can only lead to a reduction in chemical treatments, which however, remain necessary, because males of moths such as *P. interpunctella* can inseminate on average 6 females in their lifetime. Moreover, females need to be mated only once per lifetime to produce their full number of viable eggs. Consequently, a few successful copulations in an environment under MD treatment will guarantee the survival of the population. Therefore, the application of pheromone-based IPM tools should be accompanied by additional nonchemical measures, such as cleaning and sanitation, especially in critical areas, such as the inside of the machinery and around raw materials and packaging areas. More recently, it has been suggested that combining and integrating different management tools and the careful selection and timing of different approaches, together with an understanding of pest behaviour and ecology can result in greater effectiveness (Trematerra, 2013). Another approach to overcome the challenges faced by the stored-product industry due to stored-product insects is the integration of pheromone-based IPM tools with biological control, like the parasitoid wasp *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) (Ghimire and Phillips, 2010). *Habrobracon hebetor* can parasitize all larval instars of *P. interpunctella*, but significantly fewer early instars are paralyzed and reproduction requires last-instar larvae (Akinkurolere et al., 2009). In the past, various applications of *H. hebetor* in stored products have been investigated, for example, in grain spillage (Press et al., 1982), packaged products (Cline et al., 1984; Adarkwah et al., 2014), bulk peanuts (Brower, 1990) and bulk grain (Adarkwah and Schöller, 2012).

MD targets *P. interpunctella* adults, whereas the parasitoid *H. hebetor* should target *P. interpunctella* larvae. The purpose of this 4-year study (2011–2014), conducted in a chocolate factory, was to analyze the effectiveness of MD for the first time and to evaluate the combination of MD and the release as parasitoid *H. hebetor*.

Materials and Methods

The chocolate factory is located in Southern Italy, in the area of Ospedaletto d'Alpinolo (Campania region). In the factory, dried fruits were stored and processed and chocolate food was produced. The factory was constructed from concrete, and contained 3 floors with the first floor being dedicated to food production and having an area of about 2000 m2 and a height of 5 m (about 10000 m3 in total).

MD treatments

From 2011 to 2014, the pheromone component TDA [(9Z,12E)-tetradecadienyl- acetate] (Dismate, Russell IPM, Deeside, UK), was used for MD. Each cellulose acetate MD dispenser was baited with 100 mg TDA. In 2011, MD dispensers were placed in a small area of the chocolate factory (of about 320 m^2), but covered the entire chocolate factory (2000 m^2) from 2012 to 2014.

In 2011, MD dispensers were placed from May to December; in 2012 and 2013, MD dispensers were installed all-year round. In 2014, MD dispensers were put in place between July and December, during P. interpunctella adult flight activity. In 2011, 6 MD dispensers were installed. The MD dispensers were replaced with new ones every 2 months; in 2012, the number of MD dispensers was increased to 25, resulting in one dispenser per 50 m2 throughout the entire chocolate factory area.

Parasitoid release

During 2014, adults or pupae of *H. hebetor* (from Biologische Beratung Ltd., Berlin, Germany) were released within the chocolate factory. Starting from April 2014, 40 units containing 30 pupae or 30 adults each were released monthly (April 7, May 9, June 25, July 21, August 20, September 22, and October 19). Consequently, about 1200 pupae or adults were released monthly, representing a total of ca. 8400 individuals of *H. hebetor*.

Monitoring of *P. interpunctella* adult males

P. interpunctella adult males were monitored (as a control) by 7 pheromone-baited sticky traps (X-lure R.T.U. Combo 4, Russell IPM), each baited with 1 mg TDA. The traps were checked every 7 d. The TDA lures were replaced at 2 monthly intervals. The traps were placed at a height of 2.0–2.5 m above the ground. Monitoring started on March 31, 2011 and ended in December 2014. The following monitoring periods were analyzed: from April 11 to December 3, 2011; from May 14 to October 14, 2012; from June 10 to December 15, 2013; and from February 17 to November 9, 2014.

Monitoring of *P. interpunctella* oviposition

A Petri dish cup containing 15 g crushed peanuts was placed near each pheromone monitoring trap, which was used as a control oviposition trap. Seven oviposition Petri dish cups were placed throughout the entire facility (Cups: Cu1–Cu7). The cups were replaced on each trap-check date (i.e., at 7-d intervals), and the old cups were brought to the laboratory, where they were placed in incubators at 27.5 °C and 70% relative humidity after 45–60 d, the Petri dish cups were examined for emerging individuals in most cases, larval stages and usually last-instar larvae.

Data analysis

he number of *P. interpunctella* males caught in the traps during 2011–2014 was analyzed using the Duncan test and compared using Kruskal–Wallis one-way analysis of variance on ranks, and the means were separated using the Tukey's Test (P < 0.05) (using SigmaStat software, San Jose, CA, USA).

Results

The number of *P. interpunctella* males that were trapped in different areas of the chocolate factory between 2011 and 2014 is reported in Figures 1 and 2. Figure 2 and Tables 1 and 2 depict the capture trends from 2011 to 2014 in the pheromone monitoring traps. The catches obtained during the 4 years differed, and a progressive decrease in the number of *P. interpunctella* caught from the second to the fourth year of monitoring was observed. In all years of the experiment, an increase in the number of individuals caught during the hottest months (late spring and summer) was observed. During the cold months, low temperatures negatively affected the population of *P. interpunctella*, resulting in fewer trapped adults. From 2011 to 2014, the monitoring traps caught 2127 moths, and

during 2011, 2012, 2013, and 2014, pheromone traps caught 706, 784, 416, and 221 moths, respectively.

The mean catches of individual traps from 2011 to 2014 are presented in Table 3. There was a significant effect of trap location on the number of moths caught (Kruskal– Wallis one-way analysis of variance on ranks, H = 23.568, df = 6, P < 0.001). However, only traps 2 and 3 caught significantly more moths than trap 7. Trap 2 caught the highest number of *P. interpunctella* individuals (mean ± SD 127.50 ± 41.66), whereas trap 7 caught the fewest individuals (20.25 ± 1.66). This might be due to their location in the chocolate factory; trap 2 was located in the packaging area, which was characterized by windows and a door that can facilitate the migration of moths inside, which are attracted by odors of the sweet products. Traps 7 and 4 were placed in the production area of nougat, a seasonal sweet; when the machinery is operational, the temperature is high enough to impede colonization of the area by moths.

The number of *P. interpunctella* individuals trapped during the whole period of MD and MD + parasitoid experimentation in 2011–2014 decreased in all traps. The overall trend of the weekly catches from 2011 to 2014 is shown in Figure 2. Following the start of MD, a decrease in the number of *P. interpunctella* moths caught in monitoring traps was observed and the number ranged from 706 in 2011 to 416 in 2013. A further decrease in the population was observed during 2014, when MD was combined with the release of *H. hebetor* parasitoids, with 221 moths being caught in the monitoring traps.

In 2011 and 2013, the number of *P. interpunctella* individuals captured was extremely high before the beginning of the MD programme, with a rapid decrease in adults observed soon after the placement of new MD dispensers. That situation was less evident during 2014 when *H. hebetor* was released, in addition to MD activity (Figure 2).
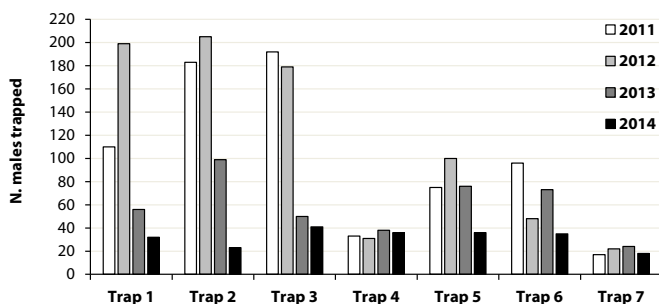

Fig. 1 *Plodia interpunctella* males caught by different pheromone traps 1-7 from 2011 to 2014.

The percentage reduction in moth captures between 2012 and 2013 reflected the increase in the number of dispensers (from 6 MD dispensers to 25 MD dispensers) and was 46.94% (Table 3). During the MD period in 2012, captures ranged from 22 (trap 7) to 205 (trap 2); in 2013, captures per trap ranged from 24 (trap 7) to 99 adults (trap 2) (Table 1). During 2014, when control with MD was only performed from July to November, and was associated with H. hebetor parasitoids, the number of trapped moths was lower, with a 46.87% reduction compared to that in 2013 (Table 4). In 2014, the moth captures per trap varied from 18 (trap 7) to 41 (trap 3) (Table 1). In 2014, the mean number of *P. interpunctella* individuals caught by each trap was significantly lower than at the beginning of the experiment (2011–2012) (Table 2).

The presence of larvae in the oviposition cups was observed occasionally during the monitoring period from 2011 to 2014. Except for positions Cu1, Cu3 and Cu5, all positions were infested at least once (Table 4).
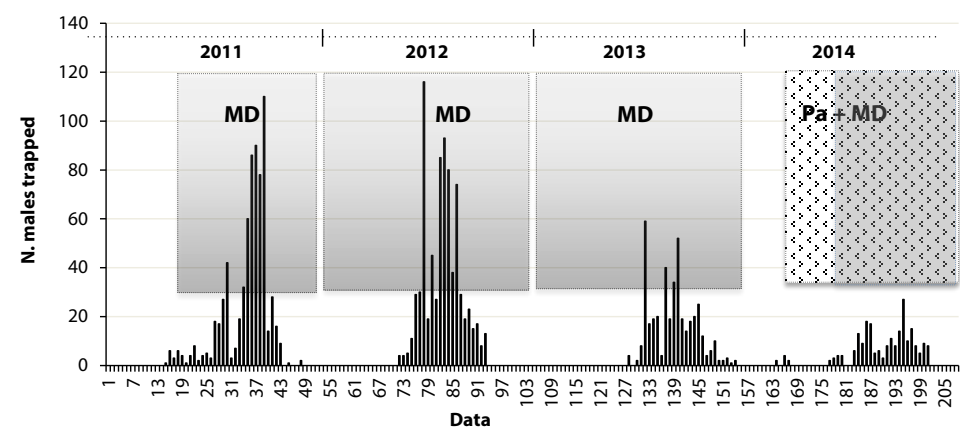
Fig. 2 Number of *Plodia interpunctella* males caught during 2011-2014 with indication of mating disruption treatments (MD) and parasitoids release (Pa).

Discussion

A reduction in the number of *P. interpunctella* moths in pheromone-baited monitoring traps was observed after placing MD dispensers at the beginning of the annual flight period of *P. interpunctella* (in May) and also after increasing the density of MD dispensers from a localized application to a general application (Figure 2). After placing the MD dispensers according to the protocol of 1 dispenser per 50 m2, the number of moths caught in the monitoring traps was drastically reduced and this trend was evident throughout the entire experimental period, particularly from 2012 to 2014. All sites showed a decrease in the number of trap catches after the introduction of MD. At 2 sites with low initial moth catches (trap 4 and trap 7 areas), the trap catches after the introduction of MD remained constant. In contrast, in areas with a higher initial population density, the reduction in trap catches was initially very low, especially in 2011, when MD dispensers were placed only in a small area of the chocolate factory. In 2013 and 2014, there was a clear reduction in the number of individuals caught in traps 1, 2, 3, and 5, especially in 2014, when *H. hebetor* parasitoids were used against *P. interpunctella* larvae, in addition to MD treatment; it is known that MD is more effective at low population densities (Trematerra et al., 2011; Trematerra, 2012).

Tab. 1 Mean number (± SD) of *Plodia interpunctella* adults caught in pheromone traps 1–7 from 2011 to 2014, per trap.

Trap	Area	Mean number of trapped moths (± SD)
1	Almond brittle	99.25 ± 37.04 ab
2	Packaging	127.50 ± 41.66 a
3	Dried fruit	115.50 ± 40.55 ab
4	Nougat	34.50 ± 1.56 ab
5	Ovens	71.75 ± 13.25 ab
6	Cooling	63.00 ± 13.54 ab
7	Nougat	20.25 ± 1.66 b

Data followed by a different letter differ significantly (Tukey's test, $P < 0.05$).

Tab. 2 Mean number (± SD) of *Plodia interpunctella* male adults caught in pheromone traps 1–7 from 2011 to 2014.

Year	Mean number of trapped moths (± SD)

2011	100.86 ± 25.58 b
2012	112.00 ± 30.71 b
2013	59.43 ± 9.57 ab
2014	31.57 ± 3.08 a

Data followed by a different letter differ significantly (Duncan test, $P < 0.05$).

Tab. 3 Changes in mean adult males trapped from 2012 to 2014 after MD and parasitoids (Pa)+MD treatments.

	Adult males trapped					
Traps		**Years**		**MD vs. MD**		**MD vs. Pa+MD**
	2012	**2013**	**2014**	**2012 vs. 2013**	**2012 vs. 2014**	**2013 vs. 2014**
1	119	56	32	- 52.94	- 73.11	- 42.86
2	205	99	23	- 51.71	- 88.78	- 76.77
3	179	50	41	- 72.07	- 77.09	- 18.00
4	31	38	36	+ 22.58	+ 16.13	- 5.26
5	100	76	36	- 24.00	- 64.00	- 52.63
6	48	73	35	+ 52.08	- 27.08	- 52.05
7	22	24	18	+ 9.09	- 18.18	- 25.00
1-7	784	416	221	- 46.94	- 71.81	- 46.87

Tab. 4 Chocolate factory 2011–2014, control dates with *Plodia interpunctella* infestation in oviposition cups (Cu1-Cu7), all other dates without infestation.

Petri dishes	Data	Cu1	Cu2	Cu3	Cu4	Cu5	Cu6	Cu7
2011	4.XI	-	-	-	yes	-	-	Yes
25.XI		-	-	-	-	-	-	-
2012	13.I	-	-	-	yes	-	-	-
17.II		-	-	-	yes	-	-	-
4.V		-	-	-	-	-	-	Yes
8.VI		-	-	-	-	-	yes	-
2013	12.VII	-	yes	-	-	-	-	-
6.IX		-	-	-	yes	-	-	-
2014	1.VIII	-	-	-	-	-	yes	-

During the MD application period (2011–2014), the moth density, overall moth activity and degree of infestation was remarkably lower than in previous years without such treatment. In addition, the chocolate factory manager reported that fewer customer complaints were made in 2014 than in 2011.

In Europe, stored-product moths are among the most serious pests in stored grain, in the retail trade, mills, the food processing industry and private households. The use of pheromones to control populations of stored-product moths has been demonstrated (Trematerra, 2012) and MD has proven successful against stored-product moths in commercial field settings (Ryne et al., 2001, 2006, 2007; Burks et al., 2011; Trematerra et al., 2011; Burks and Kuenen, 2012; Trematerra and Savoldelli, 2013). In addition, other methods that involve pheromones in mass trapping (Phillips et al., 2000; Trematerra & Gentile, 2010), attract-and-kill methods (Trematerra and Capizzi, 1991; Phillips et al., 2000; Nansen and Phillips, 2004; Campos and Phillips, 2013, 2014) and the auto-confusion system (Trematerra et al., 2011) have been successfully used to manage stored-product moths. However, in these cases, complete elimination of the moth infestations was never achieved. The monitoring of pyralid moths using pheromone traps is likely to be affected by MD. One alternative to pheromone traps for monitoring is the use of water traps; however, these have also been shown to have a control effect (Trematerra and Savoldelli, 2013). In this study, oviposition cups were used as an alternative approach to pheromone traps; however, the number of infested cups was too low to analyze the population trends based on the data.

In the present experiment, the release of the parasitoid *H. hebetor* against *P. interpunctella* larvae helped to reduce the residual moth population in the chocolate factory in 2014 (Trematerra et al., 2017).

In Italy, the use of parasitoids in food facilities is rare, and our study represents one of the first applications. In Europe, and particularly in Germany, parasitoids have been evaluated in commercial food-processing facilities since 1995, and they have been commercially available since 1998. In the United States, insect natural enemies were technically designated as insecticides, in order to be regulated, and then they were exempted from a requirement for a tolerance level in food.

Recently, Trematerra (2013) suggested that combining and integrating different management tools and the careful selection and timing of different approaches, together with an understanding of pest behavior and ecology can be more effective in pest control.

According to our results, the combination of MD and biological control can be used to control *P. interpunctella* infestation in a chocolate factory. These techniques must be considered as part of an Integrated Pest Management programme and should not be considered in isolation. MD is effective against *P. interpunctella* adults, but cannot control larval and pupal stages; in contrast, *H. hebetor* can attack larval instars, and could also be used against overwintering larvae in warehouses or food facilities, when environmental conditions are suitable.

The use of insect natural enemies and MD in an integrated approach against stored-product moths is an opportunity for the biologically based management of storage pests. MD combined with natural enemies has been shown to be promising, and might be especially relevant in the organic food industry when *P. interpunctella* and other pyralid moths are the target pests.

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Host-age preference of *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), a larval parasitoid of the lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)

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Abstract

The pteromalids (Hymenoptera: Pteromalidae) *Anisopteromalus calandrae* (Howard), *Dinarmus basalis* (Rondani), *Lariophagus distinguendus* (Förster), *Pteromalus cerealellae* (Ashmead) and *Theocolax elegans* (Westwood) are solitary larval ectoparasitoids used to suppress several species of stored-product insects that infest storage grains. We investigated host-age preference of *T. elegans* using no-choice laboratory experiments. Lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) larvae (9, 11, 13, 15, 17, 19, 21 and 23 days-old) in wheat grain kernel and cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) larvae (5–19 days-old) in cowpea beans were exposed to neonate *T. elegans* mated females to lay their eggs for two days. Our results showed that the highest number of parasitoids emerged from 23 days-old *R. dominica* larvae. The numbers of parasitoids emerged from 19, 21 and 23 days-old *R. dominica* larvae were statistically significantly different in experiments (F-test, 0.05). Progeny of *T. elegans* reared from *R. dominica* and *C. maculatus* larvae were either fully-winged (macropterous), short-winged (brachypterous) or wingless (apterous). Female *T. elegans* were rarely host-feeding on *C. maculatus* larvae. *Theocolax elegans* progeny were emerging from 14 days-old *C. maculatus* larvae only. We discussed insectary mass production of *T. elegans* for biological control.

Keywords: Biological control, *Callosobruchus maculatus*, host-age preference, *Rhyzopertha dominica*, stored-product insects

Introduction

Stored products such as grain, flour, legumes, tobacco and dried fruits have a value of more than one billion United States Dollar (USD) in developing countries (Eliopoulos *et al.*, 2002). However, these commodities frequently lose quality and quantity due to the action of stored-product insect pests. The important problems are damages by stored-product insect pests (Coleoptera) such as the larger grain borer *Prostephanus truncatus* Horn (Bostrichidae), rice weevil *Sitophilus oryzae* (L.) (Curculionidae), maize weevil *Sitophilus zeamais* Motschulsky (Curculionidae), khapra beetle *Trogoderma granarium* Everts (Dermestidae), rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Laemophloeidae) and saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Silvanidae) and some moths such as Angoumois grain moth *Sitotroga cerealella* (Oliver) (Gelechiidae), rice moth *Corcyra cephalonica* (Stainton) and Indian meal moth *Plodia interpunctella* (Hübner) (Pyralidae) (Arthur, 2010; Flinn*et al*., 2006; Hayashi *et al*., 2004; Johnson *et al*., 2000; Plague *et al*., 2010). Many countries have controlled stored-product insect pests using fumigation with methyl bromide and phosphine. However, the first mentioned compound has been banned (Credland, 2010) and resistance towards the second mentioned have been detected (Nayak*et al*., 2003).

Biological control is an alternative, environmentally friendly method of insect pest management. Parasitoids and predators are used to reduce stored-product insect pests because the natural enemies are not harmful to the environment or the user (Schöller*et al*., 2006). Nevertheless, natural enemies such as the hymenopterous parasitoids *Anisopteromalus calandrae* (Howard) and *Lariophagus distinguendus* (Förster) (Pteromalidae), *Cephalonomia tarsalis* (Ashmead) and *Holepyris sylvanidis* (Bréthes) (Bethylidae), and *Venturia canescens* (Gravenhorst) (Ichneumonidae) are well-known to suppress stored-product insect pests.

Theocolaxelegans (Westwood) (Pteromalidae) is a solitary ectoparasitoid used for biological control. *Theocolax elegans* attacks several stored-product insect pests that develop inside the grain kernel such as *S. zeamais* (Wen *et al*., 1994), cigarette beetle *Lasioderma serricorne* (Fabricius) (Anobiidae) (Hayashi *et al*., 2004) and lesser grain borer, *Rhyzopertha dominica* (F.) (Bostrichidae) (Flinn *et al*., 2006). The female parasitoid parasitizes host larvae within infested grain by using her ovipositor (Sharifi, 1972). Gordh (1979) found different morphs in *T. elegans*, i.e. winged and wingless morphs both in males and females. However, little is known concerning host-age preference of *T. elegans* with *R. dominica* and *C. maculatus* host larvae.

Materials and Methods

Mass rearing of insects

Callosobruchus maculatus, R. dominica, S. zeamais and *T. elegans* were reared at the Post-harvest and Processing Research and Development Division, Department of Agriculture, Chatuchak, Bangkok, Thailand under conditions of 24–30°C, 70–73% RH and 12L:12D/natural photoperiod. The mass-rearing method used a glass container holding 50 g of brown rice (Poaceae). One hundred unsexed adults of *S. zeamais* were placed in a glass bottle (5.5 cm diameter and 15 cm height) for oviposition on brown rice. Then the glass bottle was covered with a filter paper. After five days *S. zeamais* adults were sieved off from the brown rice. *Sithophilus zeamais* larvae (21 d old) were used as hosts for *T. elegans*. *Callosobruchus maculatus* and *R. dominica* larvae developed on cowpea bean (Fabaceae) and wheat grain (Poaceae), respectively, they were used as hosts in our further laboratory trials.

Host-age

Callosobruchus maculatus and *R. dominica* were mass reared on cowpea bean and wheat grain, respectively 50 g in each glass bottle as host species. Twenty unsexed adults of *C. maculatus* and *R. dominica* were sustained on grains in our no-choice experiment. The adults of two host species were mated and laid eggs for five days. *Theocolax elegans* parasitized different ages of *C. maculatus* larvae (5–19 d old) and *R. dominica* larvae (9, 11, 13, 15, 17, 19, 21 and 23 d old). A neonate mated *T. elegans*

female was released into a glass bottle for parasitism. The experiment was replicated 30 times. The number of progeny and sex ratio of wasps in the bottle were recorded.

Statistical Analysis

Analysis was performed using the software IBM SPSS version 20.0. The numbers of *T. elegans* progeny were fully-winged, short-winged or wingless morphs that emerged from different host-ages depending on host species was estimated using the mean±SD. The total numbers of *T. elegans* progeny were determined using one-way analysis of variance to compare mean via an F-Test in completely randomized design (CRD).

Results

Our experiments showed that *T.elegans* progeny were either fully-winged, short-winged or wingless morphs (Tab. 1). The numbers of progeny produced on *R. dominica* (9, 11, 13, 15, 17, 19, 21 and 23 d old) and on *C. maculatus* larvae (5–19 d old) was different. The highest number of *T. elegans* fully-winged progeny emerged from 23 d old *R. dominica* larvae including both females and males (Tab. 1), suggesting that ovipositing females were fertilized.

Tab. 1 *Theocolax elegans* (mean±SD) progeny emerging from different host-ages on *C. maculatus* and *R. dominica* larvae.

Host-ages in different host species	*T. elegans* ♀			*T. elegans* ♂		
	Fw	Sw	Wl	Fw	Sw	Wl
14 d *C. maculatus*	0.5±0.9	0.0±0.0	0.1±0.3	0.1±0.4	0.0±0.0	0.0±0.0
19 d *R. dominica*	1.7±2.5	0.0±0.0	3.1±4.2	0.3±1.3	0.0±0.0	1.0±2.1
21 d *R. dominica*	3.3±4.1	0.1±0.3	1.6±1.9	1.0±1.7	0.0±0.0	0.4±0.8
23 d *R. dominica*	8.3±6.7	0.1±0.4	1.1±1.5	1.9±1.4	0.1±0.4	1.3±1.2

Fw = Fully-winged, Sw = Short-winged, Wl = Wingless

The number of *T. elegans* progeny was statistically significantly different (P value<0.05) depending on host species. The highest number of progeny emerged from 23 d old *R. dominica* larvae (Fig. 1). Sex ratio of *T. elegans* progeny produced from 19, 21 and 23 d old *R. dominica* larvae was 3.7:1.0, 3.5:1.0 and 2.9:1.0, respectively. However, the results showed no progeny on 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18 and 19 d old *C. maculatus* larvae. Female *T.elegans* preferred to parasitize *C. maculatus* larvae only at an age of 14 d (Fig. 2). Sex ratio of *T. elegans* progeny emerged from *C. maculatus* larvae was 5.7:1.0.
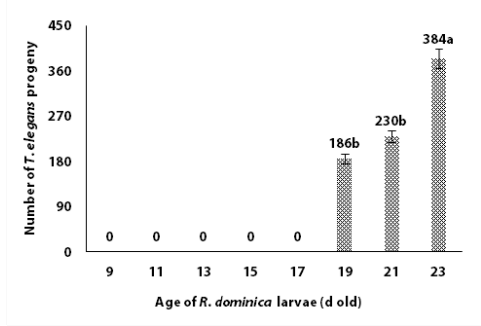

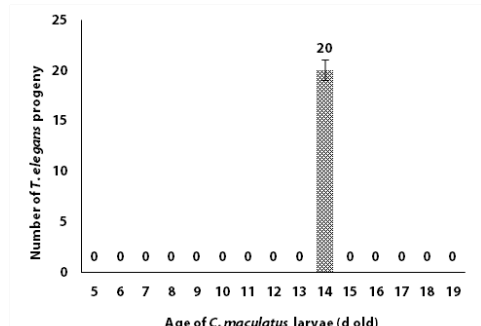**Fig. 1** Progeny of Theocolax elegans emerging from Rhyzopertha dominica larvae at different host-ages.

Fig. 2 Progeny of Theocolax elegans emerging from Callobruchus maculatus larvae at different host-ages.

Discussion

Theocolax elegans was reared on larvae of *R. dominica* and *C. maculatus*, respectively at different larval ages. Godh (1979) reported that fully-winged, short-winged and wingless morphs exist both in female and male. We found the number of *T. elegans* progeny produced on *R. dominica* larvae was higher than on *C. maculatus* larvae (Tab. 1). In this study, we infer that *T. elegans* prefers to parasitize *R. dominica* larvae compared to *C. maculatus* larvae. Similarly Shin et al. (1994) found that the Pteromalidae parasitoid *L. distinguendus* parasitized more *S. oryzae* larvae than *Callosobruchus chinensis* (L.) (Chrysomelidae) larvae. Odours may have different influence on parasitoid activity. Steidle *et al.* (2001) reported that parasitoids were affected by odours which emanate from host plants. Volatiles from faeces originating from *C. maculatus* were different to those from *Sithophilus granarius* (L.) (Dryophthoridae) and *R. dominica* (Steidle *et al.*, 2001).

The results showed that the number of *T. elegans* progeny emerged from 23 d old *R. dominica* larvae was highest (Fig. 1). The optimal host-age for *T. elegans* to parasitize was 23 d old *R. dominica* larvae. Similarly, Choi and Ryoo (2002) reported that *A. calandrae* preferred to parasitize older host larvae more than young hosts. However, our no-choice test showed that *T. elegans* females prefer to parasitize *C. maculatus* larvae at 14 d only. The other ages of *C. maculatus* larvae were never parasitized (Fig. 2). Host numbers per kernel decreased with increasing host size (Wen *et al.*, 1995). The larval instar of *S. oryzae* affects the progeny sex ratio of *A. calandrae* (Choi and Ryoo, 2002). Charnov (1982) and Ji *et al.* (2004) reported that female parasitoids assign daughters on large hosts and sons on small hosts. Host-age or host size also influenced the sex ratio (Wen *et al.*, 1995). Type of grain also influences pteromalid parasitoid growth (Smith *et al.*, 1995). The resource of food as nutritient is important to parasitoid progeny (Godfray 1994).

Acknowledgement

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Phytochemical-Based Nano Emulsions for Stored Grain Protection

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Abstract

Stored grain losses caused by pest insects contribute significantly to the global food crisis. Currently, there are two main chemical control methods against stored product insect pests: fumigation with very toxic gases and grain protection by residual contact insecticides. Today, the global tendency is to prevent/reduce the wide use of insecticides, which have high toxicity to humans and harm the environment. Therefore, there is an urgent need to develop alternative eco-friendly approaches for stored insect pest control.

Essential oils from *Micromeria fruticosa* and *Mentha rotundifolia* (Fam. Labiatae) and their main constituent pulegone which previously were shown by us as very active against stored product insect pests, were encapsulated into coarse and nano emulsions. The insecticidal activity of the developed formulations against primary internal insect rice weevil (*Sitophilus oryzae* L.) and secondary external pest red flour beetle (*Tribolium castaneum* Herbst) was evaluated in laboratory and pilot experiments.

It was found that the phytochemical-based nano emulsions offered significant advantages and provided powerful and prolonged biological activity compare with the coarse formulations. The developed nano emulsions could serve as a natural, effective, low-toxify for human, and environmentally preferred method for protection stored grain and dry food products from pest insects.

Keywords: essential oils**,** nano emulsions, pulegone, stored product insects, stored product protection.

Introduction

Insect damage in stored grain contributes significantly to the global food crisis (Philips and Throne, 2010, Nopsa et al, 2015). Today, the use of fumigants and protectants are common chemical control methods for stored product protection against pest insects. In spite of their high efficacy, both these methods have well known disadvantages (Kostyukovsky and Shaaya, 2012, Opit et al, 2012, Nayak et al, 2013, Daglish et al, 2014). The use of plant essential oils (EOs) and their constituents may be one of alternative eco-friendly approaches for stored insect pest control (Shaaya et al., 1991, 1993, Shaaya and Kostyukovsky, 2006, Kostyukovsky and Shaaya, 2011, 2012). However, for the implementation of the essential oils, suitable formulations are needed.

Encapsulation of essential oils allow even distribution of the active agents, slow evaporation of volatile compounds, and avoid the undesired odors (Onvulata et al, 2012, Majeed et al, 2015). Oil-in-water emulsions are utilized to introduce the water insoluble ingredients into aqueous solutions. In addition to the coarse emulsions, nanoemulsions can be prepared utilizing high or low energy methods. Due to a higher ratio of droplet surface area per mass unit, nano emulsions typically have increased kinetic stability and higher encapsulation capacity (Silva et al., 2012, Salvia-Trujillo et al., 2015).

Essential oils extracted from the *Micromeria fruticose* and *Mentha rotundifolia* (Labiatae) plants content 70-98% of monoterpenes (pulegone and SEM respectively), which were found to possess excellent insecticidal activity, include against stored product insect pests (Shaaya et al., 1993, Franzios et al., 1997, Kostyukovsky and Shaaya, 2011). However, their effective use is limited by the absence of suitable formulations.

In this research, we aimed to develop an appropriate formulation for effective delivery of EOs, the nature-sourced pest-managing agents for the grain storage. For this purpose, EOs were encapsulated into coarse and nano emulsions. The prepared emulsions where characterized by spectroscopic and microscopic methods and their stability and release ability at various environmental conditions were examined. The insecticidal activity against *S. oryzae*, the primary insect pest, and *T. castaneum*, the secondary insect pest, was studied under the laboratory and pilot conditions.

Materials and Methods

Oil-in-water emulsions were formed by stirring pulegone/SEM with sunflower oil in 1:1 ratio at various concentrations with Tween 80 (1.0% v/v) and double distilled water at 1000 rpm for 30 min. Microemulsions were homogenized for 3 min using Power Control Unit homogenizer (Kinematica, Switzerland). Nano emulsions were prepared by ultrasonicating the coarse emulsion with Vibracell ultrasonicator (Sonics&materials, Inc. Newtown, CT, USA) at 70% intensity for 30 min. The average droplet size and polydispercity index (PDI) were measured by dynamic light scattering (DLS) on a Zetasizer Nano ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK) working at 633 nm at 25°C and equipped with a backscatter detector (173°), which is appropriate for measuring submicron particles (Brar and Verma, 2011). Emulsions with pulegone concentrations of 1, 5, 10% and an emulsifier concentration of 1%, were placed at room temperature and checked over the period of a month to examine changes in particle size (published by Golden et al., 2018). The reported values represent an average of three measurements and standard deviation.

The adults of coleopterans *S. oryzae* and *T. castaneum* served as the test insects. The insects were reared in the Volcani Center, Department of Food Quality and Safety under the controlled climatic conditions: the air temperature 28 ± 1°C, the air relative humidity 65 ± 5% in prolonged darkness. After the laboratory treatments, the tested insects were maintained in an incubator under the same controlled conditions. The cultures of the tested insects have been reared for many years without any contact with insecticides. For rearing of *S. oryzae*, the whole wheat grains of 12% moisture content were used. *T. castaneum* was reared on the wheat flour (egg laying) and on the ground wheat grain.

The laboratory experiments were conducted in the incubator at the temperature of 28°C. The glass chambers of 600 ml volume were filled with 100 g of wheat grains with the moisture content of 12%. To each chamber, 200 µl emulsions containing pulegone/SEM at concentrations of 0.1, 1, 5, 7.5, 10 and 20% were added (2-400 ppm on the wheat kernels). Coarse and nano emulsions were examined. The chambers were closed and the grains were mixed for a few minutes. Ten unsexed adults of each test insect 10-15 days old were introduced into the chambers and the chambers were closed hermetically. The control grains were treated with an emulsion without pulegone/SEM. The insect mortality was checked weekly. The grains were sieved by the 10-mesh sieve and were re-infested with the new adults of the same species. Before grain sieving, the concentration of carbon

dioxide (CO_2) was measured by Oxybaby instrument (Witt-gasetechnik-Germany). The experiments were conducted in three replicates and were continued until the loss of the emulsions efficacy.

The pilot experiments were conducted in the bins of 60 l volume with 45 kg of the treated and untreated wheat grain. The tested insects were placed in cages with holes in three depths locations. The nano and coarse emulsions contained 10 or 20% pulegone/SEM were tested. The insect mortality was checked every two weeks and the grain was re-infested with the new adults of the same species.

Statistical analyses were done using the ANOVA and TUKEY student range test, which were performed with the JMP 13 software (Statistical Discovery™ from SAS, Cary, NC).

Results

The optimal content for the stable emulsions was istablished. The concentrations of SEM/pulegone of 5 and 10% v/v and Tween 80 of 1% v/v resulted in stable emulsions with insecticidal activity. The droplet size of nanoemulsions was depended on the pulegone concentration and stabilized after a week. The initial amount of the pulegone released from the nano formulation (after 4 days) was 3-3.5 times higher than that of the coarse emulsion. The nano emulsions provide significantly higher amounts of pulegone for prolonged period in comparison to coarse emulsion (0.15-0.35 mol/l vs 0.05-0.1 mol/l). After 200 days, in 10% pulegone at 32°C, coarse emulsions released all the pulegone whereas from nano emulsions the pulegone was still released (published by Golden et al., 2018).

The nano emulsions were found much more active compared with the coarse formulations. The total mortality of *S. oryzae* adults was recorded for nano emulsion of 10% pulegone for 5 weeks (exposure of a week) compared with one week in the coarse emulsion. The same tendency was observed in *T. castaneum*. Mortality of above 90% was observed for over 5 weeks, with 10% pulegone nanoemulsion compared with one week in the coarse emulsion (published by Golden et al., 2018).

In the case of 10% SEM emulsions, the high efficacy against *S. orysae* was recorded for 5 months with nano- and for 2 months with coarse emulsions, and against *T. castaneum* for only 1 week (nano).

In parallel to the re-infestation process, the levels of CO_2 were checked weekly. At the first few weeks, concentrations of CO_2 in the control reached 4-14%, elevating to 15% and more, that are lethal for the insects. In contrast, in the pulegone/SEM coarse and nano emulsions that caused insect mortality, the concentrations of CO_2 were at low levels of 0.1-4%.

In the pilot experiments, 10% pulegone emulsions were active against *S. orysae* for 3 months, against *T. castaneum* for a month with the advantage for nano emulsion. 10% SEM emulsions were active against *S. orysae* for 6 and 5 months (nano and coarse), against *T. castaneum* for two weeks.

Discussion

The release studies show the advantage of nano emulsions, and the effective prolonged release of pulegone. These properties are necessary when considering slow release and prolonged exposure of food storage insects to the pest-managing agent. In the most treatments, the quantity of the released pulegone/SEM from nanoemulsions were higher for prolonged period compared to the coarse emulsions. Nanoemulsions have a higher surface area in comparison to coarse emulsions and therefore can release a higher amount of pulegone. They are more stable than coarse emulsion (Arnon-Rips and Poverenov, 2016). After 200 days of application, the pulegone concentration in nanoemulsions was still high.

In the most experiments, pulegone/SEM nano emulsions provided prolonged efficacy against both primary and secondary grain pests *S. oryzae* and *T. castaneum* compared to coarse emulsions. The high efficacy of the emulsions was observed for longer periods against adult stage of *S. oryzae* compared with *T. castaneum*. This finding consist with our earlier experiments (Shaaya et al, 1993) in which *T. castaneum* was found the most tolerant insect to a wide range of essential oils and their

constituents, including pure pulegone and SEM, compared with the other stored product insect pests.

EOs-based emulsions applied to wheat grain may serve as both fumigant and protectant. The insects inhale released from the kernels fumes, contact with the treated kernels and ingest them.

EOs have good properties for fumigation and may be applied for a wide range of insect pests (Shaaya and Kostyukovsky, 2006, Isman and Akhtar, 2007, Rajendran and Sriranjini, 2008, Kostyukovsky and Shaaya, 2011, 2012). On the other hand, Germinara et al (2017) performed topical application of EO and reported a high mortality rate of adult granary weevils due to contact activity of the EOs.

The current global tendency is to decrease the wide use of toxic chemicals in food. EOs-based nanoemulsions applied to stored wheat grain may serve as an alternative eco-friendly approach for stored product insect control.

Acknowledgements

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Anti-termitic properties of Jatropha (Jatropha curcas L.) on wood termites (Macrotermes bellicosus (Smeathman))

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Abstract

The efficacy of *Jatropha curcas* in the management of wood termites, (*Macrotermes bellicosus*) was carried out in the Teaching and Research Farm of the Department of Forestry and Wildlife Resources Management. University of Calabar. The experiment consisted of 5 levels of *J. curcas* oil (0, 0.5, 1.0, 1.5 and 2.0) and a corresponding quantity in powder, replicated 4 times and arranged in Randomized Completely Block Design (RCBD). Each concentration was tested on 20 unsexed adult wood termite placed in grave yard of 8cm x 8cm. Data on mortality rate was taken at 12 hourly up to 72 hours. The result from the experiment showed that *J. curcas* oil was significantly efficacious compared with *J. curcas* powder both in the field and in the laboratory. It was observed that there was progressive increase in mortality rate due to increased concentration and time duration. The management of termite using *J. curcas* should be encouraged due to its environmental friendliness and should also be incorporated into integrated pest management (IPM).

Key words: Jatropha curcas, Macrotermes bellicosus, oil, powder, mortality

Introduction

Termites (*Macrotermes bellicosus)* are social insects living in colonies, they are sometimes called white ants but are not ants, because the true ants belong to the order hymenoptera, while termites belong to the order isoptera (Grimaldi and Engel, 2005). Termites occur in all temperate an d tropical countries of the world, many of which cause extensive damage to wooden structure and to manufactured goods made of wood, paper and cloth. Occasionally, they cause significant damage to growing trees such as teak and agricultural crops such as cotton (Solomon, 1995). The economic importance of a few pest species is so dramatic that the importance of termite breaking down woody tissue and returning nutrient to the soil is obscured (Truman and Robinson, 1982). Termites are responsible for some of the degradation of wood and other cellulose material in the terrestrial environment, mainly in the tropics and subtropics (Bulthman, 1979). Cellulose being the principal food of termites, wood and wood product such as paper, fabrics and wood structures are consumed and destroyed by termite, and hence a constant effort is directed towards their control. Field and laboratory test indicated that some woods are not resistant, but are susceptible to attack by African wood termite causing significant damage. Factors affecting wood consumption by termites are numerous and complexly related. Among the most important of these factors are: wood species, hardness of the wood Presence of toxic substance, feeding inhibitors or deterrents, Presence or absence of fungi or fungal decay, Moisture content of the wood and soil (Carter *et al.*, 1974; Peralta *et al.,* 2004).

Termites of the *Macrotermes* spp are fungus growing termite belonging to the family rhinotermitidae, they are mostly mound builders and are the largest termite species (Osipitan and Oseyemi, 2012). The species of the termite under the genus *Macrotermes*, impact the economy negatively by causing damage to various agricultural crops, rangeland, wooden portions of buildings, furniture, books, utility poles and fences in several parts of Africa (Wong *et al.,* 2001; Mitchell, 2002; Cox, 2004). It has been reported that *Macrotermes* causes a complete damage of between 80 to 100 % on stored products (Michael, 2000; UNEP and FAO, 2000; Sekematte, 2001;

Nyeko *et al.*, 2010). In some part of Africa, *Macrotermes* do cause a yield loss of 30-60% (UNEP and FAO, 2000). In east Africa, the loss caused on various crops and tree species due to termite vary ranging from 50-100% (Sekematte, 2001; Nyeko *et al.*, 2010). Pests are organism which are invasive or detrimental, notorious, troublesome, destructive to either plants or animals, or which constitute nuisance to livestock, and humans (Sharma *et al.*, 2011). Termite are serious pest of arable crops such as wheat, sugarcane, groundnut, paddy rice causing significant yield loss and also to perennial crops such as forest trees and wooden structures in buildings especially in semi-arid and sub-humid tropics of the world. They are very destructive insect as they feed on both dead wood and living plants. They can eat through the timber of wooden houses and can even attack hard wood such as *Tectona grandis*. Also, they eat furniture, books, boxes and other products of wooden origin. It has been observed that termites conveniently build their nest in fallen logs, stumps of trees, wooden buildings or pieces of wooden debris on the ground, some termite even live the heartwood of large trees (Cox, 2004).

Pesticides play an important role in the integrated pest management (IPM) on agricultural production and productivities (Logan, 1990). For controlling termite, certain synthetic termiticide such as DDT, BHC, aldrin, heptacor, and organochlorinated hydrocarbon have been used for the management of termite but were banned due to the harmful effect on humans, non-targeted spp and the environment (Mulroney *et al* 2005; Soomro *et al*, 2008; Sileshi *et al.*, 2009). As a result of the negative impact of the use of persistent and deleterious synthetic pesticide on the environment, research on the identification of eco-friendly and locally available alternative tool for the control has been the agenda of entomologist. The use of plant materials in the management of insect pest, including termite has been an old strategy in Africa and among many botanicals used in insect pest management plants such as neem (*Azadirachta indica*,), garlic (*Allium sativum*), *Clausena anisata* and have been successfully used to control termite. **(**Owusu*, 2001***;** Doolittle *et al.,* 2007; Dubley *et al.,* 2008, Muhammad, 2009).

Bio-Pesticides are pesticides that are derived from natural live forms such as plants, bacteria, fungi and nematodes and others (Copping, 2009). They are often important component of integrated pest management (IPM) and used as a component of integrated pest management program, these pesticides can greatly decrease the use of conventional pesticides hence, improve the quality of timber as well as crop production. Bio-pesticide control pests and diseases either selectively or with broad spectrum approach. Bio pesticides are generally target specific and affect only the targeted population (EPA, 2012). Control of termite has been through the use of synthetic insecticides such a DDT, BHC, aldrin, heptacor, which are environmentally hazardous. There is therefore the need to assess the efficacy of non synthetic insecticides which are environmentally friendly.

Materials and Method

Location of the study area

This experiment was conducted at the teaching and research farm of the Department of Forestry and Wildlife Resources Management, University of Calabar, Nigeria

Collection of insect sample

Plastic rubbers, 30 cm in length and 15 cm in diameter were buried in a moist soil that surrounds the termite infested trees. Soil was introduced into the plastic rubbers and pieces of rolled carton were placed inside the rubbers and the rubbers were left in the soil for 3-4 weeks. After that, the plastic rubbers were checked if they were infested with termite and the cartons containing termites were incubated under dark condition with high humidity. The termites were fed with sawdust to ensure their survival. Over 2000 population of wood termite were collected for the experiment.

Preparation of bio-pesticide

Seeds of *Jatropha Curcas* were sourced from the tree, shade-dried for two weeks and made into powder using an electric blender and stored in a cool and dry environment till when needed.

Preparation of plant extract

Alcohol extract: Fifty grams (50 g) of *J. curcas* powder each was taken using a rolled filter paper and placed in a soxhlet extractor in 50^0C with 200 ml of ethanol added to it and kept for 24 hours. This procedure was repeated many times in order to get enough amount of extract. The extract was dried in an oven in 45^0C for one hour and kept for use.

Extraction of essential oil from *Jatropha curcas:*

Fifty grams of the same powder was introduced into a flask containing 500 ml of distilled water and exposed to source of heat. The rising steam from the sample was condensed by condenser connected with a glass cylinder to collect the resultant water of the evaporation. The oil layer accumulating on the surface of water was obtained by separating funnel. The oil was kept in the refrigerator till when needed.

Experimental design

A grave yard experiment of 8 cm x 8 cm was measured, thereafter, 0, 0.5, 1.0, 1.5 and 2.0 g each of the powder and a corresponding quantity of the oil were thoroughly mixed with saw dust and introduced into the grave yard and left for one hour and then 20 unsexed adult termites were introduced into the grave yard. Each treatment and the control was replicated four times and arranged in a Randomized Completely Blocked Design (RCBD). Similar experiment was conducted in the laboratory with four replications in a completely randomized design (CRD).

Data collection

Data were collected 12 hourly in each case after administering of the bio-pesticide. Parameters assessed include mortality at 12, 24, 36, 48, 60 and 72 hours after application, respectively.

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using Statview statistical software and significant means were separated using Duncan Multiple Range Test (DMRT) at 5 % level of significance.

Result

Result of the insecticidal properties of *J. curcas* oil and powder showed significant effect on wood termites at (P< 0.05) Five levels each of *J. curcas* oil and powder 0, 0.5, 1.0, 1.5, and 2.0mls and the same concentration of the powder were applied to determine their efficacy on the mortality at 12, 24, 36, 48, 60 and 72 hours period of exposure. Generally at 12, 24, 36, 48, 60 and 72 hours of exposure, the mortality rate of termites was higher in oil when compared to powder. At 12 hours of exposure, *J. curcas* oil at 2.0 mls was highly effective compared to other levels and recorded 30% mortality rate. However, 2.0 g of *J. curcas* was as effective as 1.0mls and recorded 22.5% (Fig1). Similarly, at 24 hours of exposure, application of 2.0mls of *J. curcas* oil was effective and recorded significantly higher mortality rate of compared with other levels. Application of 0.5mls was as effective as 1.0ml of *J. curcas* oil in management wood termite. There was no significant difference that existed between 0.5, 1.0 and 1.5 g of the powder. However, the application of 2.0 g was as effective as 1.5 mls of *J. curcas* oil (Fig 2). Similar trends were observed at 36 and 48 hours of exposure. 2.0 mls of *J. curcas* oil was significantly efficacious compared to 0.5, 1.0, 1.5 mls and also the untreated. Similar result were also obtained in the application of the *J. curcas* powder with 2.0 g recording a better performance compared with other levels of application. There was no

significant difference between 1.5ml and 2.0 g (Fig 3&4). At 60 and 72 hours of exposure, application of 1.0 and 1.5 mls were as effective as applying 2.0mls of *J. curcas* oil. Application of 1.5g of *J. curcas* powder was as effective as 2.0 g at both 60 and 72 hours of exposure and were significantly different from 0.5 g, 1.0 g and the untreated. However, application of 1.5 g and 2.0 g were significantly different from 0.5 ml (Fig. 5 & 6). Generally, the mortality rate of the wood termite increased with increase in both hours of exposure and concentration of bio-pesticide.

a)

b)

c)

d)
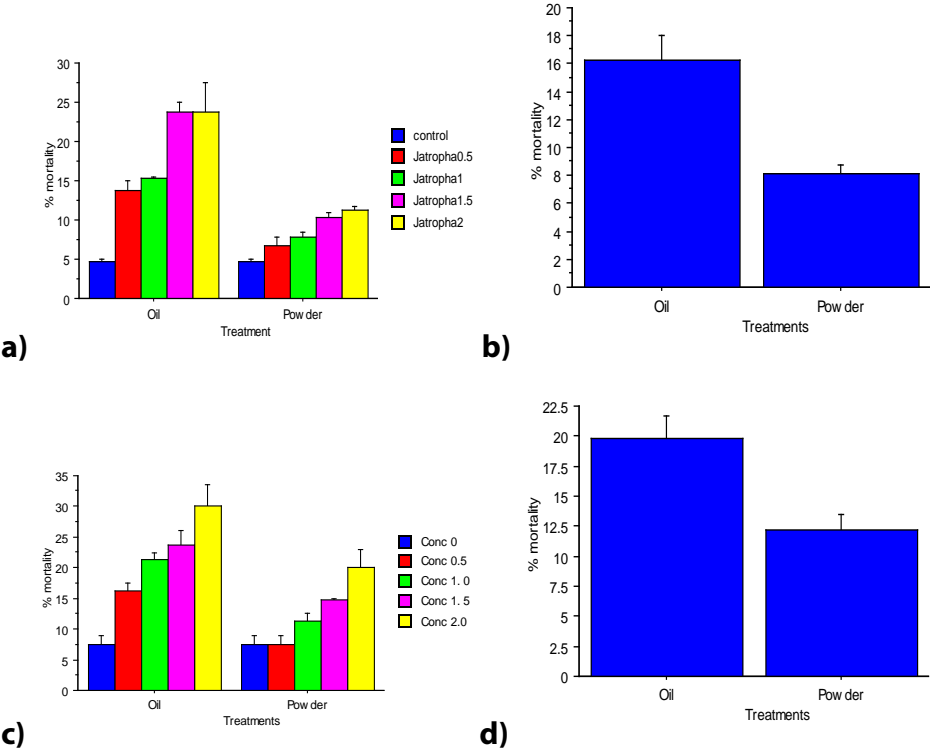
Fig 1: Effect of *J. curcas* oil and powder on percent mortality at 12 hours of exposure; a & b = laboratory result; c & d = field result
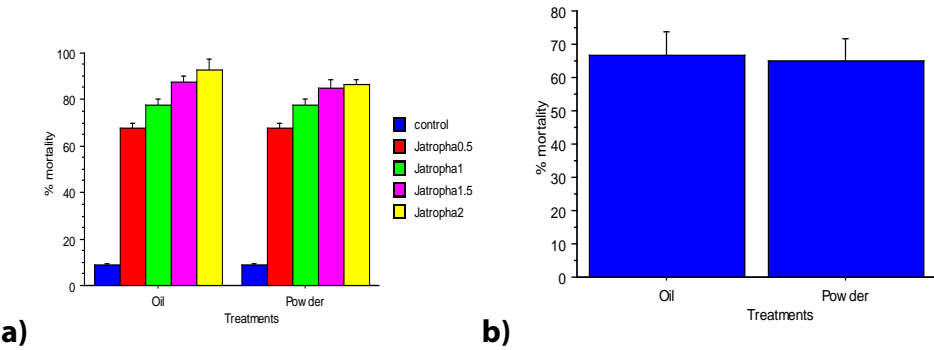

a)

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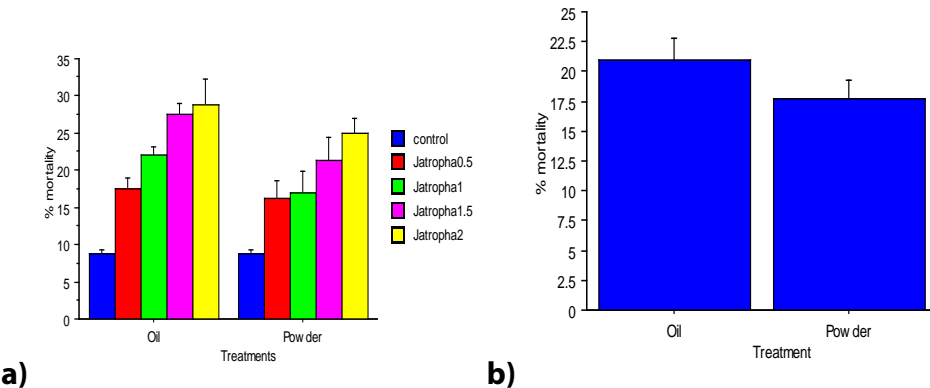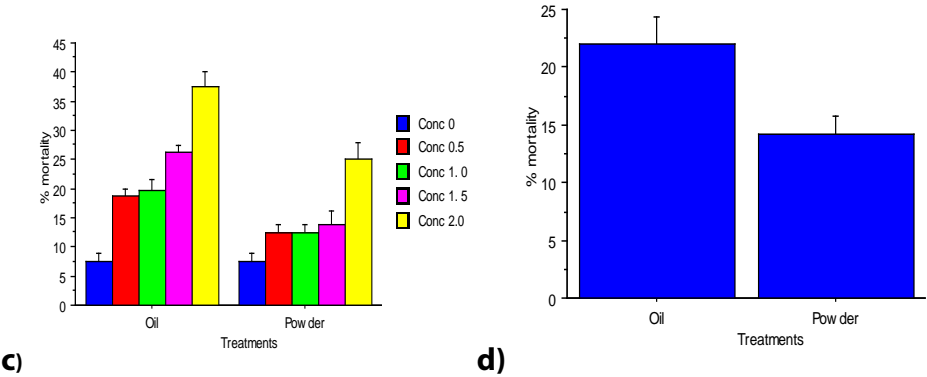
Fig 2: Effect of *J. curcas* oil and powder on percent mortality at 24 hours of exposure, a & b = laboratory result; c & d = field result

a)

b)

c)

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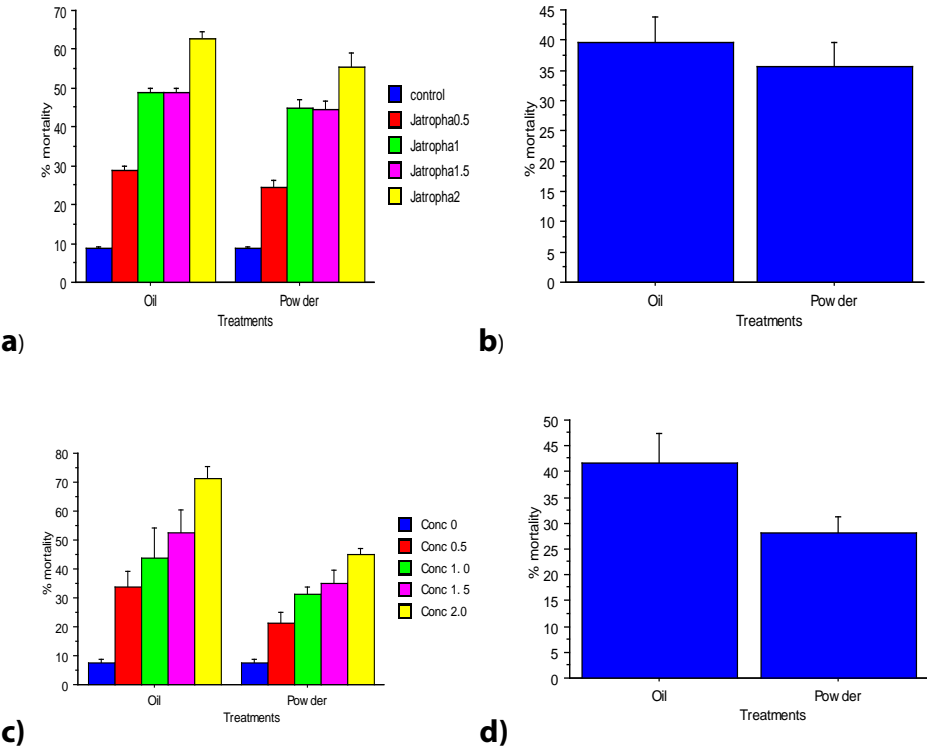
Fig 3: Effect of *J. curcas* oil and powder on percent mortality at 36 hours of exposure; a & b = laboratory result; c & d = field result

a)

b)

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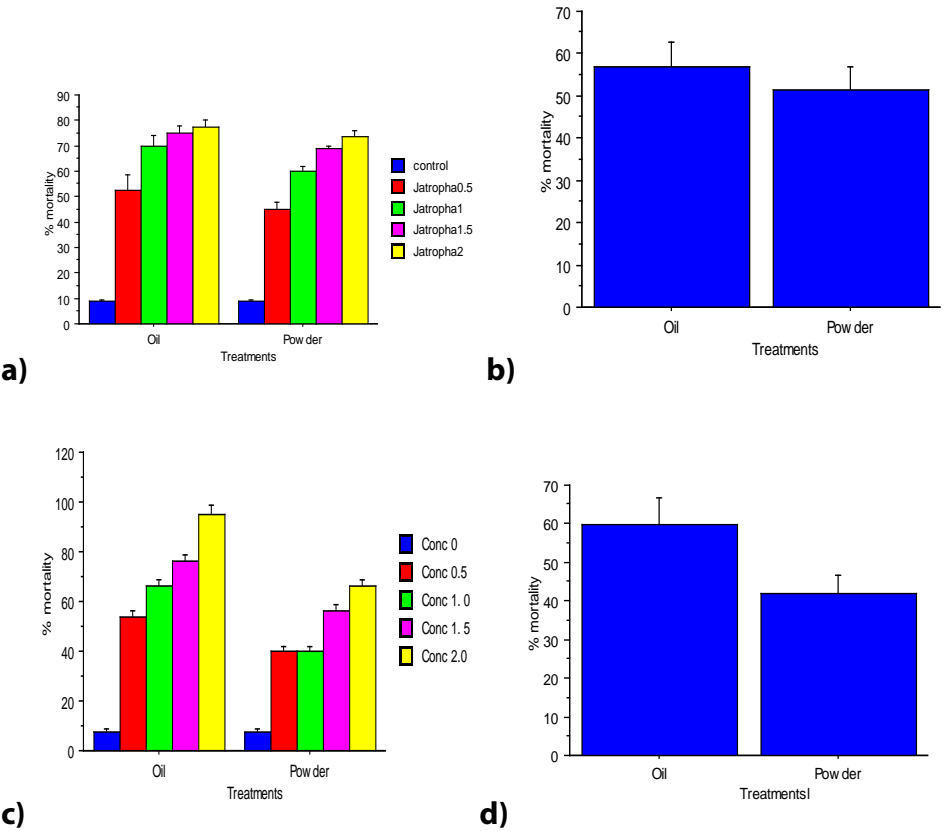
Fig 4: Effect of *J. curcas* oil and powder on percent mortality at 48 hours of exposure, a & b = laboratory result; c & d = field result

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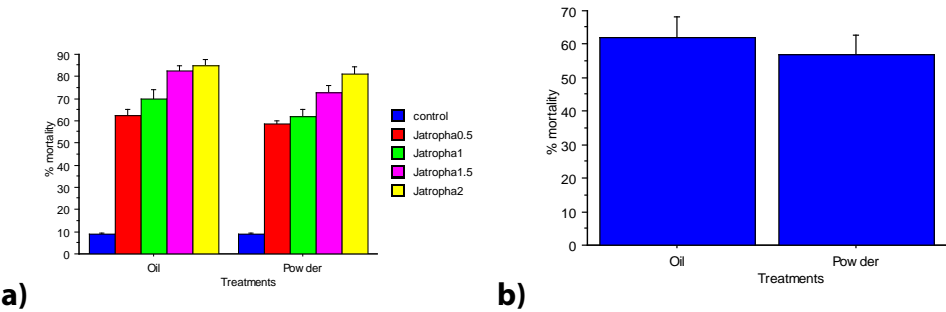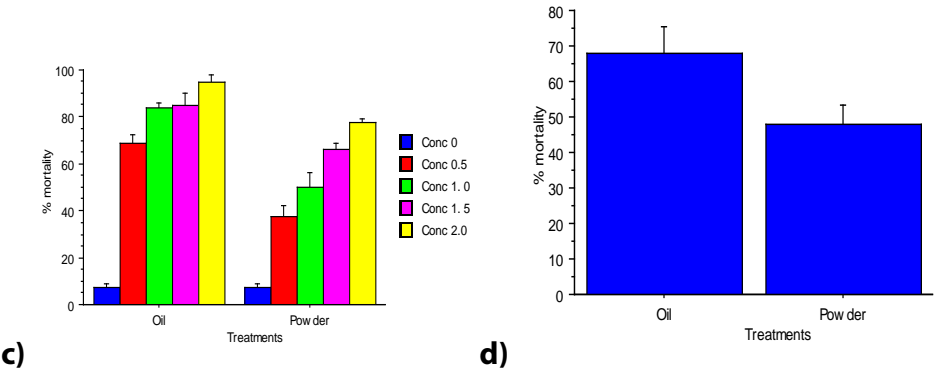
Fig 5: Effect of *J. curcas* oil and powder on percent mortality at 60 hours of exposure, a & b = laboratory result; c & d = field result

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b)

c)

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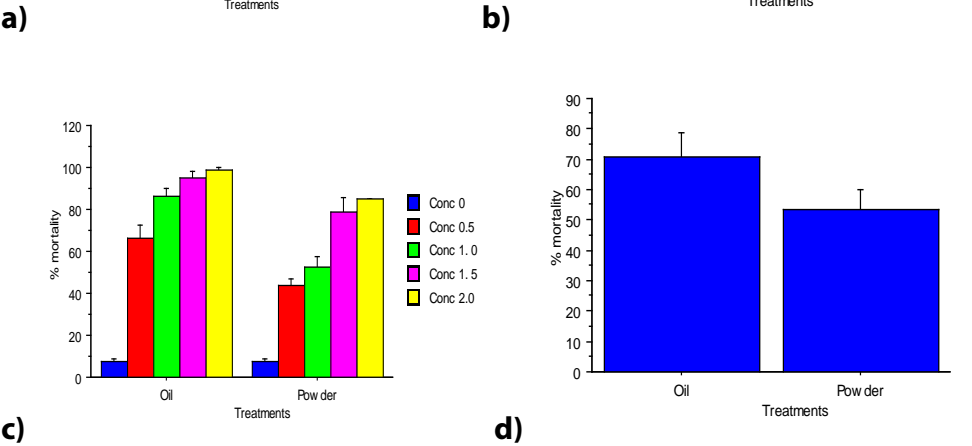
Fig 6: Effect of *J. curcas* oil and powder on percent mortality at 12 hours of exposure, a & b = laboratory result; c & d = field result

Discussion

Jatropha curas has been shown to poses bio-pesticidal properties that works against many pests. Previous works have reported the insecticidal activities of *J. curcas* oil against *Busseola fusca* and *Sesamia calamistis* (Makhar *et al.,* 2007, *Helicoverpa zea* (Olapeju *et al.*, 2008), termite (Acda, 2009), mites (Juliet *et al.*, 2012), desert locust (Bashir & Shafie, 2013) and *Sitophilus zeamais* (Ojiako *et al.*, 2014). This study demonstrated the toxic effect of *J. curcas* oil and powder in the management of

wood termite. The plant extract generally increased the mortality rate of termite and oil of *J. curcas* was found to be significantly efficacious in the management of wood termite when compared to the powder and there was an increase in the mortality rate of termite in the application of the oil than the powder. This experiment is in accordance with Habou *et al.,* (2011) who reported that *J. curcas* oil was effective against many insect pest associated with cowpea under laboratory and field condition. Also, Adebowale and Adedire, (2006) conducted a similar experiment in the laboratory on *C. maculatus* Fabr devastating insect of cowpea in Nigeria. They observed a significant reduction in egg laying of all tested concentrations and a total inhibition of eggs and larvae. The number of eggs laid by *C. maculatus* females was also reduced due to the application of *J. curcas* oil. Markkar *et al.,* (1998) reported that the *J. curcas* oil contained more phorbol esters which exerted potential insecticidal effect on *Busseola fusca* and *Sesamia calamistis.* A higher mortality rate of (70%) was recorded after 36th hour of exposure. This may be due to the breakdown of protective barriers of the insect and the active ingredient of the plant extract. Plants extract are slow to act and degrades easily in the environment. Earlier research findings therefore recommended their application at higher rates and at an increased frequency to achieve effective pest control (Ewete *et al*, (1996). At 72 hours of exposure, all the levels of *J. curcas* oil were highly effective and 2.0 ml recorded 100% mortality and this is in accordance with (Boateng, 2008) who reported that the susceptibility of *Callosobrunchus maculatus* to the *J. curcas* seed oil was highly toxic at 72 hours of exposure. *Jatropha curcas* oil being more effective than the powder and resulting in higher mortality rate in this research was due to the extraction of the bio-pesticide using ethanol. This is in conformity with the work of (Goel *et al*., 2007) who reported that enhancing the phorbol ester or curcin extract using ethanol indicated significant improvement in insecticidal and molluscicidal properties of the plant. Various methods like heat and chemical had been found to render other toxins in the plant inactive except phorbol esters. This study has revealed that treatment of wood products with bio-pesticide will protect wood from destruction by termite infestation. The bio-pesticide used in this study had a lethal effect on wood termite and has shown to be highly effective towards the management of termites in agronomic and forest crop, as well as domestic materials. *Jatropha curcas* is readily available, biodegradable and has proven to be environmentally friendly. It could serve as a valuable alternative to synthetic insecticide in the management of wood termite.

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The use of essential oils for the control of *Callosobruchus subinnotatus* (Pic) in stored *Vigna subterranea* L.

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Abstract

Studies were conducted in the Crop Science laboratory, University of Calabar to evaluate the insecticidal actions of essential oils (EOs) of *Xylopia aethiopica, Dennetia tripetala, Pysostigma venenosum* and *Senna hirsuta* in the management of *Callosobruchus subinnotatus*. The EOs were extracted using soxhlet apparatus with n-Hexane as the solvent. Four concentrations (0.25%, 0.50%, 1.00% and 2.00 %) and n-Hexane as control were laid out in completely randomized design with three replications. Parameters assessed included repellency, fumigant action, weight loss as well as Lethal concentration (LC_{50}) of the treatments to the beetles at the lowest concentration of 0.25%. The EO of *Senna hirsuta* treated samples generally resulted in significantly ($P > 0.05$) lower weight loss than n-hexane treated samples. LC_{50} computation revealed that *D. tripetala* and *P. venenosum* (LC_{50} 0.22 at 48 hrs) were most efficacious against C. *subinnotatus*. The result supports the use of the test plants by small scale farmers in the protection of stored *V. substerranea* against *C. subinn*

Key words: Insecticidal action, repellency, contact toxicity, fumigant action, LC50, weight loss.

Introduction

There are various estimates of crop losses caused by storage insect pests which range from 10 - 40% in the hot, humid regions of the world (Phillips and Throne, 2010). Losses caused by insects include not only the direct consumption of kernels, but also accumulation of exuviate, webbing and cadavers. High levels of insect detritus may result in grains that are unfit for human consumption and loss of the food commodities, both in terms of quality and quantity (Meikle *et al.*, 2002; Ukeh and Mordue, 2009). The major pests of stored grains and pulses of the sub-Saharan African region include those capable of penetrating and infesting intact seeds (grains) and have immature stages developing within the grains and secondary pests which feed on broken kernels, debris, and grains damaged by primary pests. Important among the primary pests are the pulse beetles, *Callosobruchus maculatus* and *C. subbinotatus* (Coleoptera: Bruchidae), the maize weevil, *Sitophilus zeamais* and rice weevil, *S. oryzae* etc. (Rees, 2004). The adults or larvae feed on the grains and eat the albumen or germ or both of them. The attack on the endosperm results in weight loss of the grains, reduction in nutrients and overall deterioration of their quality (Ukeh *et al.*, 2010). Synthetic insecticides are often used in controlling insect pest in stored grains (Duke *et al.*, 2003). The use of these pesticides is usually regarded as the panacea to pest problems in stored grains in order to feed the alarming human population growth. However, these insecticides pose health hazards to mammals and the environment. Their use is further limited by the lack of technical know-how of farmers, traders and consumers in handling these poisonous insecticides. There is thus, the need to search for alternative methods of pest control in all stages of agricultural production including storage. There is need for the application of less hazardous and safe alternatives that are locally and readily available in nature, cheap and affordable to farmers, simple and convenient to use, specific to the target species and are generally environmentally friendly (Isman, 2006; Umoetok *et al.*, 2009). The objectives of the study were to investigate the mode of action (eg. Repellent, contact toxicity and fumigant action) of Guinea Pepper (*Xylopia aethiopica*), Pepper Fruit (*Dennettia tripetala*), Stinking Cassia (*Senna hirsuta*), Calabar Ordeal Bean or "Eseri" Bean (*Physostigma venenosum*) against *Callosobruchus subinnotatus* in *V. substerranea* L.

Materials and methods

Description of the study location

The research was conducted under laboratory conditions in the Department of Crop Science, University of Calabar, Cross River State, Nigeria. The study area is located at geographical coordinate of Latitude 4^0 57^{1}N of Equator and Longitude 8^{0}19^{1}E of Greenwich Meridian, with an altitude of 37m a.s.l (Iloeje, 2001). Calabar is characterized by two distinct moist tropical climates of wet (April-November) and dry (December-March) seasons, respectively. It has an annual rainfall of between 2200 – 3700 mm, average relative humidity of 89%, an annual air temperature range of 26–30 ^{0}C

and lies along the humid coastal region of South Southern Nigeria (Iloeje, 2001).

Collection of plant materials and extraction of essential oils

The plant materials used for the trial were; Guinea Pepper (*Xylopia aethiopica*), Pepper fruit (*Dennettia tripetala*), Calabar bean (*Physostigma venenosum*) and Stinking Cassia (*Senna hirsuta*). The fruits of *Xylopia aethiopica D. tripetala* were obtained from Akpabuyo Local Government Area of Cross River State, while seeds of *S. hirsuta* and *Physostigma venenosum* were collected from fallow lands in Calabar Municipal area in Cross River State. The plant materials were cleaned, air dried under shade for 3 days and preserved in the freezer until they were needed for the various experiments. Fifty grams (50 g) of the dried portion of each plant material was ground and oil extracted with n-Hexane (50nml).

Fifty millimeters (50 ml) of N-Hexane was measured into 500 ml round bottom flask containing the plant powder and extracted using Soxhlet. The plant extract was put into a beaker and evaporated in water bath at 80 ^{0}C. The process was repeated until the required volume of oil needed was obtained.

Insect Culturing of insects

Callosobruchus subinnotatus was cultured on 500 g dry untreated Bambara groundnut seeds which were obtained from local farmers in Yala Local Government Area of Cross River State. The adults of *C. subbinotatus* were obtained from the laboratory stock culture maintained in the Department of Crop Science, University of Calabar, Calabar. About 100 g of Bambara ground seeds were put into kilner jars. Twenty unsexed adult *C. subinnotatus* were introduced into each jar. The covers of the jars were replaced with wire mesh to facilitate air circulation. The insect culture was maintained at room temperature in the laboratory. The adults were allowed to oviposit in the containers for 3 days, after which they were removed. The culture was left for 35 days and the new beetles emerging from each culture jars were sieved out for the experiment.

Repellence bioassays

The repellency test was adopted from the method of McDonald *et al.* (1970) as modified by Talukder and House (1995) and reported by Liu *et al.* (1999). Each plant essential oil was tested for its repellence activity against *C. subinnotatus*. Four concentrations of the four plant essential oils (0.25, 0.50, 1.00 and 2.00%) were obtained and prepared for use in the experiment by diluting each essential oil at 0.05, 0.10, 0.20 and 0.40 ml in 20 ml of n-hexane, respectively. Whatman No. 1 Filter paper (9cm diameter) was cut into two equal parts and placed in the Petri dishes (diameter 8cm) at 2 cm apart. Half part was treated with the plant essential oils and the remaining half treated with the solvent (n-Hexane). Ten (10) unsexed adults each of *C. subinnotatus* were introduced at the center of the Petri dishes, in between the filter papers and the Petri dishes were arranged on the laboratory bench in a Completely Randomised Design (CRD), replicated three times, under a relatively dark environment to minimize the effect of light and the high activity of *C subinnotatus*. The number of beetles on both sides of the filter papers were recorded from each petri dish after 30mins, 1 and 2 hrs treatment application. Based on the number of insects which stay on the treated and untreated sides of the filter, repellency was determined. Percentage repellency was calculated by the equation:

$$\text{Repellency (\%).} = \frac{(C - T)}{(C)} \times 100$$

Where C = No. of insects collected from the untreated filter papers

T = No. of insects collected from the treated filter papers

Fumigant toxicity bioassay

The various concentrations of the plant essential oils were obtained as described earlier. Strips of filter papers were soaked in the different concentrations of the four plant essential oils (i.e. 0.25, 0.50, 1.00 and 2.00% V/V) in beakers. The treated filter paper strips were allowed to dry for 3 minutes and then placed against the wall of a 100 ml flat bottom glass flask. Ten *C. subinnotatus* adults were introduced separately into the treated paper strips in the flask and the bottle sealed with screw caps. Filter papers soaked with n-hexane only served as control. The 18 treatments were laid out in a completely randomized design (CRD) with 3 replications. Mortality was determined at 1.5 hrs. and 3 hrs after treatment. Adults were considered dead if appendages did not move when probed with a Carmel hair brush. Percentage mortality was calculated and probit analysis used to estimate the lethal concentration (LC_{50}) values.

Weight loss bioasay

Hundred grams (100 g) each of maize seeds and Bambara groundnut were weighed out into separate transparent plastic containers. The seeds in each container were treated with 20 ml of each essential oil at different concentrations (0.25, 0.50, 1.00 and 2.00% V/V). The seeds and essential oil were thoroughly mixed. Twenty unsexed 3-day old *C. subinnotatus* were introduced into the admixture of oil and the seeds. Similarly, containers with seeds mixed with 20 ml n-hexane only served as controls. Thus, in each experiment, there were eighteen treatments replicated 3 times to give 54 experimental units laid out in a Completely Randomized Design (CRD). The containers were covered with nylon mesh and their perforated lids screwed in place to ensure confinement of the insects. Data on weight loss were taken cumulatively for 12 weeks. On each occasion, the insects and the produce were separated from the powder emanating from each container due to insect activities, the seeds weighed with a top load electronic weighing balance. Percentage weight loss was obtained by using the formula:

$$\frac{(W_I - W_S)}{(W_I)} \times 100$$

Where W_I = Initial weight of grain before storage
W_S = Weight of grain after storage at a specified time.

Results

Repellency of the four essential oils to *C. subinnotatus*

The repellent effects of the different plant essential oils (Eos) evaluated at different concentrations and exposure time on *C. subinnotatus* are presented in Table 1. Exposure of the beetles for 30 minutes to the different concentrations of oils resulted in an increase in repellency which was dose-dependent. It was observed that, an increase in the concentration of *P. venenosum* and *S. hirsuta* from 0.25% to 0.50% significantly (p< 0.05) resulted in higher repellence to the bean beetles. However, when the concentration was increased from 1.00% to 2.00%, *S. hirsuta* caused higher repellence than the other oils. A hundred percent (100%) repellence was obtained with *P. venenosum* at 2.00% concentration. The repellence of the beetles for an exposure period of 1 hour followed a similar trend as when it was exposed for 30 minutes. There were no significant (p>0.05) differences in repellency among the plant EOs at the lowest concentration which resulted in increase in percentage repellence with the exception of *P. venenosum*. Increase in EO concentration from 0.25% to 1.00% significantly increased percentage repellence of the bean weevil. *Xylopia aethiopica* EO at 0.50% was as effective in repelling *C. subinnotatus* as other plant essential oils at 1.00%. There were no significant (p>0.05) differences in repellence among the different oils when applied at the highest concentration of 2%. No significant (p>0.05) difference was also observed when the test insect was exposed to all the essential oils at 2 hours. Percentage mortality of adult *C. subinnotatus* exposed to varying concentrations of different plant essential oils applied as fumigant. The results of the fumigant toxicity of the plant essential oils (EOs) on the mortality of

bean weevil (*C. subinnotatus*) at different exposure period are presented in Table 2. When the bean weevil was exposed to the EOs for 1.5hrs, there was no significant (p>0.05) difference in weevil mortality between *X. aethiopica, D. tripetala* and *S. hirsuta* at both 0.25% and 0.50% concentrations respectively. The fumigant test showed that higher concentrations (1.00 and 2.00%) of the plant oils resulted in most cases to no significant (p>0.05) percentage mortality of the weevil than at lower levels of 0.25 and 0.50% respectively. No significant (p>0.05) mortality was observed when *X. aethiopica* and *D. tripetala* were applied at 0.25% and 0.50% and *D. tripetala* at 0.50% and *S. hirsuta* at 0.25%. Generally, the fumigant toxicity effect increased with increase in the period of exposure of the weevil to the different concentrations of the plant EOs. However, total mortality of the bean weevil was not achieved in any of the plant EOs within the 3.00hrs exposure period. (Table 2).

Variation in LC$_{50}$ values of essential oils by contact toxicity

The LC$_{50}$ values of the different plant essential oils against adults *C. subinnotatus* at different period of exposure by contact toxicity are shown in table 3. The LC$_{50}$ values consistently decreased with increase in the time of exposure to *P. venenosum*. However, for the other plant materials the trend was not consistent from 3 to 6 hrs exposure period but from 12 to 48hrs, there was consistent decrease in the LC$_{50}$ values. At 3 hrs of exposure, the least LC$_{50}$ values was recorded when the bean beetles were tested against *X. aethiopica*, however, at 6 hrs the essential oil of *S. hirsuta* had the least LC$_{50}$ value. At 24 and 48 hrs, *P. venenosum* EO had the least LC$_{50}$ value. Generally, at 48hrs of exposure, the LC$_{50}$ value for all the plant EOs was low with *D. tripetala* and *P. venenosum* having the lowest.

Effect of plant essential oils on weight loss of treated Bambara groundnut by *C. subinnotatus*.

The results on the evaluation of the efficacy of plant essential oils in reducing weight loss in Bambara groundnut is presented in Fig 1. The same trend were observed at 12 weeks after application (WAA) when higher percent weight loss was recorded on the control as against samples treated with essential oils. Generally, there was a reduction in percent weight loss with increase in both concentrations of the EOs.

Table 1: Repellent effects (%) of different plant essential oils (EOs) against *C. maculatus* at different time of exposure

Plant essential oils	Conc. (%)	0.5	Time of exposure (Hours)	
			1.00	2.00
X. aethiopica	0.25	33.33e	22.22e	11.11ed
D. tripetala		49.20e	22.22e	65.74abc
P. venenosum		33.33e	33.33de	73.67bc
S. hirsute		41.27e	22.22e	41.27bcde
X. aethiopica	0.5	73.67bcd	69.05abc	30.16cde
D. tripetala		69.05cd	61.11bc	84.25a
P. venenosum		49.20e	33.33de	49.20abcde
S. hirsute		49.20e	49.20cd	57.14abc
X. aethiopica	1.0	79.63abcd	75.00ab	49.20abcde
D. tripetala		84.25abcd	69.05abc	77.38ab
P. venenosum		88.88abc	69.05abc	55.16abc
S. hirsute		60,05d	69.05abc	55.16abc
X. aethiopica	2.0	96.29ab	92.59a	61.11abc
D. tripetala		96.29ab	87.96a	69.44abc
P. venenosum		100.00a	88.88a	59.79abc
S. hirsute		92.59abc	92.59a	69.05abc

Means within a column followed by the same letters are not significantly different according to Duncan's

Table 2: Percent mortality of adult *C. subinnotatus* exposed to varying concentration of different plant essential oils applied as fumigant

Plant essential oils	Conc. (%)	Time of exposure (Hours)			
		1.5		3.00	
X. aethiopica	0.25	3.33	(6.13 f)	3.33	(6.13 g)
D. tripetala		10.0	(15.00 ef)	16.67	(19.93 fg)
P. venenosum		33.33	(34.93 cde)	56.67	(48.93 cde)
S. hirsuta		13.33	(21.13 def)	20.00	(26.07 efg)
X. aethiopica	0.5	3.33	(6.13 f)	6.67	(12.27 fg)
D. tripetala		63.33	(53.07 abc	86.67	(72.80 abc)
P. venenosum		40.00	(38.87 bcd)	60.00	(51.13 bcde)
S. hirsuta		60.00	(50.87 abc)	36.67	(37.13 def)
X. aethiopica	1.0	40.00	(39.77 bcd)	63.33	(53.33 bcde)
D. tripetala		76.67	(61.73 ab)	93.33	(81.13 ab)
P. venenosum		70.00	(57.00 abc)	86.67	(68.87 abc)
S. hirsute		83.33	(66.13 a)	83.33	(70.07 abc)
X. aethiopica	2.0	66.67	(54.80 abc)	66.67	(60.00 abcd)
D. tripetala		96.67	(60.00 abc)	96.67	(83.87 a)
P. venenosum		90.00	(51.00 abc)	100.00	(90.00 a)
S. hirsute		90.00	(71.60 a)	10.00	(90.00 a)
Control		0.00	(0.00 f)	0.00	(0.00 g)
Hexane		0.00	(0.00 f)	0.00	(0.00 g)

Means within a column followed by the same letters are not significantly different according to Duncan's
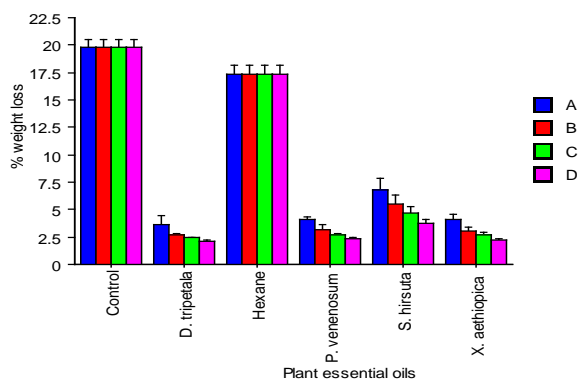
Fig.1: Effect of different plant essential oils on percentage (%) weight loss of Bambara groundnut infested with *C. subinnotatus* at 12 Weeks after application.
Key: A = 0.25%, B = 0.50%, C = 1.00%, and D = 2.00%

Table 3: Percentage variation in LC$_{50}$ values with respect to the duration of exposure of *C. subinnotatus* to the essential oils of different plants during contact toxicity test

Plant materials	Duration of exposure (hrs)				
	3	6	12	24	48
X. aethiopica	1.84	11.76	2.65	1.04	0.50
D. tripetala	2.59	19.32	5.32	0.78	0.22
P. venenosum	18.87	4.32	2.58	0.36	0.22
S. hirsute	2.53	5.17	2.29	1.69	0.37

Discussion

The results obtained from this study showed that the fruits of *X. aethiopica and D. tripetala* and the seeds of *P. venenosum* and *S. hirsuta* had varying levels of insecticidal action against *C. subinnotatus*.

When compared with the control, all the plant essential oils were effective in reducing the population and activities of *C. subinnotatus* under laboratory condition. Their effectiveness was dependent on cconcentration and exposure period except in repellence bioassay where short exposure period resulted in higher repellency than prolonged exposure. This explains why mortality was at highest concentration of 2.00% at 2hrs, for repellency bioassay and 12 wks for weight loss evaluation respectively. Dose related mortalities in similar treatments have been reported by earlier authors (Kieta *et al.,* 2000; Law-Ogbomo, 2007; Ukeh *et al.*, 2012). Ogunwenmo *et al.* (2007) reported that plants have phytochemicals that act as chemical defense against other organisms thus, the strong odour produced by the oils of these plants and their chemical composition may have been responsible for the mortalities observed on the insects (Lee *et al.,* 2007; Kouninki *et al.,* 2007). The bioactivity of these plant essential oils were due to fumigant action and contact toxicity of the oils to the insects. At higher concentrations, the oils may have blocked the insect spiracles thus, disrupting respiration and resulting in suffocation (asphyxiation) and death as reported earlier by Oparaeke and Kuhiep (2006). The results of the present study confirm reports by Lajide *et al.* (1995), Ejechi and Akpomedaye (2005), Adedire and Akinkurolere (2005), Rayapakse (2006), Kouninki *et al.* (2007) and Asawalem *et al.* (2012) that *Dennitia tripetala*, *Xylopia aethiopica* and other tropical plants species have strong anti-feedant and anti-survival effects on different storage pest including weevils and beetles. Phytochemical screening and isolation of *X. aethiopica* according to Lopez-Martin *et al.* (2002) revealed the presence of alpha-pinene, beta-pinene, 3-carene and terpinene-4-ol, while that of *D. tripetala* revealed the presence of beta-phenylnitroethane, alkaloids, dennettine, three phenanthrine alkaloids (identified as uvariopsine), stephenanthrine, argentinine, phenolics and vanillin. The presence of these anti-oxidant and semio-chemicals must have been responsible for the acute toxicity of the essential oils (EOs) to the bean beetles. These findings on the effect of contact toxicity on *C. subinnotatus* are consistent with other reports on essential oils that exhibited insecticidal activity on stored product pests (Hall and Harman, 1991; Adedire *et al.*, 2011). The results of percentage mortality of adult *C. subinnotatus* exposed to varying concentrations of plant EOs applied as fumigant revealed that, all the plant essential oils resulted in significantly (P<0.05) higher percentage mortality than the control treatment with hexane. The EOs of *D. tripetala* and *S. hirsuta* at 0.50% concentration were as effective as the highest concentration (1.00 and 2.00%) of *X. aethiopica*. These agree with other researchers on the use of plant essential oils as fumigants in the control of stored product pests (Kieta *et al.,* 2000; Law-Ogbomo, 2007; Adedire *et al.*, 2011; Ukeh *et al.*, 2012). The results of this study showed a very good potential for the use of the four plant essential oils as fumigants. Repellents in the form of essential oils, powders or distillates have the potential for the exclusion of stored-product pests from grains and they have been used to prevent insects from feeding and oviposition (Asawalem *et al.* 2012; Ukeh *et al.,* 2012). The presence of certain chemical compounds in the essential oils which altered the behaviour of *C. subinnotatus* as a result of the effect of the oils on the olfactory sensilla of the insect's antennae, Several workers including Javid and Poswal (1995), Talukdar and Howse (1995), Tapondjou *et al.*, (2002) and Ukeh *et al.,*(2009) had reported that n-hexane or ethanol extract of *D. tripetala* could individually result in 40.1–100% repellence when used in protecting stored dry fish or grains from beetles. All the plant essential oils tested in this study were highly repellent to the bean beetles. Omar *et al.* (2007) reported feeding deterrence of *Cosmopolites sordidus* due to *D. tripetala* extract while anti-feedant effect was reported by Lajide *et al.* (1995). On weight loss, results obtained from the experiment showed that there was a significant (P<0.05) increase in weight loss in the untreated (control) and hexane treated samples irrespective of exposure time.. Kieta *et al.* (2003), Tripathi *et al.* (2002) and Singh and Yadav (2003) reported that various oils used as seed treatments against storage pests are effective in reducing damage (weight loss). The different effects between the plant oils used in the present study may be due to the type of plants and the composition of their different active ingredients, but all the tested plant oils were effective in reducing weight loss of stored Bambara groundnut.

The results of the LC$_{50}$ values of different plant essential oils indicated that with prolonged exposure time, the potency of the EOs are increased. Also, it is apparent that the efficacy of the essential oils

varied among the plant materials. The active ingredients in these plant materials and the physiological mechanism of interference in the insects may possibly have accounted for this variations. From the LC_{50} values obtained, fumigation of the grains with the plant essential oils should be considered compared with spraying or just rubbing with the oils. It is possible that the volatile active components of the oils could easily permeate and penetrate the grains vis-a-avis the bean beetle, than when it is rubbed or sprayed. It was observed that the efficacy of the botanicals were dose-dependent with higher doses resulting in higher mortalities of the *C. subinnotatus*. Graphs of percentage mortality versus log of concentrations were constructed and the LC_{50} was computed for each essential oil. Results of the LC_{50} revealed that *D. tripetala* and *P. venenosum* (LC_{50} 0.22 at 48hrs) were the most efficacious against *C. subinnotatus*. Photochemical screening from literature revealed the presence of several active compounds in the essential oils of these plants and may probably be responsible for the bio-insecticidal properties of these oils and the observed mortalities.

Conclusion

Results obtained from this research revealed that the essential oils *X. aethiopica, D. tripetala, P. venenosum* and *S. hirsuta* were toxic and effective in controlling Bambara groundnut beetle, *C. subinnotatus*. They could therefore be incorporated into the integrated pest management practices by the local farmers to reduce damage caused by insect pests.

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Influence of Abiotic Factors on the Efficacy of Insect Growth Regulators Against *Trogoderma Granarium* (Everts)(Coleoptera: Dermestidae)

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ABSTRACT

Present study was designed to investigate the effects of different combinations of three temperatures (20, 25 and 30℃) and three relative humidity levels (55, 65 and 75%) on the efficacy of three synthetic IGRs i.e., pyriproxyfen, lufenuron and buprofezin at concentrations of 1, 5 and 10ppm on fecundity and adult emergence inhibition of *T. granarium* under controlled laboratory conditions. This study was conducted at Grain Research Training and Storage management Cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. All the treatments were replicated three times using Completely Randomized Design. Larvae of *T. granarium*were exposed to IGRs at different levels of temperature and relative humidity. F_1 adult emergence results showed that at temperature 20ºC, the highest percent reduction in adult emergence (84.38, 70.65 and 79.94%) was recorded after exposure to lufenuron, buprofezin and pyriproxyfen treated diet, respectively. At 75% relative humidity, lufenuron, buprofezin and pyriproxyfen caused 77.53, 80.00 and 80.32% reduction in adult emergence, respectively. Adults were exposed to IGRs at different temperature and relative humidity to evaluate the oviposition inhibition. The results revealed that at temperature 20℃, maximum percent reduction in fecundity (87.95, 80.45 and 70.55%) was recorded after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively. At 75% relative humidity buprofezin, pyriproxyfen and lufenuron caused 86.73, 83.72 and 69.11% reduction in fecundity, respectively. It is concluded that temperature and relative humidity play an important role in the effectiveness of insect growth regulators.

Key words: Temperature, Relative Humidity, *Trogoderma granarium,* Insect Growth Regulators, Efficacy

Introduction

About 9-20% post-harvest losses by stored grain pests had been reported in the developed and developing countries (Phillips and Thorne, 2010). Insect pests cause alterations in the chemical structure of the products by destroying the quality and quantity of food commodities. Among the insect pests of stored cereals, the Khapra beetle *Trogoderma granarium* (Everts) (Dermestidae: Coleoptera) is a serious pest of stored grains and their products (Burges, 2008; Ali *et al.*, 2012). In case of severe infestation, quality and quantity of grains are reduced by feeding and contamination with shed skin. Hairs of larvae may adversely affect human health (Hosseininaveh *et al.*, 2007; Ahmedani *et al.*, 2009).

Excessive use of conventional synthetic pesticides (Organophosphates, Pyrethroids) to protect stored cereals has resulted in the development of insecticide resistant strains, handling hazards, insecticide residues on food, threat to human health and serious environmental issues (Bell, 2000; Benhalima *et al.*, 2004; Desneux *et al.*, 2007). There is the need to replace synthetic chemical insecticides with safe grain protectants (Silver, 1994). Insect Growth Regulators (IGRs) are one of the best alternatives to conventional synthetic pesticides that are highly effective against pests of stored grain commodities because they have low mammalian toxicity, little environmental and health hazard effects (Kostyukovsky *et al.*, 2000; Mondal and Parveen, 2001; Ishaaya *et al.*, 2007). IGRs affect metamorphosis and molting by simulating juvenile hormone (JH, juvenile hormone agonists) or interfering JH activity (ecdysteroid agonists) or by disturbing the cuticle formation (chitin synthesis inhibitors) (Oberlander *et al.,* 1997). In contrast to traditional insecticides, IGRs are less toxic to higher animals. They inhibit the chitin synthesis of insects by causing abnormal endocuticular deposition and absorptive molting (Post and Vincent, 1973). IGRs are used to manage a wide range of insect species by interfering with their process of growth and development (Yu, 2008).

Lufenuron (CSI) is a new synthetic insect growth regulator. It is highly effective for controlling lepidopteron and coleopteron larvae on maize, cotton, vegetables, rust mites and citrus whitefly on citrus fruits. Buprofezin has become successful IGR to manage insect pests in various countries.The reproduction ability of adult females is reduced by feeding on buprofezin treated diet. (Uchida *et al.*, 1985; Izawa *et al.*, 1985; Konno, 1990). Pyriproxyfen is an IGR that strives for juvenile hormone binding position, juvenile hormone mimics and thus retaining an immature stage (Sullivan and Goh 2008). It is a safer compound for non-target organisms and used for management of public health pests (Miyamoto *et al.* 1993). Adult emergence and embryogenesis suppression are also ascribed to pyriproxyfen (Ishaaya and Horowitz 1995).

Toxicity of an insecticide is affected by several factors including temperature, insect species, insecticide type and nature of the food on which insect develops (Kljajic and Peric, 2007; Liang *et al.*, 2007). Integration of temperature with other control measures is a modern pest management strategy for stored grain insect pests (Dowdy, 1999). Similarly, temperature and relative humidity play a significant role in the efficacy of spinetoram, which becomes less effective at higher dose (Vassilakos and Athanassiou, 2013).

Keeping in view the above mentioned facts, the study sought to determine the effect of insect growth regulators on percent reduction in fecundity and adult emergence of *Trogoderma granarium*; the impact of relative humidity and temperature on the effectiveness of IGRs against test insects and the influence of relative humidity and temperature on dose and response.

Materials and Methods

Insect Rearing

Mixed population of *Trogoderma granarium* was collected from grain market and godowns of Faisalabad district, Punjab Food Department of Pakistan. Under laboratory conditions in the Stored Grain Management Cell (SGMC), department of Entomology, University of Agriculture, Faisalabad,*T.*

granarium was reared on whole wheat grains in an incubator (SANYO-MIR-254) at 30±2°C and 65±5% relative humidity according to the procedure used by Ali *et al.*, 2012. Briefly the grains (200g) were sterilized at 70°C for 15 minutes in an oven and then put in separate glass jars (250g capacity). Fifty adults of mixed sex were released into the jars. The mouth of the jars was tightly covered with muslin cloth using rubber band to prevent the escape of adult beetles. After three days the parent beetles were sieved out from culture. The wheat grains having freshly laid eggs were put into separate glass jars of 250g capacity and kept in cooled incubator (SANYO-MIR-254) at optimum growth conditions 30±2°C and 65±5% relative humidity to get homogenous population. Five days old grubs were used in further series of experiments.

Insect Growth Regulators (IGRs)

Locally available three synthetic insect growth regulators, (1) pyriproxyfen (Peradigm[R]) 10.8EC, (2) lufenuron (Lufenuron[R]) 5EC and (3) buprofezin (Buprofezin[R]) 25WP were obtained from FMC United (Pvt) Limited and used in the bioassays at the concentrations of 1, 5 and 10 ppm.

Grain treatment with IGRs

Untreated whole hard wheat (*Triticum aestivum* L.) with moisture contents 10 %, (as determined by Dickey John moisture meter) was used in the tests. Lots of 1.5 kg of grains were equally sprayed with IGRs at concentrations of 1, 5 and 10 ppm using volume at the rate of 100 ml of formulated spray per kg (150 ml of formulated spray per 1.5 kg of wheat grains). Additionally 1.5 kg lots of grains were sprayed with distilled water and used as the control treatment. After treatment application, the jars containing IGR treated diet were allowed to dry at room temperature for 30 minutes in order to evaporate the solvent.

Effect of IGRs on adult emergence of *T. granarium* at different temperatures and relative humidity levels

Five-days old larvae of *T. granarium* were placed into each plastic vial of 50ml capacity, with separate vials for the three IGRs. Different combinations of three temperatures regimes (20, 25 and 30°C) and three relative humidity levels (55, 65 and 75%) were maintained to evaluate the efficacy of pyriproxyfen, lufenuron and buprofezin, at concentrations of 1, 5 and 10 ppm on the inhibition of adult emergence of *T. granarium*. The vials were placed in separate incubators(SANYO-MIR-254) with saturatedsalt solutions at the bottom in order to maintain the relative humidity at the desirable level. After 42 days, adult emergence was observed for *T. granarium*. Percent reduction in adult emergence was calculated using the following formula (Sagheer *et al.*, 2012).

Percent reduction in adult emergence $= 100 \times (1-t/c)$

Where

t = Number of adults in treated diet

c = Number of adults in control

Effect of IGRs on fecundity of *T. granarium* at different temperatures and relative humidity levels

Three plastic cylindrical vials (3 cm in diameter, 8 cm in height) were used as replicates. Each vial was filled with 20 g of treated grain and 20 adults of *T. granarium* were placed in each vial. The vials were placed in separate incubators (SANYO-MIR-254) with saturatedsalt solutions at the bottom in order to maintain three temperatures (20, 25 and 30°C) and three relative humidity levels (55, 65 and 75 %). The relative humidity in the plastic containers was continuouslymonitored by digital Hygrometer. Fecundity (the number of eggs laid) of exposed beetles was assessed after 4 days. It was calculated using the formula (Sagheer *et al.*, 2012).

Percent reduction in fecundity $= 100 \times (1-t/c)$

Where

t = Number of eggs in treated diet

c = Number of eggs in control

Statistical analysis

Data were subjected to statistical software Statistix 8.1 for analysis of variance. The means of significant treatment were compared using Tukey's Honestly Significant Difference (HSD) test at 5% level of significance.

Results

A significant variation in the inhibition of adult emergenceof *T. granarium* was observed at different temperature regimes ($F=3.26$; $P<0.05$), relative humidity levels ($F=14.63$; $P<0.001$) and concentrations ($F=20.01$; $P<0.001$) after buprofezin treatment. The inhibition of adult emergence varied with different temperature regimes ($F=8.35$; $P<0.001$), relative humidity levels ($F=6.81$; $P<0.05$) and concentrations ($F=21.08$; $P<0.001$) after exposure to pyriproxyfen treated diet. Similarly, temperature ($F=16.82$; $P<0.001$), relative humidity ($F=21.23$; $P<0.001$) and lufenuron concentrations ($F=14.16$; $P<0.001$) caused significant variations in the reduction in adult emergence of *T.granarium*.

At temperature 20°C, the maximum percent reduction in adult emergence, (70.65, 79.94 and 84.38%) was observed after exposure to buprofezin, pyriproxyfen and lufenuron treated diets, respectively (Table 1). At 75% relative humidity, the highest inhibition of adult emergence (80.00, 80.32 and 77.53%) was recorded on exposure of buprofezin, pyriproxyfen and lufenuron, respectively (Table 1). Maximum reduction in adult emergence (81.46, 86.39 and 82.45%) was observed at 10ppm concentration of buprofezin, pyriproxyfen and lufenuron, respectively (Figure-1).

Tab. 1 Impact of temperature and relative humidity on effectiveness of insect growth regulators against percent reduction in adult emergence of *Trogoderma granarium*

	Temperature (°C)			Relative Humidity (%)		
IGRs	20	25	30	55	65	75
		Mean±S.E			Mean±S.E	
Buprofezin	70.65±2.04a	54.75±1.09b	63.15±2.55 ab	46.31±2.50c	62.24±1.91b	80.00±2.23a
Pyriproxyfen	79.94±1.57a	72.60±2.48ab	62.57±1.30 b	64.88±3.65b	69.91±2.36b	80.32±1.39a
Lufenuron	84.38±2.23a	56.01±1.66b	54.42±2.50 b	43.16±2.99b	74.12±3.92a	77.53±3.90a

Percent reduction in adult emergence is calculated by formula=100×(1-t/c), where "t" is the number of adults in treated diet, and "c" is the number of adults in control treatment.
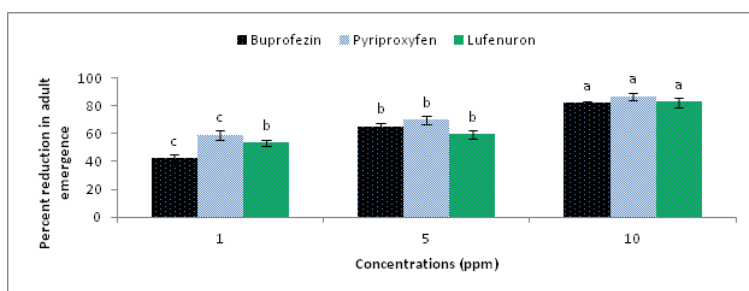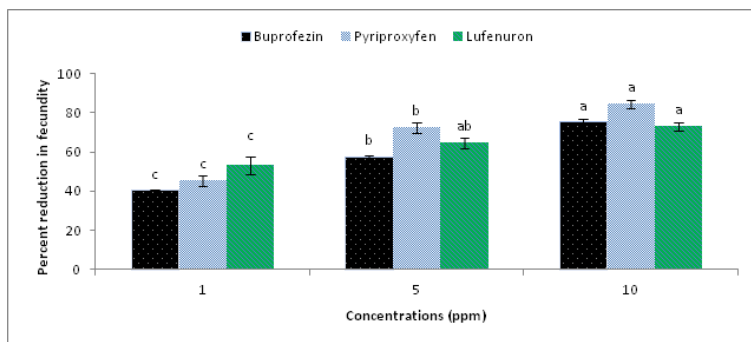

Fig. 1 Impact of various concentrations of insect growth regulators against percent reduction in adult emergence of *Trogoderma granarium*

The interaction effect of temperature and relative humidity caused maximum reduction in adult emergence (86.77%) of *T. granarium* at 25°C temperature and 75% relative humidity after buprofezin treatment (Table 2); while highest adult emergence inhibition (90.74%) was recorded in

both pyriproxyfen and lufenuron treated diet at 30ºC temperature and 75% relative humidity (Table 2).

Tab. 2 Interaction effect of temperature and relative humidity on activity of Insect Growth Regulators against percent reduction in adult emergence of *Trogoderma granarium*

IGRs	Temperature (ºC)	Relative Humidity (%)		
		55 Mean±S.E	65 Mean±S.E	75 Mean±S.E
Buprofezin	20	64.26±3.33 ab	67.02±1.76 ab	80.66±2.21 ab
	25	27.19±2.35 c	50.28±2.51 bc	86.77±1.22 a
	30	47.48±2.18 bc	69.43±3.43 ab	72.55±3.01 ab
Pyriproxyfen	20	31.33±3.56 b	35.07±2.38 b	76.07±3.96 a
	25	63.92±1.85 ab	77.72±2.43 a	80.55±2.32 a
	30	68.81±2.57 a	70.51±3.01 a	90.74±3.89 a
Lufenuron	20	27.11±1.84 b	26.29±2.82 b	76.07±1.96 a
	25	63.88±1.84 a	77.94±3.46 a	80.55±3.32 a
	30	68.51±2.48 a	73.33±2.95 a	90.74±1.89 a

A significant variation in oviposition inhibition of *T. granarium* was observed at different temperature regimes ($F=6.01$; $P<0.05$), relative humidity levels ($F=8.49$; $P<0.001$) and concentrations ($F=12.06$; $P<0.001$) of buprofezin treated diet. Reduction in fecundity varied with different temperature regimes ($F=3.72$; $P<0.05$), relative humidity levels ($F=16.57$; $P<0.001$) and concentrations ($F=12.06$; $P<0.001$) of pyriproxyfen treated diet. Temperature ($F = 15.94$; $P<0.001$), relative humidity ($F=11.53$; $P<0.001$) and lufenuron concentrations ($F=6.38$; $P<0.001$) caused significant variations in fecundity reduction of *T.granarium.*

At temperature 20ºC, the maximum percent reduction in fecundity (87.95, 80.45 and 70.55%) was observed after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively (Table 3). At 75% relative humidity, the highest inhibition in oviposition (86.73, 83.72 and 69.11%) was recorded on exposure of buprofezin, pyriproxyfen and lufenuron, respectively (Table 3). The maximum reduction in fecundity (74.78, 84.43 and 72.85%) was observed at 10ppm concentration of buprofezin, pyriproxyfen and lufenuron, respectively (Figure 2).

Table 3. Impact of temperature and relative humidity on effectiveness of insect growth regulators against percent reduction in fecundity of *Trogoderma granarium*

IGRs	Temperature (ºC)			Relative Humidity (%)		
	20	25 Mean±S.E	30	55	65 Mean±S.E	75
Buprofezin	87.95±1.24a	83.30±1.02b	86.61±1.42ab	86.29±1.22a	84.87±1.46a	86.73±1.00a
Pyriproxyfen	80.45±2.10a	75.65±2.34ab	72.66±3.49b	67.32±3.65b	77.72±1.52a	83.72±1.54a
Lufenuron	70.55±0.43a	54.40±3.35b	67.27±2.86a	55.75±2.01b	67.37±1.29a	69.11±1.44a

Percent reduction in fecundity is calculated by formula=$100\times(1-t/c)$, where "t" is the number of eggs in treated diet, and "c" is the number of eggs in control treatment.

Fig. 2 Impact of various concentrations of insect growth regulators against percent reduction in fecundity of *Trogoderma granarium*

The interaction effect of temperature and relative humidity caused maximum reduction in fecundity (91.92%) of *T. granarium* at 30°C temperature and 55% relative humidity after buprofezin treatment (Table 4); while at 20°C temperature and 55% relative humidity highest oviposition inhibition (71.66 and 70.88%) was observed in case of pyriproxyfen and lufenuron treated diet, respectively (Table 4).

Table 4. Interaction effect of temperature and relative humidity on activity of Insect Growth Regulators against percent reduction in fecundity of *Trogoderma granarium*

IGRs	Temperature (°C)	Relative Humidity (%)		
		55 Mean±S.E	65 Mean±S.E	75 Mean±S.E
Buprofezin	20	84.68±2.41 abc	88.61±2.11 ab	90.5±1.41 a
	25	82.26±1.07 bc	79.86±1.08 c	87.8±1.90 ab
	30	91.92±1.01 a	86.15±3.12 abc	81.8±1.41 bc
Pyriproxyfen	20	71.66±0.68 a	70.22±0.75 a	70.55±1.02 a
	25	32.11±3.15 b	64.72±2.15 a	68.61±2.53 a
	30	68.66±3.49 a	67.16±3.02 a	68.16±3.51 a
Lufenuron	20	70.88±0.38 a	70.52±0.75 a	70.55±1.02 a
	25	29.88±2.75 b	64.72±2.15 a	67.61±2.53 a
	30	66.50±2.62 a	64.16±3.02 a	68.16±3.51 a

Discussion

In this series of experiments, the larvae and adults of *T. granarium* were exposed to different concentration of IGRs treated diet at various levels of temperature and relative humidity. IGRs significantly prolonged the larval duration of *T. granarium* at different temperature and relative humidity levels. At 20°C temperature and 75% relative humidity the highest reduction in adult emergence was observed. Subsequent pupal development and adult emergence was completely prohibited. These results are similar to the findings of Sagheer *et al.*, (2012). It has been reported that IGRs reduced body weight of insects (Smagghe *et al.*, 1996; Parveen, 2000). Meola *et al.* (1999) reported that due to lufenuron treatment in fleas, larval hatching was prevented by raptures in the cuticle, which opened during eclosion resulting in the loss of hemolymph and desiccation of the larva.

It has been found that short term exposure to different levels of temperature had positive effects on toxicity of insect growth regulators. The lowest mortality was recorded at higher level of temperature due to decomposition of active ingredients of insecticides. These results confirm the findings on impact of high temperatures on efficacy of hydroprene applied to control *T. castaneum* (Arthur and Dowdy, 2003). Among the abiotic factors, temperature, grain moisture contents and gas compositions play a vital role in insect growth and development (Hagstrum and Milliken, 1988; Muir, 2000). The interaction of temperature and relative humidity has been studied extensively with often inconsistent results (Arthur, 1999; Fields and Korunic, 2000; Fang and Subramanyam, 2003).

All the three insect growth regulators showed reduction in fecundity of *T. granarium*. It was observed that different levels of temperature and relative humidity showed a significant variation in effectiveness of IGRs against egg laying capacity of *T. granarium*. Significant variations in response of insect were found between temperatures at different levels of relative humidity. At 20°C temperature insect growth regulators were highly effective in percent reduction in fecundity of *T. granarium* compared to other levels of temperature, while at 75% relative humidity the highest reduction in fecundity was observed. These results confirm the findings of the effect of temperature and relative humidity on the efficacy of spinetoram for the control of three stored product beetle species (Vassilakos and Athanassiou, 2013).

When the adults of *T. granarium* were released to oviposit on untreated and treated diet; fecundity was reduced significantly on treated diet compared to control treatments. These results showed

resemblance to transovarial activity of CSIs that caused reduction in fecundity in treated diets. Similar results that the adults of insects reared on treated diet lay fewer eggs compared to untreated adults have been reported by several workers (McGregor and Kramer, 1976; Nickle, 1979; Saxena and Mathur, 1981; Elek, 1998a; Parveen *et al*., 2001). It has been reported that insect growth regulators affect the embryogenesis partially or fully (Mian and mulla, 1982).

Furthermore, in this study IGRs did not kill the adults of *T. granarium* but induced suppression in egg laying capacity of treated insects compared to untreated insects. These results are similar with other findings (Carter, 1975; Faragalla *et al*., 1985; Ammar, 1988; Elek and Longstaff, 1994; Kostyukovsky and Trostanetsky, 2006). It has been found that chitin synthesis inhibitors showed a strong insecticidal activity by foliar application against Colorado potato beetle and reduced oviposition (Cutler *et al*., 2005).

Our study reveals that temperature and relative humidity have significant effecton the efficacy of the three insect growth regulators tested. Maximum control of stored grain insect pests was observed at lower temperature (20ºC) and higher relative humidity (75%), because at high temperature insecticides start to degrade. Overall control of stored grain insect pests depends on biological and physical factors such as insect species, temperature, relative humidity, dose rate and time period for which insects were exposed to insecticides. In addition, some other factors that may affect the effectiveness of insecticides are grain type, grain moisture contents and methods of insecticide application.

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Efficacy of pheromones for managing of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, in food and feed processing facilities

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Abstract

In recent years, considerable progress has been made in the monitoring and control of Lepidoptera, by pheromones also used in mass-trapping, attracticide (lure and kill), mating-disruption, auto-confusion methods. In context of IPM "insectistasis" can be readily achieved by continual supervision of environments by traps in combination with a limited number of preventive and curative measures appropriately timed. In the present paper are reported some promising results offering efficient control of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, populations in food and feed processing facilities based on pheromones and line up a number of remaining questions to be answered to improve the reliability and competitiveness of the methods used. These field researches show potential for successful pheromone-based suppression methods for Mediterranean flour moths in practical applications.

Keywords: Mediterranean flour moth, *Ephestia kuehniella*, pheromones, monitoring, mass-trapping, attracticide method, mating-disruption.

1. Introduction

Pheromones and other semiochemicals have been identified for more than 40 species of stored-product insects over the past four decades. In recent years, considerable progress has been made not only in monitoring but also in direct control of stored-product insects by different techniques (Burkholder, 1990; Chambers, 1990; Phillips, 1997; Trematerra, 2002 and 2012; Phillips *et al.*, 2000; Cox, 2004; Anderbrant *et al.*, 2007; Campos and Phillips, 2010 and 2014; Savoldelli and Trematerra, 2011; Plarre, 2013; Athanassiou *et al.,* 2016; Trematerra *et al.*, 2017).

In the present paper are reported the main results obtained in the control of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, populations by means of mass-trapping, attracticide (lure-and-kill), mating-disruption, and auto-confusion methods applied in food and feed processing facilities.

2. Mass-trapping method

As it is know any attempt to suppress the population by mass-trapping would require a sufficient number of trapped males so that nearly all females would be unmated. Theoretical considerations of mass-trapping males take into account the density of males in the population and the potential number of matings that a male is able to secure in its lifetime. If a male can mate with 6-10 females in a lifetime, as is the case of the Indian meal moth, *Plodia interpunctella* (Hübner), then up to 90% of the male population can be trapped without affecting the number of mated females as well as the subsequent larval generation (Brower, 1975).

Early attempts of mass-trapping were conducted by using pheromone blends of many target insects species. A major problem was the quantification of the number of traps necessary per unit area to achieve an effective control. Proper experiments of mass-trapping are not easy to conduct due to inadequate controls or poor replication, especially in commercial food/feed storage and processing facilities.

In practice the effectiveness of the mass-trapping technique can be reduced by factors such as: inefficient trap design, saturation of traps especially in situations of high pest density, poor pheromone release or duration, attraction of only one sex, inappropriate positioning of traps and the extensive immigration of new pests from outside the area treated with pheromones (Trematerra and Gentile, 2010).

Food lures used in combination with pheromones may offer a way of enhancing the effectiveness of mass-trapping system for stored product pests trapping males and females of a target species (Chambers, 1990; Toth et al., 2002; Cox, 2004; Trematerra, 2012).

Recent studies have investigated the potential of pheromone based mass-trapping methods to control indoor populations of *E. kuehniella* (Trematerra, 1990; Süss *et al.*, 1996; Trematerra and Battaini, 1987; Athanassiou *et al.*, 2003; Anderbrant *et al.*, 2007; Trematerra and Gentile, 2010). In

particular, Trematerra and Battaini (1987) demonstrated that integrated control of *E. kuehniella* could be achieved by mass-trapping. Furthermore, Trematerra (1990) reported results obtained by the practical application of mass-trapping to control an infestation of *E. kuehniella* in a flour-mill.

Subsequently Trematerra and Gentile (2010) presented the 5 years results of applying the mass-trapping method to contain *E. kuehniella* populations infesting a large traditional flour-mill in Central Italy. The study also investigated the effectiveness of mass-trapping, combined with other pest control techniques, at improving the procedures applied to combat *E. kuehniella* infestations using an IPM approach. Over 5 years pheromone funnel-traps baited with 2 mg of (*Z,E*)-9,12-tetradecadienyl acetate (TDA) attracted a total of 54,170 males. The constant presence of the traps caused a marked decrease of *E. kuehniella* populations. The results of the study have shown that the population density of the moth can be effectively reduced and maintained at a low level by means of mass-trapping techniques accompanied by localized insecticide treatments, and careful cleaning of various mill areas and equipment (Figure 1).
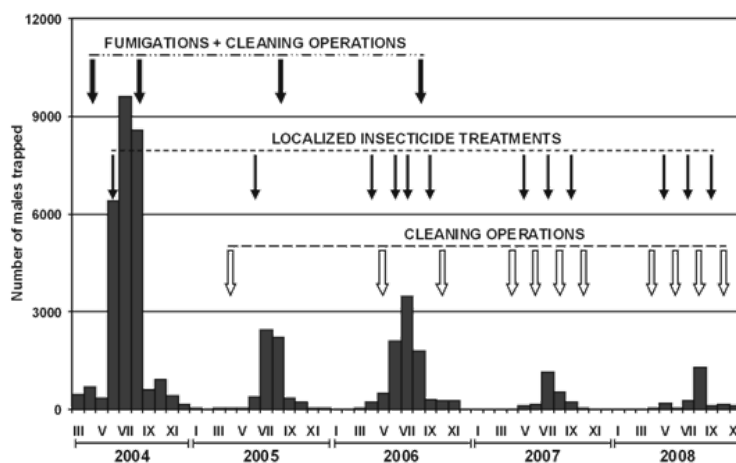
Fig. 1 Mass-trapping, cumulated montly trap catches of *Ephestia kuehniella* males inside a flour-mill.

3. Attracticide (lure-and-kill) method

Attracticide (lure-and kill) method is in some ways analogous to mass-trapping, although many more insects are affected because the attracticide is broadcast over a large area and the killing effect is not limited to individual traps. There are various promising results on the use of the attracticide concept in flour-mills and confectionary industries in the control of *Ephestia cautella* (Walker) and *E. kuehniella* in Italy (Trematerra and Capizzi, 1991; Trematerra, 1995). Preliminary results on attracticide for *Plodia interpunctella* (Hb.) also have been reported more recently in the United States (Nansen and Phillips, 2002 and 2004; Campos and Phillips, 2010, 2013 and 2014).

In Italian flour-mills, *E. kuehniella* males were successfully lured to laminar dispensers (2 cm x 2 cm), baited with 2 mg of TDA (daily release of 13 µg) and treated with 5 mg of cypermethrin; this caused a marked decrease in moth population. Trematerra and Capizzi (1991) performed behavioural tests involving olfactometer, electroantennogram, and insecticide efficacy evaluations in order to clearly determine the effectiveness of pheromone and toxicant in the attracticide method. In field tests in a practical application was conducted to determine the degree of control of *E. kuehniella*. In the olfactometer tests 80 to 90% of males responded to pheromone plus insecticide dispensers, confirming the low repellency of cypermethrin in sexual behaviour. In this context the possible interaction between optical and pheromone stimuli was also studied.

Later encouranging results were obtained by Trematerra (1995) using attracticide applications in

flour-mills placed at 220 to 280 m^3 intervals. This experiment was undertaken in Central Italy in a large mill of 16,000 m^3 that produced flour and semolina. During 2 years of application the attracticide method removed males from *E. kuehniella* populations preventing an increase in the residual population. The prolonged presence of the treated dispensers in the four-mill, particularly during periods when the moths were able to breed, led to a reduction throughout the flour-mill, including areas where no processing occured. After the two years of using the attracticide method, the usual second fumigation of mill proved to be unnecessary. The continuous presence of attracticide dispensers in the mill caused a marked decrease in the *E. kuehniella* population also during the third year.
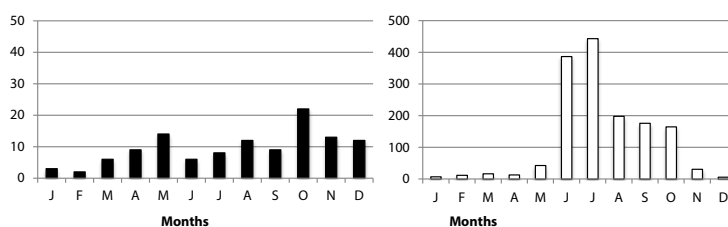
Figs 2-3 Dynamic population of *Ephestia kuehniella* males in a traditional flour-mill protected by attracticide method (2) and in untreated traditional flour-mill (3).

4. Mating-disruption methods

The mating-disruption technique generally requires use of much larger quantities of pheromones than those employed for mass-trapping and attracticide method. Therefore, the mating-disruption may be practical if the pheromones are inexpensive. In addition, there may be concerns about possible contamination of the stored products in situations where high concentrations of pheromone come in direct contact with the product. Therefore, the application of mating-disruption requires the simultaneous development of a reliable system to monitor if the method is effective for the intended time period. The response of females in the presence of high concentrations of pheromones must be evaluated. The low level of matings and the pheromonal substance present inside the treated area could induce females to leave such areas in favour of outdoor areas and could also stimulate dispersal (Trematerra, 1994; Shani and Clearwater, 2001; Trematerra *et al.*, 2013). Also, it has been suggested that insects may evade the effect of mating-disruption by progressive elevation of pheromone production and response threshold or through a change in pheromone composition over consecutive generations to compete with the background pheromone. Studies carried out in field indicate that there is a significant correlation between some of these factors and the spatial distribution of several Lepidoptera in food-processing facilities (Nansen *et al.*, 2003; Trematerra and Sciarretta, 2005; Trematerra and Gentile, 2010; Athanassiou *et al.*, 2016).

Another disadvantage of mating-disruption is that the method does not prevent mated female immigration from adjacent areas, thus oviposition and subsequent infestation are likely to still occur (Cardè and Minks, 1995; Jones, 1998). Hence, monitoring of female activity and/or oviposition is essential when developing a mating-disruption-based control program (Savoldelli and Trematerra, 2011).

The component pheromone (*Z,E*)-9,12-tetradecadienyl acetate attracts males of several Pyralid moths, thus, this 'multi-species pheromone' has been used successfully for mating-disruption in stored-product facilities. Particularly the use of mating-disruption against pyralid moths, in stored-product facilities has been evaluated, with promising results, in both laboratory and field tests. Several studies from many parts of the world have shown more or less similar results for *E. cautella*, *E. kuehniella*, and *P. interpunctella* (Phillips, 1997; Trematerra, 2002; Plarre, 1998; Ryne *et al.*, 2001, 2006 and 2007; Anderbrant *et al.*, 2007 and 2009; Sieminska *et al.*, 2009; Mueller, 2010; Trematerra *et al.*, 2011; Campos and Phillips, 2014).

However, there are consistent methodological problems with evaluating mating-disruption in practice, such as defining what a replicate is and estimation of control based on trap captures (Anderbrant *et al.*, 2009; Sieminska *et al.*, 2009). Ryne *et al.* (2007) compared two adjacent storage rooms, one that was treated with mating-disruption and one that was not, and found using electrophysiological recordings (male antennal response) that there was leakage of pheromone into the untreated room. Mating-disruption-based experiments usually use a single or low number of treatment and control rooms. Each food processing and storage facility is unique that makes finding a 'control facility' which is similar with the treated facility extremely difficult (Sieminska *et al.*, 2009). As a result, there is still inadequate information on mating-disruption effectiveness under different microclimates and in different types of facilities.

Sieminska *et al.* (2009) present results from long-term monitoring of *E. kuehniella* populations in two similar flour mills in Poland. One mill was treated with pheromone for mating-disruption for two years, whereas the other mill was untreated. Thirty pheromone dispensers (one per 100 m³ factory volume), each releasing about 2 mg TDA per day, were used. The reduction in trap catch during the mating-disruption treatment was about 90% or more, compared with the untreated mill or pre-treatment periods in the mill where mating-disruption was practiced. The reduction was larger during the second year of mating-disruption than during the first year. One of the basic drawbacks of mating-disruption method is that oviposition by mated females that enter areas under treatment from untreated areas can still occur (Jones, 1998; Athanassiou *et al.*, 2003; Campbell and Arbogast, 2004; Trematerra and Gentile, 2010). Consequently, the number of captured males in monitoring pheromone-baited traps may not be a clear indicator of mating-disruption.

To avoid these methodological issues, Trematerra *et al.* (2011) conducted a two-year, large-scale experiment that included eight facilities located in Czech Republic, Greece and Italy. The facilities were flour-mills, food and drug storage rooms, and warehouses storing organic foods, pasta, raisins, or wheat. Dispensers of cellulose pad, each with 50 mg of TDA were placed at a rate of one dispenser per 9 m² (or one dispenser per 54 m³). Based on the results reported in some storage facilities and trap-check dates, the suppression of captures in the mating-disruption-treated areas was <95% in comparison with untreated areas, suggesting that some mating may have occurred.

Generally, there is no clear indication that the moth species made a difference in mating-disruption program effectiveness, so Trematerra *et al.* (2011) proposed that the mating-disruption method had the same efficacy level for *E. cautella*, *E. kuehniella* and *P. interpunctella*. The use of a single pheromone component [(*Z,E*)-9,12-tetradecadienyl acetate] to accomplish simultaneous suppression of more than one pest species is an additional advantage for using mating-disruption in storage facilities (Anderbrant *et al.*, 2009).

In large-scale experiments with mating-disruption dispensers, the 'untreated' areas may not serve accurately as 'controls' because of the potential air permeation from the treated. Also mating-disruption may have a cumulative effect after multiple years of implementation. Historical data from previous years, concerning both adult captures and larval presence for the target facilities, may serve more accurately as 'controls' because it can also reflect seasonal patterns in activity.

Oviposition and/or immature emergence should be monitored, in conjunction with adult activity in pheromone-baited traps, to indicate if successful mating-disruption is occurring. In this regard the pheromone effect on population growth or decrease could be measured by the presence of spermatophores in females (Trematerra and Savoldelli, 2013). Also in this case, one of the most important factors impacting the efficacy of mating-disruption is the population density.

Three years of field trials (from 2007 to 2009) were carried out in Central Italy by Trematerra and Spina (2013) to evaluate MD of the Mediterranean flour moth, dispensers containing the pheromone TDA were placed in two traditional flour mills. Pheromone-baited funnel traps were used to monitor the population fluctuations of moth males throughout the entire experimental period; female oviposition was assessed by placement of petri cups containing wheat germ–semolina flour bait. According to the results, the use of MD dispensers does not interfere completely

with the reproduction of *E. kuehniella*. However, looking at the overall data, there was a significant reduction in both adults and larvae in treated mills after the MD application. According to hazard analysis and critical control point procedures (HACCP), treatment should be accompanied by general cleaning of the facilities, including corners and inside machinery, where insects can hide and reproduce.

In integrated pest management programs, the use of MD can lead to a drastic reduction in the need for chemical treatments, with improvement in food quality.

5. Auto-confusion

A particular method of mating disruption is auto-confusion, Baxter *et al.* (2008) and Hugget *et al.* (2010) reported preliminary laboratory studies to examine behavioural effects of auto-confusion on virgin male *P. interpunctella*. The method used TDA, combined with a patented electrostatic powder delivery system to disrupt mating and interrupt the lifecycle of several moth pests. Laboratory flight tunnel studies showed that contact with SP-Tab auto-confusion significantly reduced the ability of male *P. interpunctella* to locate females for up to two days.

These males could increase the confusion effect by becoming competitive attractive point sources for other males (Huggett *et al.*, 2010). Using auto-confusion Pease and Storm (2010) presented preliminary practical trials that were conducted in two flour-mills in UK and in a spice factory in Netherlands. Populations of *E. kuehniella* and *P. interpunctella* were monitored. In all cases populations were reduced compared to the same area in the previous year and compared to untreated control areas in accordance with local pest control practices.

Preliminary results of the SP-Tab auto-confusion system for mating-disruption of *P. interpunctella* in 2008 and 2010 was reported from United States by Campos and Phillips (2014).

Trematerra *et al.* (2013) applied Exosex SPTab dispensers that contained the Entostat powder, at a 5x5 m grid, in three facilities, one feed-mill in Italy and two retail stores in Greece. In the feed-mill, the most abundant pyralid species was *Ephestia kuehniella*. Monitoring through pheromone-baited traps in this facility indicated that the application of the Exosex SPTab dispensers decreased the number of captures 2 months after the initial application. In the case of the facilities in Greece, the most abundant species was *Plodia interpunctella*. In these facilities there was a continuous monitoring of moth populations from January 2008 until February 2011, with pheromone-baited traps and Petri dishes with semolina, which served as oviposition traps. In both facilities, the presence of *E. kuehniella* and of *P. interpunctella* males in the pheromone-baited traps was reduced after the placement of the Exosex SPTab dispensers, in comparison to captures for the same interval from the previous years. At the same time, the number of emerging individuals in the oviposition traps was notably reduced after the Exosex SPTab dispensers placement, in comparison to the previous monitoring interval. Our study documents that the auto-confusion system is an effective and reliable technique that can be used with success against stored-product Pyralidae, to retail stores and feed-mills (Figure 5).

6. Future prospects

As previously reported, there are consistent methodological problems in assessing the efficacy of mass-trapping, attracticide, mating-disruption and auto-confusion methods in practice. Each food storage and processing facility is unique and therefore finding a comparable 'control facility' is difficult when pheromone-based control methods are deployed. As a consequence, interpreting effectiveness of pheromone-based control measures in various facilities, at different insect densities, and microclimates becomes difficult. The available data indicate that many factors influence both male and female behaviour.
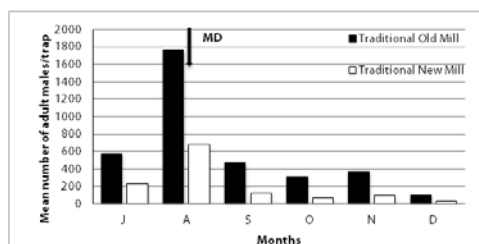
Fig. 4 Mating-disruption: Italy, traditional old and traditional new mill, 2008: mean number of *E. kuehniella* adults/trap caught in the monitoring period.
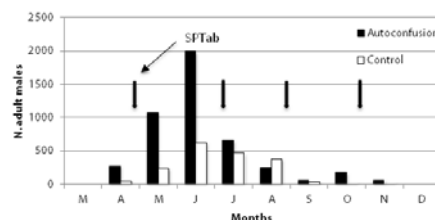
Fig. 5 Dynamic population of *Ephestia kuehniella* males in feed-mill protected with auto-confusion method.

The relative importance of these factors varies among species and among populations of the same species, undoubtedly reflecting the different ecological conditions to which they are normally subjected (Ryne *et al.*, 2007; Trematerra *et al.*, 2011 and 2012; Campos and Phillips, 2014).

In stored-product moth control, the pheromone efficacy was evaluated using the following parameters: male capture in pheromone traps, oviposition and larval emergence from eggs, incidence and frequency of mating as measured by spermatophores in females. The number of captured males or the absence of males in pheromone traps may not be a clear indicator of mating suppression and female oviposition. Oviposition and/or immature emergence should be monitored, in conjunction with adult activity. One of the most important factors, impacting the efficacy of pheromone control is the population density. Historical data from previous years, concerning both adult captures and larval presence in the target facilities, may serve as internal 'control' because such informations shows seasonal patterns of insect activity. The necessity of better controls and adequate replications need to be emphasized. In this regard the pheromone effect on population growth or decrease could be measured by the presence of spermatophores in females' *bursa copulatrix*, which is a good indicator of mating activity (Trematerra and Savoldelli, 2013). The female dissection to count spermatophores, as an estimate of mating reduction, is a more direct method than reduced trap catch or reduced oviposition in diet cups and should probably be used more.

In stored-product protection the increased use of pheromones will help reduce the number of chemical treatments with consequent economic and qualitative advantages. Pheromone-based methods need to be considered as a part of an overall IPM program in food systems (Trematerra, 2013; Trematerra *et al.*, 2017). In the future, more efficient formulations of pheromones and other semiochemicals are needed, coupled with research under real-world conditions, for effective management of stored-product insects (Trematerra and Fleurat-Lessard, 2015).

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Influence of low doses of gamma irradiation on cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)

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Abstract

Phytosanitary irradiation for food commodities has been widely accepted in recent years. Gamma irradiation has been used as a phytosanitary treatment against microbial diseases, insect infestation and food spoilage. The goal of the current study was to determine the lowest possible dose of gamma irradiation that will induce long-term sterility of insects through generations. The effect of four gamma irradiation doses examined were; 20,40, 50 and 70 Gy. Irradiated males were crossed with normal females. For the cowpea beetle *Callosobruchus maculatus*(F.), adult fecundity, hatchability, adult emergence, sterility% was investigated. 100% adult mortality was achieved by 70 Gy dose. Fecundity, hatchability, number of adults emerged, sterility% were significantly reduced when males exposed to 20, 40, and 50 Gy compared to the control. The effect of parental irradiated males exposed to 20 Gy on F2 generation was also studied. Fecundity, hatchability, number of adult emerged, sterility% were significantly reduced in F2 compared to F1 and control progeny. Interestingly, for F1 generation, the effect of gamma rays on adult emergence% exhibit a hermetic effect response although it was not significant. These results demonstrat that pulse irradiation relying on low-doses of gamma radiation induce inherited semi-sterility through generations and is a very promising phytosanitary food technology for postharvest treatments.

Keywords: inherited sterility, gamma radiation, low-dose effect, sterile male technique, sterile insect technique, hormesis.

Introduction

Inherited sterility (IS) has evovled as an alternative to the standard sterile insect technique(SIT) (IAEA, Ibrahim *et al.*2017). Male insects irradiated with sub-sterilizing doses of gamma or x-ray radiation and mated with virgin females resulted in F1 generation with more sterility than their parents. Previous sutdies have been shown that IS has a long-term impact on pest control programs against many insects such ad moths and mosquitoes (JANG *et al.*, 2012 and Shetty *et al.*,2016).

On the other hand, food irradiation has been widely used as a phytosanitary treatment and insect disinfestation methods. It is an environmentally friendly safe treatment and it has been used against many pests as an alternative to chemical control (Ibrahim, *et al.*, 2017, Ihsanullah and Rashid, 2017).

The original data for this paper has been previously published (Ibrahim, *et al.*, 2017). Here, the effect of low doses gamma radiation on the cowpea beetle *Callosobrocus maculatus* and the inherited effect on F1 and F2 generations were examined. The first objective was to determine the lowest gamma radiation dose to be used for insect disinfestation. The second one was studying the long-term effect of low doses gamma radiation on IS through generations.

This work will hopefully increase the effectiveness of using SIT and other releasing biological control programs by induced IS across different generations.

This work also expands on the importance of the potential for radiation hormesis effect that could appear as a result of using low-doses in food irradiation.

Materials and Methods

Insect rearing

The insect used was cowpea beetle, *C. maculatus*. The insect culture was maintained on cowpea seeds (*Vigna unguiculata* (L.) Walpers) in Entomology Department, Ain Shams University for many years. . Insects were maintained at 27 ± 2oC and relative humidity of 60 ± 5%.

Preliminary test

It has been reported that the highest dose of gamma radiation permitting reproduction was 50 Gy and the sterilizing dose was 70 Gy (Hasan and Khan 1998). Therefore, five doses of gamma radiation (0,20 ,40, 50, 70 Gy) were selected to determine the lowest dose that permitting reproduction and induce IS for both F1and F2 generations. No. of eggs, no. of hatched larvae, no. of adult emerged, adult emergence% were determined for F1 offsprings

From the insect rearing jar and prior to adult emergence, each 1-egg-seed was transferred to an Eppendorf tube to prevent any mating prior the experiment.

Irradiation technique

Newly emerged adult males (1-day old) were irradiated with one of five doses of (0,20 ,40, 50, 70 Gy) using ^{60}Co Indian gamma cell (Gy 4000 A), located at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The dose rate was 0.713 Rad/s.

Biological assay

For biological assay a 20 Gy dose was selected because it was the lowest dose that caused IS (Ibrahim, 2017) and at the same time produce sufficient individuals to study long-term effect in F1 and F2 progeny. After exposure to radiation, the newly emerged adult males were mated with virgin normal females in 9 mm Petri dishes with cowpea seeds. Each replicate has two males and two females. Five replicates were made for each treatment, 20 Gy and the control one. Insects were also maintained at 27 ± 2oC and relative humidity of 60 ± 5%. No. of eggs, no. of hatched larvae, no. of adult emerged, adult emergence%, sex ratio, and sterility% were recorded per each treatment for both F1 and F2 resulted form irradiated male parents.

The percentage sterility was calculated according to Chamberlain's formula (1962):

% sterility=100 -((a ×b)/(A ×B) ×100)

Where:

a = number of eggs per female in the treatment.

b = Percentage of hatched eggs in the treatment.

A = number of eggs per female in control

B = Percentage of hatched eggs in control.

Statistical analysis

For preliminary test, adult emergence % data were analysied using non-paramitric tests. Otherwise, the results were analyzed with ANOVA and followed by posthoc analysis using LSD-test with the help of SPSS program. The level of significance used was $p < 0.05$.

Results

Preliminary test

There was an overall significant effect between treatments for both number of eggs and number of hatched larvae ($P < 0.05$, Fig. 1). All doses significantly reduced the number of eggs and the number of hatched larvae compared to the control group (Fig. 1). There was also an overall significant effect between different doses for number of adult emerged ($P < 0.05$, Fig. 2). On the other hand, results of adult emergence% were not significantly different from each other ($P = 0.06$, Fig. 2). Interestingly, adult emergence% for those exposed to 20, 40, and 50 Gy seemed to be better than that of the control group although this effect was not significant ($P = 0.06$, Fig. 2).
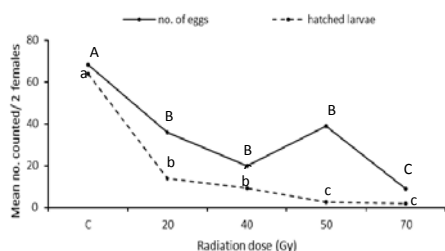
Fig. 1 Number of eggs and number of hatched larvae after crossing irradiated males of C. maculatus with normal females at 0, 20, 40 and 50 and 70 gamma radiation doses. Different letters indicate significant differences, P< 0.05. Capital letters for no. of eggs laid and small letters for no. of hatched larvae.
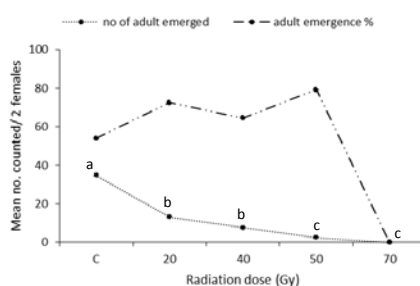
Fig. 2 Number of adult emmerged and Adult emergence % after crossing irradiated males of C. maculatus with normal females at 0, 20, 40 and 50 and 70 gamma radiation doses. Different letters indicate significant differences between no. of adult emerged among doses, P< 0.05.

Biological assay

There was an overall significant effect between treatments for number of eggs, number of hatched larvae, hatchability %, no. of adult emerged and adult emergence % (For all, $P < 0.05$, Table 1).

Tab. 1 Biological aspects of the F1 and F2 progenies resulted from irradiated parental males *C. maculatus* with 20 Gy dose of gamma radiation and crossed to normal females. Different letters indicate significant differences among different treatments, *P*< 0.05.

Conc.	No. of eggs	No. of Hatched larvae	Hatchability %	No. of adult emerged	Adult emergence %
0 GY	68.25±1.89 a	64.00±1.87 a	93.77±0.8 a	34.75±2.1 a	54.14±1.76 a
20 Gy F1	35.80±11.2 b	13.80±4.75 b	40.93±9.2 b	13.25±4.9 b	72.54±9.46 b
20 Gy F2	5.60±2.16 c	5.00±2.35 c	68.91±16.1 c	2.50±0.05 c	22.92±14.0 c

The male sex ratios were38 ± 1.00%, 42 ± 3.00%, and 17 ± 17% in the control, F1, and F2 progenies, respectively with no significant difference ($P = 0.162$). The adult percentage sterility was significant between the control (0.00 ± 0.00) and the individuals of F1 (70.87 ± 8.70) and F2 (88.31 ± 7.01), ($P = 0.000$). There was a significant difference in percentage sterility between the F1 and F2 generations ($P = 0.049$)

Discussion

Preliminary test

The present data shows that doses of 20, 40, and 50 Gy gamma radiation significantly reduce the number. of *C. maculatus* progeny compared to the control group. A dose of 70 Gy completely inhibits *C. maculatus* production. These data in agree with previous work that showed that complete sterility is achived by 70 Gy and the highest dose that permits *C. maculatus* reproduction is 50 Gy (Hasan and Khan 1998).

Data illustrating *C. maculatus* adult emergence %, demonstrate that low-doses of 20, 40, and 50 Gy seems to have a hermetic dose effect. In dose-response relationships, the hermitic effect or hormesis is a biphasic effect. Some doses of certain stimuli, whether it chemicals or radiation or even any stressors have a positive (stimulatory) effect on an organism at low doses compared to normal individuals (the control groupwhere high doses have an inhibitory effect and concurs with Baldwin and Grantham, (2015).

Biological assay

A low-dose of 20 Gy induces IS and has a long-term effect on both F1 and F2 progenies for *C. maculatus*. The same effect of low- doses of gamma or X-rays radiation has been reported for other insects (JANG *et al.*, 2012 and Shetty *et al.*,2016).

Conclusion

This paper showed that irradiated male C. maculatus with low-doses of gamma radiation induces inhirited sterility through F1 and F2 progenies. This is a very promising new technique for insect control and could be an alternative to SIT and other chemical control programs. At the same time, attention should be directed to the potential of the hormesis effect that could exist. Otherwise, low-doses induces IS might have very dramatic results instead.

Finally, hormesis effect should be investgated more in relation to different stimuli such as insecticides, radiation, physical and even environmental stressors.

Acknowledgement

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Radio Frequency Heat Treatment for Controlling Cigarette Beetle in Dried Tobacco

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Abstract

Tobacco (*Nicotiana tabacum* L.) is one of many agricultural commodities produced in Thailand. There are Virginia (flue-cured tobacco) and Burley (air-cured tobacco) typesand Cigarette beetle, *Lasioderma sericorne* F. is the most important insect pest that attacks dried tobacco. The efficacy of radio frequency (RF) heat treatment at 27.12 MHz was examined to control cigarette beetle on dried tobacco. Various growth stages of cigarette beetle were prepared within samples of dried tobacco and were exposed to RF at 55, 60, and 65 °C for 1, 2 and 3 minutes. The results showed that pupal and adult stages of cigarette beetle were the most tolerant stages to RF heat treatments. The RF treatment at 65 °C for 3 minutes is able to cause complete mortality of egg, larval, pupal and adult stages of cigarette beetle4.

Keywords: dried tobacco, *Lasioderma serricorne,* tolerant stage, heat treatment

Introduction

Virginia and Burley tobacco production in Thailand is found in 10 provinces mostly in the north and northeast of Thailand covering about 21,000 ha and is valued at approximately 2 billion US dollars in 2016-2017 cigarette sales. In Virginia and Burley tobacco storage, cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) is the most damaging insect pests of dried tobacco and grain products such as corn, beans and dried herbs in Thailand. Insecticides have been used as the main control measure for managing stored tobacco pests. To avoid chemical contamination in the commodity other control measures such as storage sanitation and pest exclusion to remove sources of infestation should be considered. Several studies have been performed using radio frequency (RF) energy to control stored product insects. Mitcham et al. (2004) tested RF energy to control Codling moth (*Cydia pomonella),* navel orangeworm (*Amyelois transitella*, and Indianmeal moth (*Plodia interpunctella),* at 55°C for 5 minutes and was able to gain complete control.. Lagunas-Solar et al. (2007) also reported that using RF at, 10 kHz to 1050 MHz resulted in >99% mortality of Anguomois grain moth (*Sitotroga cerealella*).

The use of radio frequency, as a heat treatment, has been investigated to control many kinds of insect pest associated with agricultural products (Nelson, 1996). Experiments have been conducted to control many stored product insects to determine the tolerant stages of insects to the RF heat (Table 1.). For heat treatment with radio frequency, commodities are allowed to increase temperature rapidly due to the vibrationof water molecules. Nutapong (2012) tested the efficacy of RF to kill cigarette beetle on packaged dried tobacco which was infested with cigarette beetles in all stages. The results showed that the adult stage was the most tolerance to RF heat treatment at higher temperatures (104 °C) for complete control Radio frequency also has less effect on various kinds of grains compared with conventional heat (Srikam et al., 2014; Wangsapa et al. 2016; Zhou

et al. 2015). There is very little research work of RF treatment on dried tobacco. Therefore, this experiment is aimed to examine the reduction of temperatures that causes mortality of cigarette beetle with RF to save energy costs and to reduce the effect on commodity quality.

Table 1. The exposure time of radio frequency heat on insect pests associated with various agricultural commodities which resulted for 100% morality of tolerant stages. (The Pilot scale of 27.12 MHz radio frequency was provided.)

Commodities	Insect pests	Tolerance stages	Temp (°C)	Time exposure	References
Corn	*Sitophilus zeamais*	Adult	92	4 min	Faikrajaipuen et al. (2011)
Feed	*Tribolium casteneum*	Pupa	70	1 min	Bualoi (2009)
Milled rice	*Corcyra cephalonica*[*]	Egg	60	3 min	Luechai (2008)
Milled rice	*Orhyzaephilus surinamensis*	Adult	70	2 min	Srikam et al. (2014)
Milled rice	*Rhyzopertha dominica*	N/A	70	3 min	Janhang (2005)
Milled rice	*Rhyzopertha dominica*	Adult	70	2 min 30 sec	Sumetha et al. (2009)
Mung bean	*Callosobruchus maculatus*[*]	Egg, larva, pupa	74	3 min 40 sec	Na Pijit et al. (2011)
Rough rice	*Sitotroga cerealella*[*]	Egg, pupa	50	3 min 40 sec	Buapud et al.(2012)
Rough rice	*Sitophilus oryzae*	Adult, pupa	65	2 min	Wangsapa et al.(2015)
Tobacco	*Lasioderma sericorne*	Adult	104	4 min	Nutapong (2012)

[*] Adult was not in the test
N/A =not available

Materials and Methods

Insect rearing and efficacy test of RF treatment to control cigarette beetle

Cigarette beetles were cultured with green leaf tobacco or dried tobacco in flue cured Virginia tobacco (bright tobacco) at 28-32°C with 75% rh in saturated salt solution box in Stored Product Laboratory, at Department of Entomology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. Cigarette beetle eggs at 2-4 days old while larvae, pupae, and adults were 20-25, 30-32, 35-40 days old respectively after oviposition were placed on Virginia tobacco at approximately 12% mc. Fifty insects were put in dried tobacco in small plastic cups and tempered to 75% rh to acclimate insects The cup was put in an arena test chamber that is a cylinder of 16 cm in diameter with 5 cm height and was used to be exposed to RF heat t. Pilot scale radio frequency at 27.12 MHz was used to treat all stages of cigarette beetle, at temperatures of 55, 60, 65 °C and for 0, 1,2, and 3 minutes (9 combination treatments). The pilot scale of radio-frequency unit was developed and built from the Institute of Agricultural Engineering, University of Göttingen, Germany.

The effect of RF to cause insect mortality was examined after 42, 22, 12, and 3 days on egg, larval, pupal and adult stages, respectively. The number of survivalors was calculated for the insect mortality by deduction from the total. In the untreated control, insects were not treated with RF but maintained in tobacco leaves as were those exposed to treatments. Each treatment was replicated 4 times with 50 insects per replication. Insect mortality in untreated controls was corrected using Abbott formula (Abbott, 1925). All combinations of treatments between temperatures and time exposure were calculatedusing analysis of variance and LSD test.

Results

Eggs of cigarette beetle showed 79.8% mortality at the treatment using 55°C for 1 minute. The experiment showed complete mortality using RF treatments at 65°C for 1, 2 and 3 minutes. Although in each experimental unit was run individually the longer exposure times also showed

serially increasing mortality. The mortality of eggs exposed at 65°C in 1, 2 and 3 minutes showed significantly differences from others at 55 and 60°C (Table 2.). Both temperatures alone and time exposure alone caused significantly difference of insect mortality.

Table 2. Mortality of eggs of cigarette beetle exposed to different temperatures from radio frequency at 27.12 MHz for 1,2 and 3 minutes.

Temperature from RF treatment (°C)	Average mortality of eggs			Total[2]
	1 minute[1]	2 minutes	3 minutes	
55	79.79 ± 2.76 c	86.33 ± 3.33 bc	90.65 ± 3.13 b	85.59 ± 3.16 Y
60	80.00 ± 3.87 c	88.41 ± 2.69 b	93.04 ± 1.97 b	87.15 ± 3.82 Y
65	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 X
Total[3]	86.60 ± 6.70 B	91.58 ± 4.25 A	94.56 ± 2.80 A	

[1]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=6.73).
[2]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 3.88).
[3]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 3.89).

At 1 minute exposure time of 55°C- RF heat treatment, the mortality of cigarette beetle found 41.27 % with increasing of exposure time. This heat treatment caused 100 % mortality on larvae for either of 60°C for 3 minutes or 65°C for 2 minutes. There were both showed significant difference exposure time and temperature (Table 3).

Table 3. Mortality of larvae of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes.

Temperature from RF treatment (°C)	Average mortality of larvae			Total[2]
	1 minute[1]	2 minutes	3 minutes	
55	41.27 ± 3.62 e[1]	46.89 ± 1.95 de	52.29 ± 3.66 cd	46.82 ± 3.18 Z[2]
60	56.81 ± 2.17 c	93.62 ± 0.95 b	100.00 ± 0.00 a	83.48 ± 13.46 Y
65	90.28 ± 0.78 b	100.00 ± 0.00 a	100.00 ± 0.00 a	96.76 ± 3.24 X
Total[3]	62.79 ± 14.46 C	80.17 ± 16.74 B	84.10 ± 15.90 A	

[1]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=5.67).
[2]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 3.27).
[3]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 3.27).

For pupae stage, the mortality also was checked from the survival of insect after exposing to RF heat treatment. The mortality was present in the same pattern as in larval stage but only one treatment which achieve to kill insects all compared to in larval stage. It means the pupae required higher temperature of RF heat than in larval stage. Only 65°C for 3 minute- treatment showed completely control to pupae of cigarette beetle (Table 4).

Table 4. Mortality of pupae of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes

Temperature from RF treatment (°C)	Average mortality of pupae			Tota[2]
	1 minute[1]	2 minutes	3 minutes	
55	70.19 ± 1.21 g	74.11 ± 1.17 f	91.20 ± 1.47 d	78.50 ± 6.45 Z
60	85.61 ± 1.56 e	89.80 ± 1.31 d	96.44 ± 1.19 bc	90.62 ± 3.15 Y
65	94.72 ± 0.79 c	98.37 ± 0.88 ab	100.00 ± 0.00 a	97.70 ± 1.56 X
Total[3]	83.51 ± 7.16 C	87.43 ± 7.10 B	95.88 ± 2.56 A	

[1]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=3.12).
[2]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 1.81).
[3]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 1.82).

Adult mortality was checked after insect exposed to heat for 3 days. The results were similar to those in pupal stage. Only 65°C for 3 minute- treatment showed completely control to pupae of cigarette beetle (Table 4). There were significant differences among temperature treatments (55, 60 and 65°C).

Table 5. Mortality of adults of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes

Temperature from RF treatment (°C)	Average mortality of adults			Total[2]
	1 minute[1]	2 minutes	3 minutes	
55	51.38 + 2.87 d	81.17 + 2.78 b	87.16 + 2.26 b	73.24 + 11.06 Z
60	69.07 + 5.79 c	88.26 + 2.65 b	98.93 + 0.57 a	85.42 + 8.74 Y
65	81.09 + 4.71 b	98.95 + 0.56 a	100.00 + 0.00 a	93.35 + 6.14 X
Total[3]	67.18 + 8.63 C	89.46 + 5.17 B	95.36 + 4.11 A	

[1]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=8.66).
[2]Means in the same column followed by the same letter are not significantly different at P≥0.05 LSD = 5.00).
[3]Means in the same row followed by the same letter are not significantly different at the P≥0.05 (LSD = 5.00).

4. Discussion

Yu et al. (2011) and Conyers and Collins (2006) reported that eggs of cigarette beetle were the most tolerant to air dry heat and complete mortality of eggs required 50°C for 18 hours. Thus RF heat treatments would be an alternative control measure for solving the problem of using large amounts of energy. Nutapong (2012) demonstrated, larval and egg stages of cigarette beetle were more susceptible to RF heat than pupal and adult stages exposed to the radio frequency at 420 watts for 60 seconds. The rank of RF tolerance is: adult>pupal>larval= egg stages. Other stored product insects such as adult of *S. oryzae, S. zeamais* and *Rhyzopertha dominica* have demonstrated tolerance to RF heat (Faikrajaipuen et al. 2011; Sumetha et al., 2009; Wangsapa et al., 2015). On the other hand, eggs of Angoumois grain moth, *Sitotroga cerealella* (Buapud et al., 2012) and the cowpea weevil, *Callosobruchus maculatus* (Na Pijit et al., 2011*)* which both lay eggs on rice kernels and mung beans are RF heat tolerance and is likely due to their adherence to the seeds.

The treatments; 60°C for 3 minutes, and 65°C for 2 and 3 minutes caused 100% mortality of larvae. These results can be associated with greater water content in larvae which would cause higher temperature to kill insects due to high oscillation of water molecules or polar molecules as presented by Wangsapa (2016) who reported that moisture contents of *S. oryzae* larvae (66.53±0.8% mc) was greater than pupae(64.21±0.9%) and adults (46.96±0.4% mc), respectively.

Adult of cigarette beetle showed 51% mortality when exposed to RF at 55°C for 1 minute while the pupal stage showed 71.19%, but both adult and pupal stage were completely killed when exposed to treatments of 65°C for 3 minutes. This experiment would confirm that pupal and adult stages of cigarette beetle are the most tolerant to RF heat treatment. From previous experiment done by Nutapong (2012) the result also showed adult and pupal stages were the most tolerant to heat greater than egg and larval stages compared to the heat of 104°C for 180 seconds. Since the treatment of 65°C for 3 minutes caused 100% mortality of tolerant stage it can be recommended for commercial scale treatment. Thus, this result was able to minimize the heat of RF on controlling of all stages of cigarette beetle with 100% of the insect control.

While no work on the effects on progeny were performed in this work, Wangsapa (2016) found that thirty rice weevil, *S. oryzae* treated with 27.12 MHz in laboratory condition were able to produce 13.25±4.5 insects compared with the untreated control insects that produced 26.75±0.8 offspring. Srikam et al. (2014) also determined the effect of RF treatment on sawtoothed grain beetle, *O. surinamensis* infested rice. Their results found that when insects were treated with RF, the number of progeny produced from survivors was significantly less than in untreated controls. If adult of *O. surinamensis* were completely killed by RF heat treatment, there was no progeny production found.

This indicates that even if insects have the opportunity to lay eggs before the heat treatment was completed, all adults and their eggs would die when exposed to heat.

In dried tobacco process, there is the lamina re-drier process of tobacco which consisted of 4 continuous drier chambers at 80-88°C for 3 min in each chamber (personal contact), so this treatment temperature would have less effect ton tobacco quality. The data was supported by research done in Nutapong (2012) which treated dried tobacco with 27.12 MHz of RF energy at 104°C for 3 min caused slightly changed for Burley green leaf tobacco (B2F grade). In dried tobacco treated with RF the nicotine content was 4.13% significantly higher than in untreated control (3.18%). The chemical and physical properties of RF-treated tobacco were remained in Burley green leaf tobacco standard. According this experiment, the death profile of cigarette beetle with 65°C for 3 minutes has potential to apply on dried tobacco with less effect to tobacco quality.

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Lethal effects and mechanism of infrared radiation on *Sitophilus zeamais* and *Tribolium castaneum* in rough rice

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Abstract

The objective of this study was to investigate the characteristics of adult *Sitophilus zeamais* and *Tribolium castaneum,* and the 21.1% dry base (d.b.) MC of rough rice by ATR-FTIR spectra, and determine the theoretical optimum infrared (IR) heating temperature of the tested samples. In laboratory experiments, a ceramic IR drying device was used to heat infested rough rice to research the mortality of *Sitophilus zeamais* and *Tribolium castaneum*, the drying characteristics of rough rice, and milling quality. The theoretical calculation optimum temperature of IR heating was 300 ˚C according to the results of FTIR spectra. In addition, the effects of the different IR radiation intensities and heated rough rice temperatures on mortality of insects, moisture removal, and milling quality were determined in this text. A high insect mortality, heating rate and corresponding high moisture removal were achieved by using IR heating. After heating, tempering process significantly increased insect mortality when the heated tempered rice temperature was less than 55 ˚C, and improve moisture removal and milling quality of rough rice during nature air cooling. When the rice heated under the IR radiation intensity of 2780 W/m^2 for 110 s, the rice temperature reached 60.2˚ ± 0.5˚C, 100% mortality of *S. zeamais* and *T. castaneum,* and 3.97 percentage points of moisture removal during the heating period after tempering and natural cooling. In addition, the high rice milling quality can be achieved after tempering treatment. Therefore, it can be concluded that the optimum conditions of simultaneous disinfestation and drying were 60 ˚C rice temperature under the IR radiation intensity of 2780 W/m^2, followed by tempering and natural cooling.

Keywords rough rice; infrared radiation; *Sitophilus zeamais*; *Tribolium castaneum;* disinfestation; milling quality

Introduction

Rough rice is a major source of food for both human and animals as one of the three main grain varieties. During storage, infestations by stored grain pests may occur if the internal and external conditions are suitable (Lee et al., 2001). It was estimated that 10-40% of worldwide annual production of grain (Mishra et al., 2013) and 27% of the milled rice was lost due to the infestation of pests (Alfonso-Rubí et al., 2003). Various methods of pests control have been implemented to protect the rough rice, in which the chemical fumigation is the most widely used method for disinfestation (Ogendo et al., 2010). However, the effect of chemical fumigation is becoming less effective due to the increase of resistance of insect pests. Chemical application may affect the environment and grain, which could potentially affect the health of human beings (Vadivambal, et al., 2010). Therefore, it is urgent to research and develop alternative technology to chemical fumigation methods.

Infrared radiation (IR) is an efficient and safe physical process method, with wavelengths range from 0.75 to 100 μm. IR can be directly transferred to the material without medium, and converted into heat after the absorption of the electromagnetic wave. In the 1940s, gas-fired IR technology was firstly used for grain disinfestation. Frost et al. discovered that the mortality rate increased with the temperature rise of insect pests (Frost et al., 1944). In the 1960s to 1980s, gas-fired IR method began to be investigated to kill different immature stages and adults of stored-grain pests (Cogburn 1967; Cogburn et al., 1971; Kirkpatrick and Tilton, 1972b; Tilton et al., 1983). Tilton et al. (1963) exposed three species of insects commonly found in grain to IR and achieved 100% of mortality with the temperature of grain ranging from 65 ˚C to 70 ˚C. It was also reported that adult *Rhyzopertha dominica* was killed by IR at 57 ˚C (Kirkpatrick et al., 1972a). The infrared generators in the above

study had an open flame with temperatures exceeding 926 °C (Kirkpatrick and Cagle, 1978). Such high temperatures are not suitable for grain processing because of potential safety hazard. In recent years, a sort of flameless catalytic infrared technology was developed for various drying applications, and these catalytic emitters generated temperatures of less than 500 °C. Khamis et al. (2010) treated *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* by the flameless catalytic infrared, and proved that there was significant correlation between mortality and temperatures of the 3 pests according to logistic regression statistics. Pan et al. (2008) concluded that rough rice was heated to 60 °C followed by tempering and slow cooling can achieve simultaneous drying and disinfestation with high rice milling quality. However, the effects of different IR intensity on the mortality of insects and its theoretical mechanism of insect lethality were unclear. Therefore, the objectives of this research was to investigate the effect of IR on rice disinfestation and rice quality, and analyze the differences of rice and insect heating rate to reveal the relevant mechanism.

Materials and methods

Preparation of insect and rough rice samples

As one of the main cultivated japonica rice in Jiangsu province, freshly harvested No.5 Huaiyin japonica rough rice was selected for this study, which was obtained from Shibuqiao Grain Reserve Depot, Nanjing, Jiangsu province. The moisture content of rough rice was 21.1 ± 0.5% in dry basis (d.b.). All moisture content was determined by the standard air oven method at 130 ± 2 °C for 24 h (ASAE, 1995) in an electric dry oven (Model 101-3AS, Sujin Instrument Factory, Shang Hai, China) and was expressed as percentage in dry mass basis with triplicates.

Two major stored-grain insects, *Tribolium castaneum* and *Sitophilus zeamais* obtained from Chengdu Grain Storage Research Institute, Chengdu, Sichuan province, were used for the study. The *T. castaneum* samples were grown in laboratory on wheatmeal and yeast extract (Oxoid Lid, Wade Road, Basingstoke, Hants, UK) and maintained at 28 ± 2 °C and a relative humidity (RH) of 64 ± 3% (Kirkpatrick 1975). The *S. zeamais* was maintained on organic whole wheat (Beidahuang Qinmin Organic Foods CO., LTD) in incubator at a temperature of 30 ± 2 °C and a RH of 70 ± 2%.

ATR-FTIR spectra of insects and rough rice

IR heating results in the vibration and rotation of atoms and molecules and leads to a rise in sample temperature. In order to research the IR absorption characteristics of insect and rough rice, the spectral information of live adults and rough rice were collected by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) (Tensor 27, Bruker Corporation, Germany). A single adult or rough rice kernel was placed at the center of the ATR ZnSe crystal (Pike, USA) using a pair of forceps. The background spectrum of the air was scanned before determination of the sample. Through ATR-FTIR application, the spectral region of sample ranged from 4000 to 600 cm^{-1} The number of scans was 64, and the resolution was 4 cm^{-1}. All reported data were means of triplicates. The results of samples exposed to ATR-FTIR spectra were calculated to evaluate the optimum radiation temperature during IR heating following Wien displacement law with equation (1).

$$T = 2898/\lambda_{max} \qquad (1)$$

Where T is blackbody temperature, K. λ_{max} is the peak wavelength of blackbody radiation energy.

Infrared heating treatment

A laboratory-scale ceramic infrared drying device provided by Maybo Innovation (MB-EHR12/10KW, Zhen Jiang) was used to treat the rough rice and insect samples. The infrared drying device consists of an IR emitter, a circulating fan, a sample holder and a control panel. The IR emitter was the source of IR radiation (HTS, Elstein-Werk, Germany), and it had a surface temperature of about 630 °C and

corresponding peak wavelength of 3.2 μm, assuming a blackbody. The sample holder was a steel reticulation of dimension 50 cm ×35 cm.

The samples, made up of a mixture of rough rice and insects were placed in a single layer on a tray with a loading rate of 2.08 kg/m^3 at a 20 cm distance under the IR emitter. The mixture samples were separately heated to 50, 55, 60 and 65 ˚C under different IR emitter temperatures (200, 300, 400, and 500 ˚C), which corresponds to the IR radiation intensity of 2125, 2780, 3358 and 3974 W/m^2. The temperature of IR emitter was controlled using the control panel. The radiation intensity of heated rice was measured with Ophir thermal excimer absorber head (FL205A, Ophir, Washington, USA) under the different temperature of IR emitter. The temperature of heated rice was measured by using a type-K thermocouple (RDXL4SD, OMEGA Engineering Inc. Stamford, USA), which can be monitored in real time by contact of the thermocouple to the rough rice. The experiments were implemented using rough rice samples and insects with a proportion of 50 insects/100 g rough rice (Yan et al., 2014). The single layer of infested rough rice was uniformly distributed on the holder, insects were randomly distributed.

Long period of IR heating without tempering would affect milling quality of rough rice due to the high heating rate of rice (Ding et al., 2015a). To improve the rice milling quality, treated rough rice was immediately transferred into sealed containers and placed in an incubator with the same temperature of heated rice for 4 hours (Khir et al., 2011). The tempered and non-tempered samples were prepared for analyzing the influences of tempering on disinfestation and milling quality. After the tempering or no-tempering treatment, the samples were placed on a laboratory bench for 40 mins of natural cooling to room temperature of 23 ±1 ˚C.

Determination of temperature distribution of infested rough rice

Water molecules are highly polar and easily absorb the radiation energy into heat. The moisture content of insects is much higher than that of rough rice, and results in the heating rate of insects higher than that of rough rice under IR treatment. In order to analyze the temperature distribution of rough rice and insects, a series of temperature monitoring experiments were conducted. Based on the theoretical calculation optimum temperature of IR heating was 300 ˚C. This is according to the results of FTIR spectra, and the spectra of adult *S. zeamais* and *T. castaneum* were similar. Thus, the adult *T. castaneum* samples were placed in the circular area uniformly in a thin layer (3 mm thick). The mixture of rough rice and adult *T. castaneum* were placed in single layer on tray at a 20 cm distance under IR emitter, and were separately heated under the IR emitter radiation intensity of 2780 W/m^2 for 160 s. After IR heating, the temperatures distribution photographs of heated rice and insects were captured by infrared thermal imager (TiX580, Fluke Corporation. USA). The image results could be analyzed and processed by its own software (FLUKE SmartView 3.7).

During the IR heating experiments, the insects number were much lower than rough rice and they tend to move to the bottom of rice layer, resulting in the temperature determination of insects difficultly. To determine the temperature distribution of insects by IR heating, the heated insects were concentrated in a circle area without rice samples, and the radius of the circle area was about 3 cm.

Assessment of mortality of Sitophilus zeamais and Tribolium castaneum

Treated rough rice samples were placed in jars and placed in incubator at 28 ˚C and 70% RH to allow development of any surviving *S. zeamais* and *T. castaneum*. Adult mortality was determined after 24 h. Mortality of eggs, larvae and pupae was based on the number of adults that emerged from those stages (Khamis et al., 2010). The values were compared with adult emergence in untreated rough rice samples.

Determination of rice milling quality

Milling quality is is an important factor for the processing and sale of rice, the most important rice milling quality indices are total rice yield (TRY) and head rice yield (HRY). To evaluate the effects of IR heating treatment, the 400 g rice samples were dehulled and milled using Yamamoto Husker (FC-2K) and Yamamoto Rice Mill (VP-222N, Yamamoto Co. Ltd., Japan). The rice samples were milled three times to achieve well-milled rice as defined by the standard GB 1350-2009. The settings of throughput and whitening were 1 and 4 during the first two millings and 1 and 5 during the third milling process (Ding et al., 2015b; Pan et al., 2007). TRY was the percentage of milled rice weight divided by the weight of untreated rough rice (GB/T 5495-2008). HRY was determined by GB/T 21719-2008. All reported data of milling quality indicators are means of triplicates.

Statistical analysis

Analysis of variance (ANOVA) was performed in a completely randomized design, data of the mortality of insects and rice milling quality were statistically analyzed with PASW 18 (IBM SPSS Statistics, Chicago, IL, USA) at a 95 % confidence level. One-way ANOVA with Duncan's multiple comparisons test were applied to compare the data among different radiation intensity and different exposed time. Significance was reported at $p < 0.05$ for all data.

Results and Discussion

Characteristics of the ATR spectra of insects and rough rice

The average ATR-FTIR spectra of adult *S. zeamais*, *T. castaneum*, and rough rice as shown in Fig. 1. The spectra form of adult *S. zeamais* and *T. castaneum* were similar. There are two significant absorption region of adult *S. zeamais* and *T. castaneum*, that was mainly distributed in the wavenumber range of 3600-3000 and 1800-800 cm^{-1}. The rough rice has a similar significant absorption in the wavenumber range of 3600-3000 cm^{-1}. The spectrum of the rough rice also has a higher absorption in the wavenumber range of 1250-800 and 1700-600 cm^{-1}, which was different from the fingerprint region in 1800-800 cm^{-1} of the *S. zeamais* and *T. castaneum*. Since the *insects* consist of higher amount of proteins and lipids than rough rice that mainly absorb the IR with wavelength ranges from 5.71 to 10 µm (corresponding wavenumber were 1751 to 1000 cm^{-1}) (Pan and Atungulu, 2011). Therefore, the spectra of adult *S. zeamais* and *T. castaneum* could absorb higher IR within 1800-800 cm^{-1}.

The absorption peak around 3600-3000 cm^{-1} is related to vibration of O-H, with the relevant component of O-H being water and/or carbohydrates in the samples (Ionel 1992). The maximum absorbance of adult *S. zeamais* and *T. castaneum* in this range was 0.23 and 0.16, which are higher than that of the rough rice (0.05). This is may be due to the higher MC of adult *S. zeamais* and *T. castaneum* than that of the rough rice. The corresponding temperature of the spectra region were range from 869 ˚C to 1043 ˚C according Wien displacement law, and this is too high for large-scale commercial processes.

The maximum IR absorbances of adult *S. zeamais* and *T. castaneum* were 0.2 and 0.3 at 1632 cm^{-1}, and were significantly higher than that of rough rice (0.15 at 1020 cm^{-1}). Obviously, the IR absorption ability of insects is higher than that of rough rice. To improve the efficiency of IR drying and disinfestation, the strong absorption wavelength range should be applied during IR treatment. According Wien displacement law, the corresponding temperature of the wavenumber of 1800 cm^{-1} was 248 ˚C. As practical materials, the IR absorption rate of *S. zeamais*, *T. castaneum* and rough rice were less than that of blackbody. To facilitate the experiment operation and commercial process requirements, the IR radiation temperature of 300 ˚C should be more effective with low energy consumption.
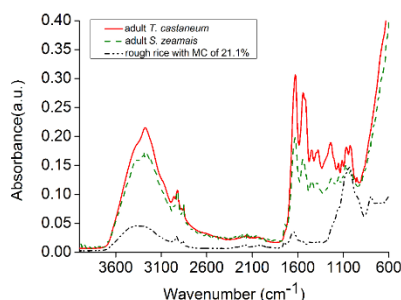
Fig. 1 Average attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of adult *S. zeamais*, *T. castaneum* and rough rice with MC of 21.1%.

Mortality of insects

Mortality of Sitophilus zeamais

The mortality of egg, larvae, pupae, and adult stages of *S. zeamais* is given in table 1. The control mortality of the four stages of *S. zeamais* was zero. After the rice samples were heated to temperatures of 50, 55, 60 and 65 °C, the mortality of adult *S. zeamais* was 81±4.6, 98±3.5, 99±0.6 and 100% at the radiation intensity of 2125 W/m^2 with non-tempering treatment, respectively. The mortality of *S. zeamais* was positively correlated with the heated rice temperature, and above 96 % when the rough rice temperature exceeded 55 °C.

After the rough rice temperature heated to 50 °C, regardless of tempering, the four stages of *S. zeamais* were still living. The results revealed *S. zeamais* could not reach the complete death when the rough rice temperature heated to 50 °C in a short time (110 s). However, the mortality of adult *S. zeamais* at the radiation intensity of 2125, 2780, 3358 and 3974 W/m^2 was 81±2.6, 76±3.5, 73±3.5, and 93±3.1 % when the rough rice temperature of 50 °C without tempering, respectively. Only 7±3.1 % adult *S. zeamais* survived at the radiation intensity of 3974 W/m^2 without tempering treatment. Similar ascend trends of the mortality of four stages of *S. zeamais* were observed along with the increase of IR intensity at same heated rough rice temperature. The *S. zeamais* temperature rose faster at higher radiation intensity, and the temperature of *S. zeamais* increased rapidly in a short time, which could result in dramatic changes in *S. zeamais*. It was also shown that the mortality of adult *S. zeamais* was lower than that of other stages at the same experimental condition when the heated rice temperature was 50 °C without tempering treatment. For example, the mortality of *S. zeamais* egg, larvae, pupae, and adult stages was 87±4.6, 83±4.0, 81±4.5 and 76±3.5 % at the radiation intensity of 2780 W/m^2 and the heated rice temperature of 50 °C without tempering treatment, respectively. This might be due to the fact that the adults move to the lower temperature area when heated by IR.

The mortality of all life stages of *S. zeamais* was 100% when the rough rice was heated to 65 °C, regardless of tempering, and 55 °C with tempered rice that achieved 100% mortality. The results obtained agreed with reported results that the time to mortality of the insect was less than 40 s when they heated to temperature above 65 °C (Kirkpatrick and Tilton, 1972b). In addition, the non-tempered samples, especially at low rice temperature, had fewer insects survived than the samples with tempering. This could be because the lethal temperature of *S. zeamais* was about 56 °C (Schroeder and Tilton, 1961), the lethal rate would significantly increase when *S.zeamais* for a long time (4h) at the rough rice temperature beyond 50 °C.

Mortality of Tribolium castaneum

The mortality of egg, larvae, pupae, and adult stages of *T. castaneum* is given in table 2. The control mortality for the four stages of *T. castaneum* was zero. There are many similarities to the results of *S.*

zeamais mortality. For instance, the mortality of *T. castaneum* adult is the lowest among the four stages. In addition, at the 65 °C of the rice temperature, regardless of tempering, 100% mortality were found in all the samples under such treatment. Moreover, tempering treatment could effectively improve the mortality of insect. However, the mortality of egg, larvae, pupae, and adult stages of T. *castaneum* was 75±2.1, 73±2.6, 64±3.2 and 56±2.1 % at the rice temperature of 50 °C without tempering, respectively, which was lower than that of four stages of *S. zeamais* (by 87±4.6, 83±4.0, 81±4.5 and 76±3.5 %) at the same treatment conditions. The results were especially obvious when heated rice temperatures were 50 and 55 °C. This shows that *T. castaneum* has a stronger heat resistance than *S. zeamais* at these temperatures. In addition, 100% mortality was observed in all samples for the four stages of *T. castaneum*,at rice temperatures of 60°C.

Based on these results, it can be concluded that insects tested can be effectively killed by using IR, especially with the tempering treatment. It is recommended that the rice temperature of IR heating be controlled at close to 60 °C, and followed tempering treatment, which could ensure that the mortality of *S. zeamais* and *T. castaneum* was 100%.

Temperature distribution of rough rice and insects

The rough rice sample temperatures changes during different heating durations under different radiation intensities were shown in Fig. 2. It was proposed that the heated rice temperature of 60 °C was the suitable drying parameter of IR drying for rough rice (Ding et al., 2015b). In this research, after 30 s of IR heating with intensity of 3974 W/m^2, the temperature of the single layer of rice samples with MC of 21.1±0.5% achieved 60.5±1.8 °C. For the IR intensity of 2125, 2780 and 3358 W/m^2, the rice temperatures increased to 10.7+23.2 °C, 20.5+23.2 °C and 39.0+32.2 °C after 60 s of heating, respectively. It was obvious that the IR heating could rapidly increase the rice temperature. Because IR is an electromagnetic wave with wavelength of 0.75 to 1000 µm (Carlomagno et al., 2002). When the infrared electromagnetic wave acts on the rice surface, the electric, vibrational, and rotational states of atoms and molecules in rice will be changed accordingly, and the IR energy absorbed by rice will be transformed into thermal motion of molecules allowing rice to heat and evaporate the water for drying (Matsuoka 2011).

Under the same loading capacity and thickness of rough rice, when the radiation intensity was 2125 W/m^2, the temperature rough rice temperature rose to 60 °C after 260 s exposed time. In general, the rough rice temperature was linear with the exposed time, it means that the average heating rate of rough rice was 0.14 °C/s. Similarly, under the radiation intensity was 2780 and 3358 W/m^2, the temperature rose to 60 °C after 110 s and 60 s exposed time, and the corresponding average heating rate of rough rice was 0.34 °C/s and 0.62 °C/s. The results revealed that the heating rate of rough rice and radiation intensity is positively correlated, and suggests that IR intensities may achieve a higher heating rate for rough rice. This is because that the higher energy could cause stronger vibration of molecules in rough rice, which leading to faster heating rate of rough rice. However, when the radiation intensity was 3974 W/m^2, the temperature of rough rice was 72.3±1.3 °C after 40 s of heating, and the average heating rate of rough rice was 1.23 °C/s, the heating rate of rough rice was high and easily cause gelatinization and denaturation of rough rice starch and protein, even lead to the burning of heated rough rice. Therefore, in order to avoid the harm of high IR radiation intensity and improve heating efficiency for commercial drying processing of rough rice, the radiation intensity of 2780 and 3358 W/m^2 were more operable and reasonable among the four radiation intensities.

Table 1 Mortality of life stages (egg, larvae, pupae, adult) of *S. zeamais* expose to IR radiation intensity of 2125, 2780, 3358 and 3974 W/m^2 with or without tempering.

Data (mean ± SD) in each column with different letters have significant differences (p<0.05).

Radiation intensity(W/m²)	Rice temperature (°C)	Tempering	Mortality of different life stages of *S. zeamais* (%)			
			egg	larvae	pupae	adult
2125	50	Yes	94±2.5ac	95±2.0a	92±2.3a	92±4.5a
	50	No	88±3.2b	86±3.2b	82±2.6b	81±4.6b
	55	Yes	100d	100e	100d	100c
	55	No	100d	100e	100d	98±3.5c
	60	Yes	100d	100e	100d	100c
	60	No	100d	100e	100d	99±0.6c
	65	Yes	100d	100e	100d	100c
	65	No	100d	100e	100d	100c
2780	50	Yes	93±2.6a	91±3.8c	88±2.3c	83±4.6b
	50	No	87±4.4b	83±4.0d	81±4.5b	76±3.5d
	55	Yes	100d	100e	100d	100c
	55	No	100d	100e	99±0.6d	98±2.5c
	60	Yes	100d	100e	100d	100c
	60	No	100d	100e	100d	99±1.5c
	65	Yes	100d	100e	100d	100c
	65	No	100d	100e	100d	100c
3358	50	Yes	96±2.1c	94±3.6a	89±2.6c	82±3.2b
	50	No	87±3.0b	85±3.1bd	78±2.1e	73±3.5d
	55	Yes	100d	100e	100d	100c
	55	No	100d	100e	100d	96±2.9c
	60	Yes	100d	100e	100d	100c
	60	No	100d	100e	100d	100c
	65	Yes	100d	100e	100d	100c
	65	No	100d	100e	100d	100c
3974	50	Yes	100d	100e	100d	100c
	50	No	100d	100e	95±1.2f	93±3.1a
	55	Yes	100d	100e	100d	100c
	55	No	100d	100e	100d	98±2.6c
	60	Yes	100d	100e	100d	100c
	60	No	100d	100e	100d	100c
	65	Yes	100d	100e	100d	100c
	65	No	100d	100e	100d	100c

Table 2 Mortality of life stages (egg, larvae, pupae, adult) of T. castaneum expose to IR radiation intensity of 2125, 2780, 3358 and 3974 W/m² with or without tempering.

Radiation intensity(W/m²)	Rice temperature (°C)	Tempering	Mortality of different life stages of *T. castaneum* (%)			
			egg	larvae	pupae	adult
2125	50	Yes	84±3.6ab	82±2.5a	78±3.8a	74±5.7a
	50	No	67±4.4c	68±2.5b	57±6.5b	52±9.5b
	55	Yes	100f	100h	96±3.6cd	92±3.5cd
	55	No	92±4.6d	93±3.2c	88±4.6ef	80±3.2ef
	60	Yes	100f	100h	100d	100g
	60	No	100f	100h	98±1.5cd	93±2.1cd
	65	Yes	100f	100h	100d	100g
	65	No	100f	100h	100d	100g
2780	50	Yes	91±2.1d	85±1.5d	78±4.0a	72±7.6a
	50	No	75±2.1e	73±2.6e	64±3.2g	56±2.1b
	55	Yes	100f	100h	100d	100g
	55	No	98±1.5f	96±1.7f	91±1.5fh	84±3.2f
	60	Yes	100f	100h	100d	100g
	60	No	100f	100h	100d	96±2.5dg
	65	Yes	100f	100h	100d	100g
	65	No	100f	100h	100d	100g
3358	50	Yes	87±2.5b	87±3.5d	79±3.2a	75±2.3a
	50	No	78±2.9e	75±1.2g	67±4.0i	62±2.6h
	55	Yes	100f	100h	100d	96±2.1dg
	55	No	100f	96±1.7f	94±2.1ch	89±3.2c
	60	Yes	100f	100h	100d	100g
	60	No	100f	100h	98±1.5cd	97±2.6dg
	65	Yes	100f	100h	100d	100g
	65	No	100f	100h	100d	100g
3974	50	Yes	92±3.6d	91±1.5c	85±3.2e	78±5.9ae
	50	No	83±4.6a	78±3.8g	72±3.8j	65±5.5h
	55	Yes	100f	100h	100d	98±1.7dg
	55	No	100f	100h	96±2.3cd	93±1.5cd
	60	Yes	100f	100h	100d	100g
	60	No	100f	100h	100d	100g
	65	Yes	100f	100h	100d	100g
	65	No	100f	100h	100d	100g

Data (mean ± SD) in each column with different letters have significant differences (p<0.05).
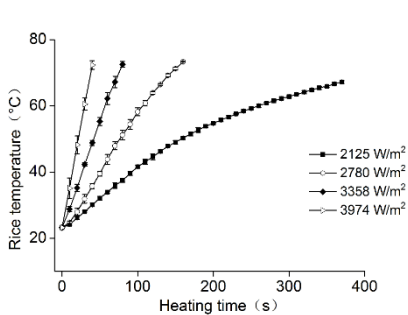
Fig. 2 Heating curve of rough rice under IR radiation intensity of 2125, 2780, 3358, 3974 W/m².
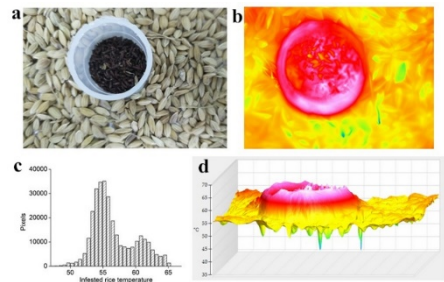
Fig. 3 Thermal (a) and visual (b) images of infested rough rice heated for 160 s under IR radiation intensity of 2780 W/m². The rectangle area represented rough rice, and circular area represented adult *Tribolium castaneum*.

Thermal and visual images of infested rough rice heated for 160 s under IR radiation intensity of 2780 W/m2 were shown in Fig. 3a and Fig. 3b. In thermal image of infested rough rice, the red part indicated that the temperature was high, and the temperature range from 50 ˚C to 80 ˚C. The adult T. castaneum temperature was 74.3±2.4 ˚C while the rice temperature was only 71.6±3.6 ˚C. Based on the analysis of FTIR results, when the IR radiation intensity was 2780W/m^2, the maximum IR absorbance of adult *T. castaneum* was 0.3 at 1632 cm^{-1}, and was higher than that of rough rice (0.15 at 1020 cm^{-1}). Obviously, the IR absorption ability of adult *T. castaneum* was higher than that of rough rice. In addition, the temperature of rough rice heated using IR was uniform in Fig. 3a. The results also confirmed the previous finding that the uniformity of IR heating was higher than that of microwave heating (Kirkpatrick et al., 1972).

Moisture removal

The MC of rice samples during IR heating after tempering and non-tempering with different radiation intensities were shown in Fig. 4. The MC of the rough rice that heated to 50 ˚C under 3358 W/m^2 had decreased 1.1 percentage point, which was the least moisture removal (MR) samples of all. Even though, IR heating could effectively remove the moisture in rough rice. The reason for high drying efficiency of IR was that the moisture molecules in rough rice could absorb IR easily and transfer the electromagnetic energy into intermolecular friction, which may lead to the temperature rising and moisture evaporation. Ding et al. (2015b) reported that the drying rate of studied IR heating process for rough rice was 21 and 186 times of that of hot air drying and ambient air drying, respectively.

For the rice samples dried to the same temperature under IR heating, higher moisture removal was usually achieved by those dried with lower IR intensity, which may due to the low energy efficiency of process under high IR intensity. The MR of rice was strongly positively correlated with heated rice temperature. Under the radiation intensity of 2125 W/m^2, the MC of non-tempering samples varies 19.3% to 15.6% in the tested temperatures range from 50 ˚C to 65 ˚C. The higher rice temperature means that the more IR energy were absorbed, which could result in the molecule friction and moisture evaporation of rough rice.

It was also found that the MR after tempering treatment was higher than that without tempering. For instance, when the rice was heated to 60 ˚C, the MR of rice after tempering was 1.2, 1.2, 1.3, and 1.5 percentage points lower that the heated rice after non-tempering, which showed that tempering treatment significantly improved the MR during cooling. The tempering process could reduce the moisture gradient in rice kernels and allow the moisture to distribute uniformly before natural cooling. For the heated rice without tempering treatment, there was a significant moisture gradient in the rough rice kernels and low MC near the rice surface, which led to less moisture removal during cooling. Therefore, tempering process is an essential step to increase the moisture removal during cooling, especially when the rice was heated to a high temperature. The results were similar to those reported by other researchers (Aquerreta et al., 2007; Dong et al., 2010; Pan et al., 2008).

Rice milling quality

The TRYs of infrared dried rough rice were shown in Fig. 5a. Compared with the untreated rough rice, the infrared drying without tempering have negative effects on the milling quality of rough rice. In contrast, the TRYs of tempered rice by using IR were higher than that of non-tempered rice. For instance, when the radiation intensity of 2780 W/m^2, the TRYs of rice dried by IR heating followed with tempering treatment were 2.6 percentage points higher than the untreated rough rice when the rough rice heated to 60 ˚C. The TRYs of the rough rice heated to 60 ˚C under IR intensity of 2125, 2780, 3358 and 3974 W/m^2 with tempering were 69.4±0.5%, 70.2±0.7%, 69.5±0.6% and 68.6±0.9%, which were higher than other groups of rough rice that heated to 50, 55 and 65 ˚C. However, the rice samples treated under 3974 W/m^2 with tempering had lower TRYs than the other rice samples

heated to 50, 55 and 65 °C with tempering, which revealed that the high radiation intensity would decrease the TRY of rough rice.
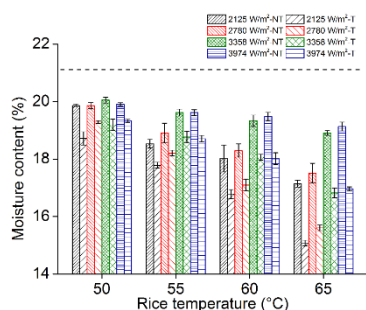
Fig. 4 Measured moisture content under IR radiation intensity of 2125, 2780, 3358, 3974 W/m² with and without tempering for rough rice after heating to various temperatures. (T- Tempering, NT- No tempering.) In addition, the dotted line indicated the control group value (untreated samples).
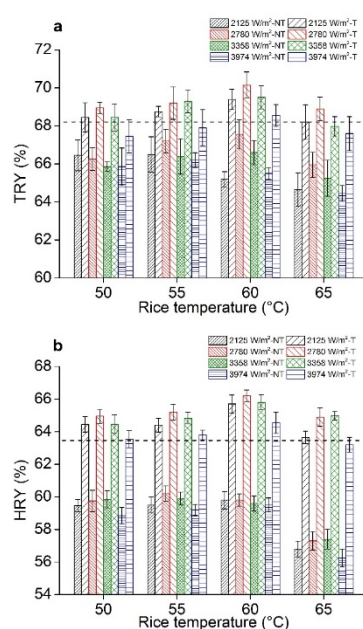
Fig. 5 Total rice yields (TRY) (a) and head rice yields (HRY) (b) of rough rice under IR radiation intensity of 2125, 2780, 3358, 3974 W/m² for rough rice after heating to various temperatures. (T- Tempering, NT- Non tempering.) In addition, the dotted line indicated the control group value (untreated samples).

Similar results were also found for the HRYs in Fig. 5b. The rough rice heated using IR with non-tempering had significantly lower HRYs than the untreated rough rice. For example, the HRYs of non-tempered rice that heated to 65 °C were 6.2 to 7.4 percentage points less than the untreated rice. However, the HRYs of the rice heated using IR with tempering were higher than untreated rice except for the rice sample dried under the IR intensity of 3974 W/m². The maximum HRY was 66.2±0.4% that was heated to 60 °C under the intensity of 2780 W/m² with tempering.

Based on the milling quality results, the highest milling quality was achieved by IR heating to 60 °C followed with tempering and natural cooling. Conssen (2002) reported that the glass state in rice may transfer into a rubbery state at 60 °C, and may result in a large amount of moisture removal and well maintaining of milling quality. Since the IR could penetrate the single layer of rough rice, the internal moisture in rice kernels could uniformly absorb IR as the moisture distributed close to the surface. Therefore, the temperature and moisture gradient were kept in same direction (from inside to outside) which could reduce the moisture gradient increase between internal to surface in rice kernels, and contribute to the good rice milling quality. In addition, tempering treatment plays an essential role for maintaining the rice milling quality. During cooling process, based on the glass transition hypothesis, the surface temperature and moisture of rice changed first and starch reached glass state during cooling. However, the center temperature and moisture in the rice kernel were still in rubbery state. Due to the different stage of starch in the rice kernel, stress and fissure were generated that resulting in low TRY and HRY. Therefore, tempering process is important for rough

rice IR drying that could maintain the uniformity of temperature and moisture in the rice kernel and preserve the quality effectively.

Conclusions

IR heating was used as an alternative disinfestation method of *S. zeamais* and *T. castaneum* in rough rice. The theoretical calculation optimum temperature of IR heating was 300 ˚C according to the results of FTIR spectra, which corresponding to the IR intensity of 2780 W/m^2. After the IR radiation intensity of 2780 W/m^2 for 110 s with tempering, the mortality of *S. zeamais* and *T. castaneum* achieved 100 % while the rice temperature reached 60.2˚ ± 0.5 ˚C. Besides, 3.97 percentage points of moisture were removed and rice milling quality were well maintained. When the rough rice was heated to 65 ˚C, the insects could be completely killed regardless of tempering in all samples. Although the higher mortality of insect could be achieved with the increase of heated rice temperature, the higher IR heating intensity and long exposure time might reduce the rice milling quality. Therefore, the tempering treatment after IR heating is important to achieve high insect mortality and moisture removal with good rice milling quality. It can be concluded that the rough rice heated under IR of 2780 W/m^2 to 60 ˚C with tempering and cooling is a feasible processing method for rough rice disinfestation and drying.

Acknowledgements

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Effect of passing *Beauveria bassiana* through alkane based media on the adult mortalities of *Rhyzopertha dominica* and *Sitophilus oryzae*

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Abstract

Entomopathogenic fungi have been investigated for management of stored product pests as alternatives to chemical control. *Beauveria bassiana* is commonly considered and thus increasing its efficacy has also been studied. The purpose of this study is to evaluate the effect of passing two *B. bassiana* cultures (wild and single-spore cultures) through n-hexadecane and n-octocasane based media on *Rhyzopertha dominica* and *Sitophilus oryzae* adult mortalities. For each Petri plate, 2 ml of 10% alkane was spread, let to evaporate and fungus was inoculated. After sporulation, spores for pathogenicity tests were produced by solid fermentation method on rice. Pathogenicity tests were conducted by application of 500 ppm (w/w) spores in wheat on 20 adults at $25\pm2°C$, 65 ± 5 r.h. in darkness with five replications. The efficacy of wild culture towards *R. dominica* adults was enhanced in both treatments. Mortality in 7 days increased from 35% to 55 and 69% when n-hexadecane and n-octocasane were used, respectively. Similarly, these treatments increased 14-day mortalities from 65% to 77 and 87%, respectively. Treatment of single-spore culture, however, either showed no change or reduced mortality. Passing both cultures through both alkane based media did not statistically affect the activity against *S. oryzae*. This study illustrated that increasing the virulence of *B. bassiana* is possible for *R. dominica* and increase depends both on the starting fungus culture and alkane used. Starting with a wild fungus culture with a wider genetic diversity, and using n-octocasane can produce a better enhancement.

Keywords: microbial control, biological control, virulence, entomopathogen.

1. Introduction

Cereals are produced throughout the world as nutrition for both humans and livestock. These commodities generally require storage for at least a short time and need to be protected against

insect and mite pests. Unprotected stored grains usually lead to quantitative and qualitative loss of grain and reduction of seed germination (Moino et al., 1998; Padin et al., 2002; Haq et al., 2005; Stejskal et al., 2015). Although synthetic insecticides have been used to control stored product pest populations (Athanassiou & Palyvos, 2006), they have various negative consequences such as residue accumulation in products (Ferizli et al., 2005), hazardous effects to humans and the environment (Michalaki et al., 2007), and pest resistance (Arthur, 1996). Therefore, there have been increasing efforts to find environmentally friendly and nontoxic ways to control these pests. Entomopathogenic fungi have been one of the considered alternatives (Moino et al., 1998; Michalaki et al., 2007; Sewify et al., 2014; Wakil & Schmitt, 2014) because they are natural and safer for humans and the environment (Moore et al., 2000). Bioinsecticide potential of entomopathogenic fungi against various insect pests of stored products have been established with a number of studies (Cherry et al., 2005; Wakil & Ghazanfar, 2010; Shams et al., 2011; Barra et al., 2013; Khashaveh & Chelav, 2013; Sewify et al., 2014). They have also been considered potential in combination with diatomaceous earth (Athanassiou & Steenberg, 2007; Athanassiou et al., 2008; Wakil et al., 2011; Riasat et al., 2011, 2013; Shafighi et al., 2014). Enhancing the pathogenicity of a potential fungal isolate would increase its value as biocontrol agent and has been the subject of many studies (Ortiz-Urquiza et al., 2015). One way of doing this is the modification of culturing media by using alkanes as carbon source (Crespo et al., 2002; Pedrini et al., 2011; Barra et al., 2015). In this study, the effect of passing two *B. bassiana* cultures (wild and single-spore cultures) through n-hexadecane and n-octocasane based media was tested to increase their efficacy against *Rhyzopertha dominica* and *Sitophilus oryzae* adults.

Materials and Methods

Insect cultures

Rhyzopertha dominica and *Sitophius oryzae* cultures have been maintained in our laboratory. Starting insects had been originally obtained from surrounding storage facilities. Durum wheat with 12% moisture content was used for the cultures. Glass jars of 1 Lt capacity with 250 gr of wheat were used. Adults of mixed sex were placed into the jars and kept for three days for oviposition. After removing the adults, the cultures were incubated for the emergence of new generation adults. One week old adults were used for the bioassays. All the cultures were maintained at 26±2 ºC and 65±5% relative humidity in darkness.

Fungus cultures and spore production

In the study, two *B. bassiana* cultures were used; one wild culture (151138) and another one that was obtained after single-spore selection (5-4) (Er et al., 2016). The fungi were grown on potato dextrose agar and their spores were suspended in %0.02 Tween 80. After determination of concentration by using Neubauer hemacytometer, spore concentration was adjusted to 10^6 spores/ml by dilution. 200 µl of spore suspension was spread on deficient media agar (DMA) containing alkane (Crespo et al., 2002). 10% n-hexadecane and 10% n-octacosane were prepared using hexane and 2 ml of required alkane was spread on DMA and evaporated prior to spore inoculation. In order to see any effect of the solvent hexane, DMA with only hexane was also tested. These cultures were kept at 25±2ºC for 14 days and grown fungi were used for spore production following mass production procedure described by Barış (2016). 100 g of rice was soaked overnight with tap water and the excess water was drained. The rice supplemented with 1.5 gr of $CaSO_4$ and $CaCO_3$ was sterilized in a polyethylene bag (25 cm x 38 cm). After cooling, it was inoculated with 10 ml of spore suspension ($2x10^7$ spores/ml) and sealed. Following fungal growth at 25±2℃, 12/12 photoperiod for 14 days the culture was dried at 25±2℃. Spores were separated from the substrate by using a 500 µm sieve.

Pathogenicity tests

Centrifuge tubes of 50 ml capacity each with 40 g of wheat were used for the tests. Wheat in each tube was mixed with 20 mg of spores producing a final concentration of 500 ppm (w/w) by shaking for 5 minutes. Twenty adults were released in each tube and kept at 25±2ºC, 65±5% relative humidity in constant darkness. Wheat kernels without spores were used as control. The experiment had five replicates.

Results

The efficacy of wild culture (151138) of *B. bassiana* towards *R. dominica* adults was enhanced when the fungus was passed through both n-hexadecane and n-octocasane based media. Mortality in 7 days increased from 35% to 55 and 69% when n-hexadecane and n-octocasane were used, respectively. Similarly, these treatments increased 14-day mortalities from 65% to 77 and 87%, respectively. Treatment of single-spore culture (5-4) of *B. bassiana*, however, either showed no change or reduced mortality. Passing both *B. bassiana* cultures through both alkane based media did not statistically affect their efficacies against *S. oryzae* adults. Using hexane alone did not change the effects of the *B. bassiana* to either of the species.

Discussion

This study illustrated that increasing the virulence of *B. bassiana* against *R. dominica* adults is possible by passing the fungus through media having n-hexadecane or n-octocasane as carbon source. Increase in mortality was reported by Crespo et al. (2002), Pedrini et al. (2011) and Barra et al. (2015) when fungi were grown on media containing these alkanes. In the case of *S. oryzae*, this procedure did not enhance the efficacy. This may be due to differences in the cuticular components of two insect species. In the previous studies, host insects were treated with spores that had been harvested directly from media with alkanes. However, in the present study the fungi were passed through media with alkanes and then cultured by mass production procedure using rice as substrate. Therefore, the results of this study indicate that the increase in virulence against *R. dominica* adults was due to a selection of fungi that can use n-hexadecane or n-octocasane as carbon source. This was also supported by the mortality levels caused when single-spore culture (5-4) of *B. bassiana* was used after passing through the alkanes. Starting with a wild fungus culture with a wider genetic diversity, and using n-octocasane can produce a better enhancement.

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Bio-nanosilver synthesized by the entomopathogenic nematode-symbiotic bacterium as bio-insecticide for the red flour beetle (*Tribolium castaneum*)

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Abstract

Biological control can be another important way to manage post-harvest insect pests. Some organisms that showed biological control activity against some soil pests are insect-parasitic nematodes. There are two different species of nematodes, steinernematids and heterorhabditids, who carry within their bodies insect-pathogenic bacteria. *Xenorhabdus* spp are bacteria which infest steinernematids and *Photorhabdus* spp. bacteria infect

heterorhabditids. The study aimed to develop pesticide alternatives by synthesizing silver bio-nanoparticles (AgNPs) using *Xenorhabdus indica* bacterial filtrate. The nanoparticles synthesized by the bacterial strains were purified and its cytotoxicity and bioactivity was examined against the larvae of the *Tribolium castaneum*. AgNPs were characterized by Scanning Electron Microscopy and X-Ray diffraction analysis, and the results revealed that the obtained nanoparticles are nanosilver with sizes ranging from 30 to 70 nm, with spherical shape and non-smoothed surface. Insect larvae were initially exposed to descending concentrations (100, 50, 25, 10 and 5 µg/ml) of the biosynthesized nanosilver for 48 hours. Results of the bioassay showed that mortality of treated larvae was concentration-dependent with LC_{50} of 25 µg/ml. Higher mortality percentage (89%) was observed with the concentration 100 ug/ml and the lower one was obtained by the concentration 5 ug/ml (60%). Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates. However, testing other types of bio-synthesized nanomaterials, and its vital effect as bio-insecticide for storage insect species are still under investigation.

Keywords: Entomopathogenic bacteria, biocontrol, nanosilver, *Tribolium* sp., *Xenorhabdus* sp.

Introduction

Stored commodities are vulnerable towards attack of insects (Ukeh *et al.*, 2012) and a possible infestation can deteriorate the quality as well as the quantity of the attacked commodity (Nadeem *et al.,* 2012). This will resulted in significant decrease in volume, nutritional value, substantial weight loss and reasonable germination damage (Nadeem al., 2012, Phillips et al., 2010). It was reported that *Tribolium castaneum* is a common pest found in granaries, mills, warehouses, especially in wheat flour, which causes serious damages to all kinds of stored grain products (Prakash *et al.,* 2008), but also feeding on different stored-grain and grain products (Weston and Rattlingourd, 2000). Being polyphagous and cosmopolitan, a number of insecticides had been used for successful control of this pest (Islam and Talukdar, 2005).

Synthetic insecticides have been successfully used to protect stored grains from insect infestation (Sighamony. *et al.*, 1986). *T. castaneum* is affected by both the quantity and quality of synthetic insecticides such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin and the fumigant phosphine. These are currently the main products used to protect stored grains from insects (Bond, 1984). Increased public concern over the residual toxicity of insecticides applied to stored products, the occurrence of insecticide-resistant insect strains, and the precautions necessary to work with traditional chemical insecticides stress the usage of e.g. botanicals to control insects of stored product (Su, 1991).

In the present decade, nanotechnology is a promising field that introduces an excellent chance for research and is expected to give major impulses to technical innovations in a variety of industrial sectors in the future. Benelli (2016) reported that the biosynthesis of AgNPs is an arising tool for fighting mosquito vectors. Nanoparticles of noble metals like silver and gold exhibited remarkable physical, chemical and biological properties from their bulk counter parts (Priya and Santhi, 2014).

Microbial and endo-toxin insecticides based on *Bacillus* spp. bacteria, as well as decreasing of breeding habitat can achieve considerable IPM program goals (Rydzanicz *et al.*, 2009). In this context, Adams and Nguyen (2002), found that *Xenorhabdus* and *Photorhabdus* gram negative symbiotic bacteria accompanied with the entomopathogenic nematodes *Steinernema* and *Heterorhabditis*, are injected into the haemocoel of target insect hosts. A variety of these toxins have been characterized and classified into four major groups (Rodou *et al.*, 2010). The toxin complexes (Tcs) are one of these four major groups which attracted attention from the fact that some of their complexes showed a high potential toxicity towards insects after oral application, suggesting potentiality as insecticides (Waterfield *et al.*, 2001).

Results and Discussion

The results revealed that the obtained nanoparticles are nanosilver with sizes ranging from 30 to 70 nm, with spherical shape and non-smoothed surface. Insect larvae were initially exposed to descending concentrations (100, 50, 25, 10 and 5 µg/ml) of the biosynthesized nanosilver for 48 hours. Results of the bioassay showed that mortality of treated larvae was concentration-dependent

with LC$_{50}$ of 25 µg/ml. Higher mortality percentage (89%) was observed with the concentration 100 ug/ml and the lower one was obtained by the concentration 5 ug/ml (60%). Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates.

The cause of insect death could be *via* binding the nanoparticles to proteins containing sulphur in the intracellular space or phosphorus in the DNA, which leads to enzyme and organelle degradation. Basically, cell death is mainly caused by decreased membrane permeability and disturbed proton motive force which leads to cellular function loss. The pathogenicity of these toxin complexes upon releasing into the host heamolymph causes histopathological lesions and septicaemia leading to host death. Moreover, the high larvicidal activity of AgNPs can be attributed to their lower particle size which increases the surface area to volume ratio, and thus, increases its action against insect.

Conclusion

Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates. However, testing other types of bio-synthesized nanomaterials, and its vital effect as bio-insecticide for storage insect species still under investigation.

Tab 1: The effect of the culture filtrate and the biosynthesized nanosilver on insect mortality compared with a commercial insecticide.

		Mean mortality± SE					
	Concen-tration (%)	Time (*hrs*)					
		3hr	*6hr*	*12hr*	*24hr*	*48hr*	*72hr*
Water	Cont.	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*
Culture	1	*0.33±0.577*	*15.0±10.0*	*23.33±12.58*	*30.0±10.0*	*35.0±15.0*	*50.0±10.0*
filtrate	10	*0.00±0.00*	*26.67±2.9*	*31.667±2.89*	*41.67±2.89*	*51.667±2.89*	*65.0±5.0*
	20	*66.67±18.9*	*75.0±22.9*	*78.33±20.82*	*81.667±15.*	*83.33±12.58*	*90.0±10.0*
Insecticide	1	*0.0±0.0*	*1.67±2.89*	*5.0±0.0*	*13.3±2.887*	*16.667±2.89*	*26.67±2.9*
	10	*0.0±0.0*	*3.3±2.887*	*13.33±7.638*	*23.33±2.887*	*30.0±0.0*	*50.0±5.0*
	20	*56.667±16.*	*66.67±16.*	*75.0±17.32*	*78.3±15.28*	*80.0±18.028*	*86.7±12.5*
	1	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*6.67±2.887*	*11.667±2.89*	*21.67±2.9*
Bio-synthesized nano silver	10	*11.667±7.6*	*23.33±10.*	*35.0±13.229*	*53.33±10.4*	*60.0±13.229*	*73.33±10.*
	20	*90.0±5.0*	*90.0±5.0*	*93.33±2.887*	*95.0±5.0*	*98.33±2.887*	*100.0±0.0*

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Insecticidal Effect of Central Anatolian Region Diatomaceous Earths Against Confused Flour Beetle (*Tribolium confusum* Du Val.) on Stored Paddy

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Abstract

In this study, insecticidal efficacy of different local diatomaceous earth (DE) deposits obtained Central Anatolian Region in Turkey and commercial DE deposit (German origin), Silicosec® were evaluated against substantial pest on stored grain as *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) at five different concentrations of 100, 300, 500, 900 and 1500 ppm on stored paddy. Mortality of the exposed adults was assessed after 7, 14 and 21 days of exposure. Also progeny productions were assessed after 65 days The tests were carried out at 25±1 oC temperature, 55±5% R.H. under dark conditions. The most effective DE in a short time were assessed AG2N-1 which caused 97% mortality of *T. confusum* adults at 1500 ppm concentration after 7 days of exposure in paddy. Complete mortality of *T. confusum* adults was recorded on AG2N-1 at 900 ppm for 14 days and treatments of AG2N-1, BGN-1, CBN-1 for 21 days at 500, 900 and 1500 ppm respectively whereas 87% mortality rate was determined for 21 days exposure of Silicosec® at the highest concentrations on paddy. In conclusion, this study indicated that Turkish DE deposits, AG2N-1, BGN-1 and CBN-1 had high insecticidal efficacy in comparison with the commercial Silicosec® and would have potential to be used against insects in the pest management of stored paddy.

Keywords: Turkish diatomaceous earths, *Tribolium confusum*, toxicity, paddy, Silicosec

1. Introduction

Currently, the control of insect pests in durable stored food products, such as grains and legumes, is based on the use of chemical methods such as fumigants and residual insecticides. However, the use of these substances is directly related with toxic residues on the final product, as well as serious environmental hazards. These factors, along with the consumers' demand for residue-free food and the development of resistance by several insect pests, have made essential the evaluation of alternative, low-risk and environmentally-friendly control methods. One of the most promising alternatives over the use of traditional pesticides in durable stored products is the use of diatomaceous earths (DEs). DEs are composed by the fossil skeletons of phytoplanktons, also known as diatoms, which occur in fresh and salt water since the Eocene period and produce a soft sedimentary rock, which is composed mainly by amorphous silica ($SiO_2 + H_2O$). The DEs currently mined vary remarkably in their insecticidal activity, depending upon species composition, geological and geographical origin as well as certain chemical characteristics, such as SiO_2 content, pH and tapped density (Korunic 1997). DEs are probably the most efficacious natural resource-based dry materials that can be used as insecticides (Korunic 1998). DEs act in the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss. They are non-toxic to mammals (rat oral LD50>5000 mg/kg of body weight), leave no toxic residues on the product and

according to the US EPA they are classified in the category of GRAS (Generally Recognised As Safe) since they are used as food or feed additives (FDA 1995). Regarding their insecticidal use, DEs can be applied with the same application technology with traditional grain protectants, which means that no specialized equipment is required (Athanassiou et al. 2005). Several DEs, based on natural deposits, are now commercially available, and have proved very effective against stored grain pests (Subramanyam and Roesli 2000, Athanassiou et al. 2011). However, the investigation for newer, naturally-occurring DEs that are more effective in insect control is still in progress, especially in areas rich to silicaceous rocks. Based on the first evidence and preliminary samplings, it seems that Turkey is considered rich to natural DE deposits, and there is clear evidence for the existence of large DE deposits at some areas (Özbey and Atamer 1987, Mete 1988, Sıvacı and Dere, 2006, Çetin and Taş 2012). Diatomite reserve of Turkey is 125 million tons. However, there is limited information on the efficiency of local DEs from these areas in Turkey against stored grain insects. In this study, efficiency of three local diatomaceus earth formulations against Confused Flour Beetle (*Tribolium confusum* Du Val.) on paddy, was investigated under laboratory conditions.

2. Materials and Methods

2.1. Test Insects

Confused Flour Beetle adults used in the bioassays were taken from a culture that was kept at the Namık Kemal University, Department of Plant Protection, Toxicology Laboratory on whole wheat at 26 ± 1ºC, 65 ± 5 %. All individuals used in the tests were <2 wk old.

2.2. Diatomaceous earth formulations

The three DE formulations used in biological tests were of three Turkish diatomaceous earth formulations (AG2N-1, BGN-1 and CBN-1) and commercial diatomaceous earth (Silicosec®). Turkish diatomaceous earth formulations were collected from diatomite reserve at middle Anatolia of Turkey.

2.3. Experimental procedure

Exposure studies were carried out at 25 ± 1ºC, 55 ± 3 % RH, and five dose rates of four Turkish diatomaceous earth formulations and commercial diatom earth (Silicosec®) (100, 300, 500, 900 and 1500 ppm) on paddy (*Oryzae sativa* L. variety of Osmancık 97) with %13 moisture content. For each trial (DE formulation-dose combination), five samples of 50 g paddy were taken. Each sample was placed in a small glass jar that was closed, and that was covered with organtine for sufficient ventilation. The samples were treated individually with the respective DE quantity and then shaken manually for 5 min to achieve equal distribution of the dust in the entire product quantity. Five additional tubes, containing untreated paddy, served as control in each case. Subsequently, 20 adults of *T. confusum* were introduced into each tube. The tubes were then placed in incubators, set at above mentioned temperature and relative humidity level. Dead adults of both species were counted after 7, 14, 21 d. After the last count for mortality, all adults (dead and alive) were removed from the DE-treated jars, and the jars were left in the incubators for an additional period of 65 d. Then, the emerged *T. confusum* individuals were counted.

2.4. Data processing and analysis

Generally, the control mortality was very low, but where it was considered necessary the mortality counts were corrected by using the formula of Abbot (1925). The data were analyzed, separately for each species, by using the Anova test of SPSS (SPSS,2009), with insect mortality as the response variable and type of DE formulation and dose rate, as the main effects. For the progeny production counts, with number of progeny as the response variable and type of DE formulation, and dose rate as main effects. Means were separated by using the Anova test at $P<0.05$.

3. Results

Efficacy of Turkish DE's represented different values according to exposure times and concentration at the end of the experiment (Table 1,2 and 3). Complete mortality of *T. confusum* adults was recorded after 14 days of exposure at 900 ppm treated with AG2N-1 (Table 2) and 21 days of exposure at 500, 900, 1500 ppm treated with AG2N-1, BGN-1 and CBN-1 respectively whereas 87% mortality rate were recorded for Silicosec° after 21 day exposure at the highest concentrations on paddy (Table 3). According to the results of the biological tests carried out on the paddy, F1 values of *T.confusum*, including the control group, was not seen in the adult exit. This result wold be caused by confused flor beetle one of the important secondary insect species in the stored product pests and was not able to eat whole kernel of the paddy.

Table 1. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 9 DEs after 7 days

Concentration (ppm)	Silicosec®	BGN-2	BHN-1	AG2N-1	CBN-1	F value	P value
1500	3±2 Ac*	25.3±4.3 Ab	0±0 Ac	96.9±2.1 Aa	19.2±4.5 Ab	F₄,₂₀=105.52	P<0.0001
1000	1±1 Ac	16.2±2.6 Ab	1±1 Ac	82.3±4.5 Ba	1.8±1.8 Bc	F₄,₂₀=87.38	P<0.0001
500	0±0 Ac	5.3±1.7 Bb	0±0 Ac	54.2±5 Ca	0±0 Bc	F₄,₂₀=111.33	P<0.0001
300	0±0 Ab	2.4±1 Bb	1±1 Ab	32.3±4.4 Da	1.6±1 Bb	F₄,₂₀=32.96	P<0.0001
100	0±0 Ab	1.6±1 Bab	1±1 Ab	4.2±2.1 Ea	0±0 Bb	F₄,₂₀=3.99	P=0.015
Control	0±0	1±1	0±0	4±1.9	1±1		
F value	F₄,₂₀=1.708	F₄,₂₀=15.303	F₄,₂₀=0.5	F₄,₂₀=74.503	F₄,₂₀=17.621		
P value	P=0.188	P<0.0001	P=0.736	P<0.0001	P<0.0001		

Table 2. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 9 DEs after 14 days

Concentration (ppm)	Silicosec®	BGN-2	BHN-1	AG2N-1	CBN-1	F value	P value
1500	39.4±4.2 Ad	89.8±4.6 Ab	15.2±2.9 Ae	100±0 Aa	80.6±4.1 Ac	F₄,₂₀=77.96	P<0.0001
1000	5.5±2.7 Bd	66.3±9.2 Bb	4.4±2 Bd	100±0 Aa	25.5±4.4 Bc	F₄,₂₀=72.54	P<0.0001
500	4.6±3 Bc	21.4±4.7 Cb	0±0 Bc	94.3±1.8 Ba	1.2±0.7 Cc	F₄,₂₀=97.46	P<0.0001
300	2.6±1.8 Bc	16.3±6.4 Cb	3.6±2.7 Bc	72.7±4.5 Ca	3.9±1.8 Cc	F₄,₂₀=30.01	P<0.0001
100	1.8±1.8 Bb	2.4±0.6 Dab	1.6±1 Bb	8.4±3 Da	1.2±0.7 Cb	F₄,₂₀=2.21	P=0.103
Control	1±1	2±1.2	1±1	12±3.7	2±1.2		
F value	F₄,₂₀=13.74	F₄,₂₀=33.82	F₄,₂₀=7.33	F4,₂₀=135.58	F₄,₂₀=75.73		
P value	P<0.0001	P<0.0001	P=0.001	P<0.0001	P<0.0001		

Table 3. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 5 DEs after 21 days

Concentration (ppm)	Silicosec®	BGN-2	BHN-1	AG2N-1	CBN-1	F value	P value
1500	86.9±3.4 Ab	100±0 Aa	56.6±3.4 Ac	100±0 Aa	100±0 Aa	F₄,₂₀=140.39	P<0.0001
1000	29.3±6.6 Bc	100±0 Aa	23.2±8.2 Bc	100±0 Aa	72.6±3.1 Bb	F₄,₂₀=75.52	P<0.0001
500	11.3±4.6 Ccd	83.3±1.9 Bb	5.7±3.8 Cd	100±0 Aa	14.7±5.9 Cc	F₄,₂₀=78.53	P<0.0001
300	2.6±1.8 Cc	50±8.2 Cb	5.5±2.7 Cc	94.2±2.6 Ba	7.4±2.7 Cc	F₄,₂₀=51.93	P<0.0001
100	2.6±1.8 Cb	4.2±1.3 Db	2.4±1 Cb	33.3±7.4 Ca	1.1±1.1 Db	F₄,₂₀=15.76	P<0.0001
Control	1±1	4±1.9	1±1	13±4.1	5±2.7		
F value	F₄,₂₀=44.42	F₄,₂₀=181.57	F₄,₂₀=15.41	F₄,₂₀=70.61	F₄,₂₀=140.14		
P value	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001		

*Two-way variance analysis (ANOVA) was applied to the data and the differences between the averages were based on the 5% significance level. The different uppercase letters in the same column and the different lowercase letters in the same line are statistically different.

Discussion

The most effective DE in a short time were assessed AG2N-1 which caused 97% mortality of *T. confusum* adults at 1500 ppm concentration and 82% at 900 ppm concenatrition after 7 days of exposure on paddy. Other diatoms showed low toxicity on all concentrations. Complete mortality of *T. confusum* adults was recorded on AG2N-1 at 900 ppm for 14 days and treatments of AG2N-1,

BGN-1, CBN-1 for 21 days at 500, 900 and 1500 ppm respectively whereas 87% mortality rate was determined for 21 days exposure of Silicosec® at the highest concentrations on paddy. Athanassiou et all. (2004), reported that Silicosec was't reached complete mortality at 750,1000 and 1500 ppm concentration on rye, oats and triticale againt *T.confusum* after 21 days application but decrased to number of insect on F$_1$. In a similar study conducted on rice with *T. confusum* adults, Alagöz (2016) found that Silicosec® commercial diatom was found to be 20% at the end of 7th day, 75% at the end of 14th day and 99% at the end of 21st day, at the end of the day, did not find a new generation of adult outbreaks, including the control group, in all diatoms used in experiments. Ziaee et al. (2012), a study conducted on wheat with *T. confusum* adults, found 51% mortality with Silicosec after 2days at 2000ppm concentration and complete mortality was recorded at 1000,1500 and 2000 ppm after 7 days and more. In conclusion, this study indicated that Turkish DE deposits, AG2N-1, BGN-1 and CBN-1 had high insecticidal efficacy in comparison with the commercial Silicosec® and would have potential to be used against insects in the pest management of stored paddy.

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Twelve years (2005-2017) of scientific and professional work in the field of stored products pests protection in Slovenia

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Abstract

Scientific and professional work in the field of stored products pests protection in Slovenia began in 2005, when we tested the efficacy of entomopathogenic nematodes against the granary weevil (*Sitophilus granarius*) and the sawtoothed grain beetle (*Oryzaephilus surinamensis*) adults under laboratory conditions. In 2007, we participated as partners in the project SEE-ERA.NET "Development of a non-toxic, ecologically compatible, natural-resource based insecticide from diatomaceous earth deposits of South Eastern Europe to control stored-grain insects pests" (coordinated by C. Athanassiou), and we thus became acquainted with the research work in the field of investigation the efficacy of diatomaceous earth in controlling beetles from the *Sitophilus* genus. We have continued the research of different aspects of diatomaceous earth (the influence of geochemical composition and abiotic factors on its efficiency, the effects of individual and combined application, the effects on various harmful insect pests, etc.). In search for comparable substances to diatomaceous earth (regarding the efficacy), we have studied insecticidal effects of quartz sand and entomopathogenic nematodes from Slovenia,

plant powders and essential oils on various harmful beetles. In the recent years, our research work has been mainly dedicated to studying the efficacy of wood ash and zeolites as natural insecticides, which have demonstrated sufficient efficiency in suppressing *Sitophilus* beetles. In the same period, we studied the seasonal dynamics of the Indian mealmoth (*Plodia interpunctella*), the Mediterranean flour moth (*Ephestia kuehniella*) and the Angoumois grain moth (*Sitotroga cerealella*) in cereal stores, where we were also searching for possible indigenious natural enemies of stored product insects pests. We have confirmed the occurrence of two parasitoids, *Anisopteromalus calandrae* and *Dibrachys microgastri*. In 2017, we have organized the 11th Conference of the IOBC/wprs Working Group on Integrated Protection of Stored Products (Ljubljana, 3-5 July), which was attended by 136 participants from 25 countries. We also transfer knowledge to Slovenian agricultural specialists about the harmfulness and possible ways of controlling stored products insects pests. In 2014, we have organized a workshop on this topic ("From Technological Maturity to Storing of Cereals and Legumes"). In 2015, we have hosted C. Athanassiou as an invited lecturer at the 12th Slovenian Conference on Plant Protection with international participation in Ptuj. In recent years, we have been working with experts from other countries with the aim of studying the efficacy of environmentally acceptable insecticides (spinosad, spinetoram) and the influence of cereal production technologies on grains' susceptibility to attack by *Sitophilus* beetles. Furthermore, we participate in the research regarding the efficiency of new formulations of insecticidal preparations. The paper presents the chronology of activities in this area of our work.

Keywords: stored products pests, beetles, inert dusts, essential oils, biological control, Slovenia

1. Introduction

In Slovenia, the systematic research and professional work in the field of stored product pest control began in 2005, when in laboratory conditions we exposed the selected stored products beetles to entomopathogenic nematodes, whose effects were then tested on different species of insect pests. In the next 12 years, also with the help of foreign experts, we expanded the scope of stored product pest research to include other fields, primarily the fields of natural products, and physical and other techniques for stored products pest control. Bellow, we present the results of our work and the complete overview of references in this field.

2. Chronology of scientific and professional work in the field of stored products pests protection

2.1. First attempts or entomopathogenic nematodes against stored products beetles

Four entomopathogenic nematode species (*Steinernema feltiae, Steinernema carpocapsae, Heterorhabditis bacteriophora*, and *Heterorhabditis megidis*) were tested in a laboratory bioassay with the aim of studying their efficacy in control of the adults of two stored grain pests, *Sitophilus granarius* and *Oryzaephilus surinamensis*. Activity of the biological agents studied was determined at three different concentrations (500, 1000, and 2000 infective juveniles [IJs] per adult) and temperatures (15, 20, and 25°C). The granary weevil mortality rate was higher than the mortality rate of the saw-toothed

grain beetle. *Heterorhabditis megidis* proved to be the least efficient in control of both pests, while no significant differences were recorded between any of the other three nematode species. The experiment demonstrated that the entomopathogenic nematodes were most efficient in the control of *S. granarius* at 20°C (LC$_{50}$ after 7-day exposure 803-1195 IJs/adult) and 25°C (LC$_{50}$ 505-1175 IJs/adult). A satisfactory level in control of the pest *O. surinamensis* was reached at 20°C (LC$_{50}$ 921-1335 IJs/adult). The concentration of the suspension used in our experiment was shown to be a less important factor affecting the biological activity of nematodes against the adults of both stored grain pests. Though the use of entomopathogenic nematodes for control of the tested pests is not possible at the present time, it may be possible to combine this approach with some other (biotechnical) methods in the future (Trdan et al., 2005; Trdan et al., 2006).

The efficacy of three new strains (B30, B49 and 3162) of the entomopathogenic nematode *Steinernema feltiae* in controlling rice weevil (*Sitophilus oryzae*) adults was tested in a laboratory bioassay. The aim of the study was to determine the activity of selected biological control agents

against one of the most important primary stored product pests to prevent the occurrence of rice weevil resistance to insecticides. The pathogenicity of biological agents was studied at four different temperatures (15, 20, 25 and 30°C) and for five concentrations of nematode suspension (125, 250, 500, 1000 and 2000 IJs per adult). Beetle mortality was determined on 4, 6 and 8 days after treatment. The results showed that all studied strains were most pathogenic (42-72% mortality) at 25°C and the highest concentration of the nematode suspension, while the lowest pathogenicity (from 6 to 11%) was found at 30°C and the lowest concentration of the nematode suspension. Besides, at higher concentrations the suspension of entomopathogenic nematodes can be an effective biological agent in controlling adult rice weevils. The lowest LC_{50} value (1165 IJs/adult after an 8-day exposure) was obtained for the Hungarian strain 3162 at 25°C, while the highest (2533 IJs/adult after an 8-day exposure) was obtained for the Slovenian strain B30 at 30°C (Laznik et al., 2010; Laznik and Trdan, 2010).

2.2. Seasonal dynamics of stored products lepidopteran pests

In the period 2004-2006 seasonal dynamics of Mediterranean flour moth (*Ephestia kuehniella*), Indianmeal moth (*Plodia interpunctella*) and Angoumois grain moth (*Sitotroga cerealella*) was studied in the mills and grain warehouses in central Slovenia. For this purpose pheromone traps were used from April until December, and the males of all three lepidopteran pests were counted in two week intervals. The three insect pests under investigation developed two peaks in capture per year that might represent two distinct generations per year. In the maize open air storage *Ephestia kuehniella* was the most numerous, while *Plodia interpunctella* was more frequent in the closed storage in mills and warehouses, *Sitotroga cerealella* was slightly less common in these latter closed warehouses (Trdan et al., 2010).

2.3. Diatomaceous earth, quartz sand, plant powders, wood ashes, and zeolites in single and combined use

Laboratory experiments were carried out to evaluate the impact of diatomaceous earth (DE) samples of different origin with their insecticidal properties to control *Sitophilus oryzae*. We tested the efficacy of three local DEs, from Serbia, Greece and Slovenia, and commercial formulation SilicoSec against the adults in stored wheat. The experiments were carried out at three temperatures (20, 25 and 30 °C) and two relative humidity (RH) levels (55 and 75 %). Mortality of pest was counted 7, 14 and 21 days after exposure (DAT) at the following DE dose rates: 100, 300, 500 and 900 ppm. The mortality of adults normally increased with increasing dose rates and DAT. In all samples the mortality of rice weevil adults (dose rate 900 ppm, 21 DAT) was above 90 %, except at Slovenian DE (at 20 °C and 55 % RH) and Greek DE (at 25 °C and 75 % RH), when the mortality was 85.3 and 67.6 %, respectively. With 100 % mortality (14 DAT and at 900 ppm) the most effective was SilicoSec. Slovenian DE was more effective at 55 % RH than at 75 % RH (7 DAT at all temperatures) (Rojht et al., 2008, 2010a; Rojht et al., 2012a).

Laboratory experiments were done to determine the effect of geochemical composition of diatomaceous earth (DE) on insecticidal activity of DE against adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Samples of DE were mined from DE-deposits in Slovenia, Greece, and Serbia. In addition, a commercially available DE formulation (SilicoSec®) was used in the tests and served as a positive control. The bioassays were carried out at temperatures 20, 25, and 30°C, relative humidity levels of 55 and 75%, and at application rates of 100, 300, 500, and 900 ppm. Adult mortality was recorded after 7, 14, and 21 days of exposure. Prior to bioassays with *S. oryzae*, the geochemical composition of all DEs that were used in the tests was determined by whole rock ICP geochemical analyses. Silica (in the form of SiO_2 or opal-A) was the DE ingredient that was significantly correlated with efficacy in most of the bioassays. Some weak positive correlation was observed between *S. oryzae* mortality and MnO or CaO content. All significant correlations between mortality and Al_2O_3, Fe_2O_3, K_2O, TiO_2, Cr_2O_3, P_2O_5, and MgO content were negative, while correlation between Na_2O content and mortality was generally not significant (Rojht et al., 2010b).

The efficacy of Slovenian quartz sands admixed with stored wheat was tested against rice weevils (*Sitophilus oryzae*) in laboratory conditions. Five different samples of quartz sand of different ages were tested. Samples from the location Raka-Ravno (with admixture and clean) and the location Moravče (with admixture and clean) and commercially available cleaned quartz sand from the locality of Puconci (Plantella) each were used at six concentrations: 100, 300, 500, 900, 1200, and 1500 ppm. The amount of SiO_2 in all sand samples was high and varied from 91.52 to 99.24%. For each dose rate, the treated wheat grains were placed at four temperatures (20, 25, 30 and 35°C) and at 55 and 75% relative humidity level. After 7, 14 and 21 days of exposure dead adults were counted. All samples showed only a slight insecticidal effect on adults of rice weevil and are not appropriate for wider use against rice weevil adults in stored wheat. The highest mortality (15%) of rice weevil adults was confirmed 21 days after treatment at 900 ppm, 30°C and 55% RH level for quartz sand with admixture from the Moravče location (Rojht et al., 2010c; Rojht et al., 2011).

In the search for an effective and sustainable control method against the bean weevil, *Acanthoscelides obtectus* (Say), three different powders were tested against adults under laboratory conditions. The three powders were diatomaceous earth (DE) (commercial product SilicoSec®), common lavender (*Lavandula angustifolia*) powder and field horsetail (*Equisetum arvense*) powder. The substances were tested at five temperatures (15, 20, 25, 30, and 35°C), two relative humidity levels (RH) (55 and 75%), and four concentrations (100, 300, 500, and 900 ppm). The mortality of adults was measured after the 1st, 2nd, 4th, and 7th days of exposure. The efficacy of the powders increased with the temperature, whereas in general, RH did not have a significant effect on the adults' survival. According to common practice of storing common beans, we recommend the use of DE against the pest in question, as this inert powder showed the highest efficacy at lower temperatures and concentrations. Concerning the wider use of common lavender and field horsetail powders, we suggest studying their combined use with other environmentally friendly methods with the aim of achieving the highest synergistic effect possible (Trdan and Bohinc, 2011; Bohinc et al., 2013).

In the search for an effective and sustainable control method against the maize weevil (*Sitophilus zeamais* Motschulsky), an important insect pest affecting stored grain, different inert dusts were tested under laboratory conditions. We treated wheat grains with quartz sand, zeolites, and diatomeaceous earth. Inert dusts of different origins were used, namely diatomaceous earth from Slovenia and SilicoSec, quartz sands from two locations from Slovenia, and three different zeolites (two types of natural zeolite from location in Slovenia, and synthetic zeolite Asorbio®). Untreated winter wheat grains served as control treatment. The substances were tested at three different temperatures (15, 20 and 25 °C) and two different relative humidity levels (55 and 75%). Mortality was measured 7th, 14th and 21st day after exposure. Inert dusts were applied at two different concentrations, 450 and 900 ppm. The analysis of pooled results provoked significantly the highest mortality of beetles in treatments with SilicoSec® (52.31 ± 2.07%), and in treatment with one type of Slovenian zeolite (31.48 ± 1.42%). The lowest mortality was recorded in treatments with quartz sands from both Slovenian locations, Moravče (18.84 ± 1.31%), and Raka (9.12 ± 0.66%). Mortality of *S. zeamais* was significantly the highest in treatments exposed to 25 °C (28.32 ± 1.16%), and in treatments exposed to higher concentrations (900 ppm) of inert dusts (27.30 ± 0.87%). The use of diatomaceous earth is well established in stored products pest management, however the knowledge on the efficacy of zeolites is very week and offers a lot of opportunities for future researchers (Trdan et al., 2015).

The effectiveness of three different wood ashes from black locust (*Robinia pseudoacacia*), beech (*Fagus sylvatica*), and Norway spruce (*Picea abies*) were evaluated on maize weevil (*Sitophilus zeamais*) regarding adult (2-4 weeks old) mortality. Diatomaceous earth served as positive control. We have tested wood ashes as surface treatment (10 and 20 g/m2) and as admixtures (2.5 and 5 w%). Mortality of weevils, when wood ashes were applied as surface treatment was evaluated every day till 7th day of application, and every day till 14th day of application (as delayed mortality). When wood ashes were admixed, we have evaluated mortality after 7, 14 and 21st day. Research was

performed at two different relative humidty values (55 and 75%) and at three different temperatures (15, 20 and 25 °C). Based on the results of our survey we conclude that mortality of *Sitophilus zeamais* adults was influenced by wood ash species, air temperature and relative humidity. As surface treatment, 99.69 ± 0.31% mortality was achived at treatment with Norway spruce on day 7 at 25 °C. When admixed, 100% mortality was achieved on day 14, when Norway spruce's wood ash has been applied at 25 °C. Use of wood ash as stored product protectant proved to be efficient in our survey, although additional reseach should be made (Bohinc et al., 2017a).

Laboratory experiment was carried out to evaluate the impact of zeolites of different origin on the mortality of the maize weevil (*Sitophilus zeamais* Motschulsky) adults. We have tested the efficacy of natural zeolites (Slovenian and Serbian) and synthetic zeolites ('Asorbio'). Diatomaceous earth (product SilicoSec® was used as positive control). We have applied zeolites as surface treatment (at concentrations 10 and 20 g/m2) and as admixtures (at concentrations 450 and 900 ppm). Mortality of weevils, when zeolites were applied as surface treatment was evaluated everyday till 7th day after application, and everyday till 14th day after application (as delayed mortality). When zeolites were admixed, we have evaluated mortality after 7th, 14th and 21st day. Research was performed at two different relative humidty values (55 and 75%), and at three different temperature (15, 20 and 25 °C). We conclude that mortality of maize weevil adults was influenced by higher temperature values and lower relative humidity value. When we have applied 'Zeolite Slovenia' (at 900 ppm, 15 °C, 55% Rh) as admixture we have recorded 69.69 ± 7.04% after day 21, meanwhile mortality reached 83.66 ± 3.21% after day 21, when 'Zeolite Slovenia' was applied at 25 °C. 100% mortality of maize weevil adults was recorded, when 'Zeolite Slovenia' (after day 7 at 25 °C) was applied at surface. There was no impact of zeolite's dose on mortality of maize weevils. Mortality of weevils was alike in two natural zeolites (Slovenian and Serbian), meanwhile mortality of maize weevils was the lowest in treatments with 'Asorbio'. Use of natural zeolites proved to be efficient as stored product protectant in our research, although additional surveys should be made (Bohinc et al., 2017b).

Laboratory experiment was carried out to evaluate the insecticidal efficacy of different environmentally acceptable substances on the mortality of the granary weevil (*Sitophilus granarius*) adults. We treated wheat grains with diatomaceous earth (commercial formulation SilicoSec®), quartz sand, leaf powder of neem tree (active ingredient azadirachtin, commercial formulation Neem listni prah®), and wood ash. Wheat grains were also treated with combination of diatomaceous earth and wood ash, combination of leaf powder and wood ash, quartz sand and wood ash and with a combination of four different substances (diatomaceous earth, wood ash, leaf powder and quartz sand). Substances were applied at different concentrations. Mortality of the granary weevil adults was tested at 3 different temperatures (20, 25 in 30°C) and at 2 different relative humidity levels (55 and 75%). Mortality was evaluated 7, 14 and 21 days after exposure. We have detected significant impact of different substances on the mortality of the beetles. Significantly the highest mortality of the beetles was evaluated in treatments with wood ash in single or combined use, i.e. individual use of 2,5 w% wood ash (69.73±2.52%), and combined uses of diatomaceous earth (450 ppm) and 2.5 w% wood ash (71.94±2.40%), quartz sand (450 ppm) and 2.5 w% wood ash (68.72±2.80%), and diatomaceous earth (225 ppm), wood ash (1.25w%), leaf powder (0.625 w%), and quartz sand (225 ppm) (68.76±2.75%). We established that wood ash in single or combined use can perform environmentallyacceptable alternative to synthetic insecticides in controlling granary weevil adults, however for final confirmation of this thesis we have to study the activity of the substances against the eggs and the larvae of the pest (Trdan and Bohinc, 2013, 2014; Bohinc and Trdan, 2017).

2.4. Essential oils and helbal extracts

Fumigant toxicity of essential oils from *Rosmarinus officinalis, Salvia officinalis, Laurus nobilis, Citrus bergamia*, and *Cinamomum camphora* against *Acanthoscelides obtectus* adults reared on common bean seeds was assessed. Properties of essential oils were tested at two different dose rates (245 and 980µl/l). Insecticidal efficacy was tested at five different temperatures (15, 20, 25, 30, and 35°C)

and two relative humidity (RH) levels (55 and 75%). Responses varied with type of essential oil, time of exposure, dose of essential oil, as well as with temperature and relative humidity levels. Three days after treatment over 90% adult mortality was achieved. An essential oil from rosemary gave over 94% efficacy after three days. At 75% relative humidity essential oils were significantly more effective than at 55% relative humidity level. The plant essential oils described in this paper could be useful for managing populations of *A. obtectus* in warehouses (Trdan and Bohinc, 2012; Bohinc and Trdan, 2013).

A trial was conducted to assess the fumigant toxicity of the essential oils from *Rosmarinus officinalis* L., *Salvia officinalis* L., *Lavandula angustifolia* Mill. and *Mentha balsamea* Willd. against the adults of *Sitophilus granarius* (L.). The relationships between the time after treatment (1, 2, and 3 days), temperature (20, 25, 30, 35, and 40°C), concentration of essential oils (2.4 and 7.4 ml/L air) and mortality were investigated. In the experiment, the efficacy of the essential oils at 40°C was 95%, whereas their efficacy was considerably lower at lower temperatures (from 12 to 36%). Throughout the experiment, the essential oil of rosemary proved to be the most effective fumigant, causing more than 60% mortality of the granary weevil adults. When applying the essential oil of rosemary, more than 50% mortality in the adults of granary weevil was attained at 35°C (89%) and 40°C (99%). A satisfactory efficacy of the other essential oils, common lavender (90%), peppermint (97%) and common sage (94%), was attained only at the highest temperature. The activities of the essential oils were better at higher concentrations (36%) than at lower concentrations (32%). When assessing the effect of the concentration on the adult mortality, we achieved more than 50% efficacy only with rosemary (2.4 ml/L of air, 58%; 7.4 ml/L of air, 63%). The data for the other essential oils ranged between 19% (peppermint, 2.4 ml/L of air) and 34% (common sage, 7.4 ml/L of air). The calculated values for the LC_{50} and LC_{90} showed that only rosemary produced satisfactory fumigant activity on the adult granary weevils, especially in relation to the temperature. However, the positive efficacy identified in our laboratory experiments needs to be validated under conditions similar to those of the applied conditions, that is, warehouses (Laznik et al., 2012).

Ethanol extracts of *Rosmarinus officinalis*, *Lavandula angustifolia* and *Ruta graveolens* were tested against adults of *Acanthoscelides obtectus*, an important insect pest affecting stored common beans and other legumes. Using a newly developed computer tracking system, a choice test revealed that all of the extracts have a repellent action. The highest repellent activity against the bean weevils adults showed the ethanol extract of rue. We suggested that a cocktail of volatile components in the ethanol extracts was responsible for the observed repellent action. All three of the extracts have insecticidal effects on bean weevils, reducing F1 adult emergence, with no side effects on the germination of the bean plants (Rojht et al., 2012b).

2.5. Biological control agents of stored products pests

In Slovenia, biological control agents, whose introduction, rearing and use are permitted according to Rules on biological control of plant pests (Official Gazette RS No 45/06), are classified in the List of indigenous biological control agents. Since 2006, when the first List was composed, until 2015 the number of indigenous biological control agents increased for 11 species (to present 25 species). The knowledge about occurrence and distribution of indigenous natural enemies is the key factor for their implementation in food and ornamental plants production systems (Trdan and Bohinc, 2016).

In 2012, we established the first record of parasitoid wasp *Dibrachys microgastri* (Boche, 1834) in Slovenia. The wasp was detected in the Laboratory of Entomology (Biotechnical Faculty in Ljubljana) in the rearing containers filled with wheat grains, which are used for reproduction of population of the granary weevil (*Sitophilus granarius*) (Trdan et al., 2013). During 2013 and 2014, we first recorded nine beneficial organisms in Slovenia, among them also parasitic wasp *Anisopteromalus calandrae* Howard. In our opinion this insect species has the potential for future implementation in plant production, since it is already used in some European countries in biological control of some stored products beetles like *Sitophilus* species and *Rhyzopertha dominica* (Bohinc and Trdan, 2015).

2.6. Environmentally acceptable insecticides

The efficacy of spinetoram and spinosad against 2-4 weeks old *Sitophilus* adults has been tested under laboratory conditions. Spinetoram and spinosad were applied at three different dose rates (0.5., 1 and 2 mg/kg). Experiment was performed at 25 °C and 65% rh, on four different winter wheat varieties. Mortality counts were assessed on day 7, day 24 and day 21. Our research demonstrated impact of grain type, dose of exposure, day of evaluation and *Sitophilus* species on mortality of weevils. Mortality of weevils was higher in treatments treated with spinetoram (90.19 ± 0.48%). After day 21, spinosad caused 91.64 ± 0.93% mortality, meanwhile 96.13 ±0.51% mortality was detected in spinetoram treatment after day 21. When applied spinosad as 2 mg/kg, 96.35 ± 0.44% was detected. Spinetoram caused 96.79 ± 0.38% at 2 mg/kg. Efficacy of spinosad (69.47 ± 1.87%) and spinetoram (78.23 ± 0.83%) was the lowest on variety 'Fidelius'. Spinosad caused the highest mortality in treatments with Serbian maize weevil (96.64 ± 0.31%), meawhile spinetoram prooved to be the most efficient in treatments with Serbian rice weevil (94.58 ± 0.77%) and Serbian granary weevil (94.07 ± 0.64%). Our resarch on the efficacy of spinetoram and spinosad against stored product insect pests is the first one for Slovenian agriculture. It presents good basics for further studies on implementation of tested insecticides as protectant of stored grains in Slovenia (Trdan et al., 2017).

2.11 Other types of investigation

In a study the effects of the production systems of wheat from different production systems on the mortality, progeny production and preference of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) were evalueted. The factors tested were production system (integrated [INT], organic [ORG], biodynamic [BD] and control), which differed in plant protection and fertiliser procedures during plant growth and development; exposure interval (7, 14 and 21 d); relative humidity (r.h.) (55% and 75%) and temperature (20°C, 25°C and 30° C). Mortality after 7 d increased with the temperature increase and decreased with the increase in r.h. in most of the tested combinations. The mortality of weevils was higher in ORG compared to INT-produced wheat after 7 d. Progeny production was recorded 56 d after removal of parental adults and was higher at 75% r.h. in comparison to 55% r.h. At 55% r.h. and 20°C, progeny was 60.8% higher when *S. zeamais* were exposed to ORG in comparison to INT-produced wheat. Wheat from different production systems influenced mortality rates which were higher in alternative compared to INT production systems under optimal conditions for wheat storage (low temperature and r.h.). The reverse was recorded for temperature and r.h. increase. Progeny was not affected by wheat from various production systems. Significantly more *S. zeamais* adults were found in traps containing wheat from BD and control in comparison to INT. An understanding of the agricultural processes, biotic and abiotic factors which alter the post-harvest response of storage pests could be useful for the development of efficient post-harvest strategies for ORG and BD farms and the processing industry (Turinek et al., 2016).

2.8 Organization of workshops for agricultural specialists and IOBC Conference

In 2017, we have organized the 11th Conference of the IOBC/wprs Working Group on Integrated Protection of Stored Products (Ljubljana, 3-5 July), which was attended by 136 participants from 25 countries (Trdan and Trematerra, 2017; Trematerra and Trdan, 2018). We also transfer knowledge to Slovenian agricultural specialists about the harmfulness and possible ways of controlling stored products insects pests. In 2014, we have organized a workshop on this topic ("From Technological Maturity to Storing of Cereals and Legumes") (Trdan, 2014ab), however even three years before we transfered our knowledge in the field of stored pests protection to Slovenian agricultural specialists in a siminal event (Trdan and Bohinc, 2011). In 2015, we have hosted C. Athanassiou as an invited lecturer at the 12th Slovenian Conference on Plant Protection with international participation in Ptuj (Athanassiou, 2015; Trdan, 2015ab).

2.9 Cooperation with foreign experts

In 2007, we participated as partners in the project SEE-ERA.NET "Development of a non-toxic, ecologically compatible, natural-resource based insecticide from diatomaceous earth deposits of South Eastern Europe to control stored-grain insects pests" (coordinated by C. Athanassiou), and we thus became acquainted with the research work in the field of investigation the efficacy of diatomaceous earth in controlling beetles from the *Sitophilus* genus (Athanassiou et al., 2009, 2011).

The opportunity to reduce the amount of pirimiphos-methyl applied to grain by formulating it in an electrostatic powder was investigated in a study of international research group. The insecticidal efficacy of pirimiphos-methyl in EC formulation or formulated using electrostatic powder (EP) as an inert carrier was investigated against *Sitophilus oryzae* (L.), *Oryzaephilus surinamensis* (L.), *Rhyzopertha dominica* (F.) and *Tribolium confusum* Jacquelin du Val. Furthermore, the adhesive properties of EP to rice, corn and wheat, together with the effect on bulk density and bread- and pasta-making properties, were investigated. The results showed that pirimiphos-methyl formulated with EP provided better efficacy against adultswhencompared with EC formulation for O. surinamensis and T. confusum, but there was no difference for R. dominica. Progeny production was consistently lower in grain treated with the EP formulation than in grain treated with the EC. Tests showed that EP adhered to the kernels for longer on hard wheat than on maize or rice. In most commodities, EP did not alter the bulk density. Finally, the addition of EP did not affect flour- and bread-making properties, nor the pasta-making properties. The results of the present study suggest that an EP could be used to reduce the amount of pirimiphos-methyl applied to grain for effective pest control, with no detrimental effects on grain quality (Athanassiou et al., 2016, 2017).

The study of Slovenian-Serbian research group focused on examining of spinosad and spinetoram efficacy after 21 days of *S. granarius* and *S. oryzae* adults exposure in treated wheat grain and their influence on weevils offspring production and wheat grain damage rates. Investigation was conducted under laboratory conditions at 25±1ºC and 70±5% r.h. Both insecticides were applied to untreated wheat grain with 12.3±0.1% of m.c. at the rates of 0.5, 1.0 and 2.0 mg a.i./kg for both weevil species. Then, 25 adults were added to each plastic dish containing 50 g of treated wheat, in six replicates, for each insecticide/species tested. Mortality of weevils was determined after 21 days, and the effect on progeny production was determined seven weeks after parental exposure. When the offspring were counted, damage caused by the weevils were also assessed on 100 randomly selected kernels. Spinosad and spinetoram demonstrated the highest mortality (96-100%) of *S. granarius* and *S. oryzae* parents after 21 days of contact with 1-2 mg/kg and 2 mg/kg, respectively. The highest *S. granarius* offspring reduction (>90%) was found in wheat treated with 2 mg/kg spinosad and 1-2 mg/kg spinetoram, while S. oryzae offspring reduction was the greatest in wheat treated with 2 mg/kg spinetoram. In these experimental conditions, the percentage of grains damaged by *S. oryzae* was ≥50% in wheat treated with 0.5-1 mg/kg spinosad and 0.5 mg/kg spinetoram, while grain damage below 5% was found only in wheat treated with 2 mg/kg spinetoram. The results show that spinetoram was more effective than spinosad. Also, *S. granarius* was more susceptibility to both insecticides than *S. oryzae*. Under these experimental conditions, spinosad and spinetoram can be successfully used to control both weevil species at the rate of 2 mg/kg (Andrić et al., 2016, 2017).

Discussion

In 12 years of our work in the field of stored product pest control, our laboratory studies most often included three species from the genus *Sitophilus* (*S. granarius, S. oryzae* in *S. zeamais*) and the bean weevil (*A. obtectus*), i.e. harmful organisms which are in Slovenia of great economic significance. The economically harmful butterflies (E. *kuehniella, P. interpunctella* and *S. cerealella*) have been so far addressed in only one study. The main body of our research work included studying insecticidal properties of diatomaceous earth and other inert dusts, e.g. quartz sand, plant powders, wood ashes and zeolites. Diatomaceous earth was due to its efficiency often used as a positive control when studying the efficiency of other inert dusts. Considerable fumigant effects on harmful beetles were

in our laboratory experiments displayed also by some essential oils, particularly rosemary essential oil. In our professional work, in which we have been systematically sampling autochthonous natural enemies for more than 10 let years, we found two parasitoids of stored products beetles. In our view, the more important of these two is *Anisopteromalus calandrae*, which is already being systematically introduced in cereal storages in some European countries, while the Slovenian legislation in the field of biological control do not yet enable its use in practice. By organising expert meetings for the needs of the domestic experts who study the significance and control of stored product pests, we provide implementation of expert knowledge into practice, and we were delighted when the IOBC-WPRS entrusted us with the organisation of the 11th Conference of the IOBC/wprs (OILB/srop) Working Group on Integrated Protection of Stored Products (Ljubljana, Slovenia, 3-5 July 2017), which is also a result of our recognisability and our good cooperation with foreign experts. Studies in the field of monitoring and control of stored product pests will remain a part of our research-professional activities, as this group of harmful organisms will be also in future, due to the increasing world population, intense international trading in plant materials, climate changes and some other factors, one of the economically most important groups of plant pests.

Acknowledgement

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Investigations on the efficacy of Turkish diatomaceous earth comparing with SilicoSec? against the stored grain pests

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Abstract

In this research, both Turkish diatomaceous earth from Central Anatolia and SilicoSec? was evaluated against *Tribolium confusum* Jacquelin du Val (Tenebrionidae: Coleoptera), *Rhyzopertha dominica* (F.) (Bostrychidae: Coleoptera) and *Sitophilus granarius* (L.) (Curculionidae: Coleoptera) adults. Different rates (0, 250, 500, 750, 1000, 1500 ve 2000 mg/kg wheat) of diatomaceous earth from both sample mixed with wheat were evaluated for 1, 2, 3 and 4 -weeks exposure period at 55% r.h. and 25 adult/vial insect density in ten replicates. Mortalities were determined at the end of exposure, whereas F1 adult production were determined at 8 weeks after mortality

observation.   Mortalities and F1 adults of *T. confusum*, *R. dominica*, and *S. granarius* was significantly changed by the rates of diatomaceous earth. In untreated wheat mortality was less than 10% for three species. Mortalities at 2000 mg/kg wheat applications at the end of 4-week of exposure against *S. granaries* were found to be 95.6% and 98% for SilocoSec and local DE, respectively. For *R. dominica* adults, mortalities were recorded as 90% and 92.4% for SilocoSec and local DE, respectively. Additionally, mortality of *T. confusum* at 2000 mg/kg wheat was 100% for both DE samples. Results showed that local DE and SilocoSec are equally effective against three major stored grain pests.

The Effectiveness of Silicosec, Diatomaceous Earth Against the Lesser Grain Borer, *Rhyzopertha dominica* (L) (Coleoptera: Bostrichidae)

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Abstract

In this research, the efficacy of SilicoSec was assessed in two sets of experiments. In the first set of experiment, the efficacy of SilicoSec at the rates of 0, 250, 500, 1000, 1500, 2000, 2500 and 3000 mg/kg wheat were evaluated against *Rhyzopertha dominica* adults at 55% r.h and 25°C. Insect counts were performed at the end of 3 week of exposure for the mortality. F1 progeny assessment were made after 8 weeks.

In the second set of experiment, the efficacy of SilicoSec against the same insect pest was evaluated at dose rates of 0, 250, 500, and 1000 mg/kg wheat for three months of exposure. In each experimental vial there were 10 adults per 250 g wheat with 10 replicates. Insect counts as dead and alive were made at the end of three months of exposure. For each vial, progeny production was determined as total insects excluding 10 adults introduced at the beginning of the experiment.

According to results, increase in dose rates increased the adult mortality, while it decreased the progeny production. As the exposure time increased, mortality rates were also increased. At the dose of 2000 mg/kg, 98.5% adult mortality and 10.55 adult progeny per vial were obtained.

For three months exposure, population development was inversely proportional to dose rates. According to results, increase in dose rates increased the adult mortality, while it decreased the progeny production. Population confinement was achieved at 1000 mg/kg dose rate of SilicoSec for three months of exposure.

Key words: Diatomaceous earth; *Rhyzopertha dominica*; exposure interval; progeny production; mortality

Host-preference and parasitic capacity of five *Trichogramma* species (Hym.: Trichogrammatidae) against some stored product moth pests

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Abstract

Most stored product insects are either beetles or moths. Moth pests are important hazards to the storage of a wide variety of products. Natural enemies are applied commercially against stored-product moths in Central Europe .So, the host-preference and parasitic capacity of four local *Trichogramma* spp.(*T. bourarachae*, *T. cordubensis*, *T. euproctidis* and *T. cacoeciae*) , towards four species of stored product moth eggs were investigated in laboratory experiments in order to select new candidate species for use in mass rearing and biological control against moths in storages. The results were compared with *T. evanescens,* the common wasp used commercially for biological control. The naturally occurring *Trichogramma* species were collected for the first time in Egypt from two representative olive growing areas in arid area (170 km south of Alexandria) and semi-arid area (60 km west Alexandria, near the coast). All these wasps were also bred from naturally parasitized host eggs during

favorable and even at unfavorable temperature conditions of June-August. The presence of warm weather wasp-strains may suggest the existence of well-adapted wasp species or strains which may be appropriate candidates for the control of stored product pests. The strains had also been collected in late winter and summer, thus demonstrating activity also during less favorable weather conditions, raising again the possibility of using these egg parasitoids as an inundative biological control agent in stored products.

Experiments were carried out by offering eggs of the Indianmeal moth *Plodia interpunctella* (Hübner), the Mediterranean flour moth *Ephestia kuehniella* Zeller, the warehouse moth *E. elutella* (Hübner), and the almond moth *Cadra cautella* (Walker) in choice and no-choice assays to a single female parasitoid. Two different choice experiments were used to certify the same conclusion in both methods. The bioassay for host-preference of *Trichogramma* spp. was carried out by offering a single female wasp the choice between equal numbers of host eggs on square cards "Petri dish tests "and /or strip cards "strip card tests". In both methods ,counting the number of *Trichogramma* developing in the host eggs (parasitism) show the preference of the wasp for ovipositing and indicated the ability of the parasitoid to develop in these eggs (i.e., host suitability).

In Petri dish tests, *E. kuehniella* was a highly accepted host species for *T. bourarachae*, *T. euproctidis*, and *T. cacoeciae* wasps while *E. elutella* and *C. cautella* eggs were more accepted by *T. evanescens* and *T. cordubensis,* respectively. In the strip card tests, *E .kuehniella* eggs were highly accepted by *T. bourarachae, T. cacociae* and *T. evanescens.* Eggs of *E. elutella* and *C. cautella* were more acceptable for *T. euproctidis* and *T. cordubensis,* respectively. Furthermore, a comparative study of the parasitic capacity of the *Trichogramma* spp. was carried out under 'no choice conditions' by exposing a freshly emerged single wasp to an unlimited number of host eggs. Significant differences were found among the parasitic capacity of the tested *Trichogramma* spp.: *T. bourarachae* showed a good parasitic potential against *S. cerealella* and *E. kuehniella*; *T. evanescens and T. cacoeciae* against *S. cerealella*; *T. cordubensis* against *S. cerealella* and *P. interpunctella* and *T. euproctidis* against *P. interpunctella*. However, dissection of host eggs with wasp-emergence holes showed that all tested wasps had a propensity to superparasitize the host eggs. Results of the present work suggest that the test wasps failed to discriminate parasitized hosts eggs among a large number of non-parasitized eggs, thus superparasitism occurred. Also, both of Petri dish and strip cards methods may underestimate the actual parasitization capacity due to self-superparasitism and mortality in black eggs that suffered desiccation during the early stages.

T. cordubensis, *T. euproctidis* and *T. bourarachae* showed promise for further investigation into selecting new biological control agents against some stored product lepidopterous pests.

Keywords: Stored product moths; *Trichogramma* spp.; host preference; parasitization capacity; superparasitism.

Monitoring of the Indian meal moth and its parasitoids in long-term grain storage

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Abstract

The Indian meal moth *Plodia interpunctella* became a major pest in bulk grain storage in Germany in recent years. Monitoring with adhesive pheromone-baited traps revealed a dependence of the number of generations of the moth from the temperature conditions in store, which themselves depend on insulation of the storage structure. The larval parasitoid *Habrobracon hebetor* was monitored with the help of cone traps placed in the grain. Baiting these traps with moth webbings significantly increased the number of female wasps trapped in 5 cm depth in wheat. Field trials showed both the pest and the beneficial can be monitored in stores, but more research is needed to develop a biological control strategy for *P. interpunctella*.

Keywords: stored products, bulk grain, Pyralidae, Trichogrammatidae, Braconidae

Introduction

Monitoring of pest populations is a basic prerequisite for biological control of stored-product pests (Zimmermann 2004), but difficult in large quantities of bulk grain. Within the frame of a project on the application of beneficials in long-term grain storage of grain, the phenology oft the Indian meal moth *Plodia interpunctella* (Hübner, 1813) (Lepidoptera, Pyralidae) was studied in different grain flat

stores. In German grain storage, large populations of the Indian meal moth were observed in the past six years resulting in significant damage and repeated control activities, consequently new control options were evaluated. The Indian meal moth is laying up to 400 egges close to the stored product. The emerging larvae typically develop through five instars in grain. A first signal of infestation are webbings on the grain surface produced by the larvae, produced presumably for protection. The last larval instar shows increased mobility in order to find a pupation site, and is consequently called wandering larva (Mohandass et al., 2007).

For the development of a biological control strategy a monitoring oft he beneficials is helpful. The larval parasitoid *Habrobracon hebetor* (Say, 1836) is naturally occuring in Central Europe, among its hosts are the Indian meal moth, the warehouse moth *Ephestia elutella* (Hübner, 1796) and the flour moth *E. kuehniella* Zeller, 1879 (Prozell & Schöller 2001).

In this study, the effectiveness of different monitoring techniques for parasitoids is described and the potential release of benefials within the frame of an integrated control concept discussed.

Materials and Methods

Monitoring of the Indian meal moth *P. interpunctella*: The phenology was studied in different flat grain stores. Various methods were applied, i.e. adhesive surfaces for larvae and adults, artificial pupation aid structures for larvae, and pheromone-baited traps for adults (Fig. 1).

Monitoring of the larval parasitoid *H. hebetor*: 20 kg grain, wheat or oats, respectively, were filled in a Hobbock. Per Hobbock, one cone trap was placed (Fig. 1). The cone trap has two components, a vial and a perforated lid. The lid is regularly vaulted, this is where the insect enter. The diameter of the holes in the lis is approximately 2 mm. Liquid teflon is added to the upper rim oft he vial, in order to hinder the insects to move back tot he lid. The cone trap was placed either on the grain surface, or 5 cm deep in the grain. The cone traps on the surface were carefully placed in one level with the grain surface. The cone traps were either unbaited, or baited with 5 g of webbings of the flour moth. Per Hobbock, 50 *H. hebetor* were released, i.e. 25 females and 25 males. The Hobbocks were closed and stored for 14 days at 20.8°C ± 0.95°C (mean ± SD) and 43.6% ± 5.5% RH (mean ± SD). After that period, the cone traps were removed and the traped *H. hebetor* counted. The sex ratio of *H. hebetor* was determined. Moreover, six cone traps each were placed in different grain flat stores (3000–4000 metric tons wheat) and removed ca. four weeks after parasitoid release, again the number of insects traped was counted.

The number of trapped insects were compared with the help of a t-test, in case data were not normally distributed, the Mann-Whitney Rank Sum Test was applied. Statistical analyses were performed using the software package SigmaStat 3.1.

Fig. 1 Cone trap used for monitoring of *Habrobracon hebetor* (a) and adhesive trap baited with sex pheromone (ZE-TDA) (b) for monitoring of *Plodia interpunctella* in flat grain storage.
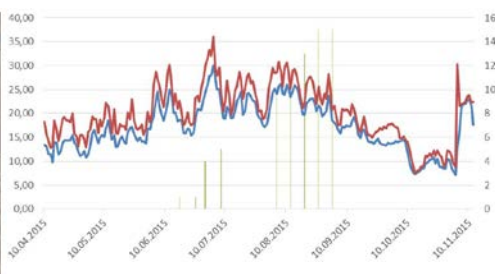
Fig. 2 Records of adult *Plodia interpunctella* (green bars) in a non-isolated store in Northern Germany, 2015. Left axis: surface temperature [°C], right axis: no. of moths. Blue line: mean temperature, red line: maximum temperature.

Results

Monitoring *of P. interpunctella*: In a non-isolated storage building in Northern Germany, adult moths were recorded already in June, 2015 (Fig. 2), while in a well isolated storage building in Southern Germany, adult moth activity started in August (Fig. 3). At that time, a second generation of adults developed already in the non-isolated store (Fig. 2). A peak in the number of adult moths in the non-isolated store was reached in August.

While the F_2-moth progeny in the non-isolated store overwintered in the last larval instar, only one moth generation was recorded in the well-isolated grain store (Fig. 3).

Monitoring of Habrobracon hebetor:

(1) Unbaited cone traps: both in wheat (t-Test, t = 8.061, DG=4, P<0.001) and in oats (t-Test, t = 8.061, DG=4, P<0.001), significantly more wasps were traped in 0 cm compared to 5 cm depth (Fig. 4). On the surface, significantly more wasps were traped in wheat compared to oats (t-Test, t = -3.742, DG=4, P = 0.020). In 5 cm depth, no difference in recapture was detected comparing wheat and Oats (t-Test, t = -0.707, DG=4, P > 0.05).

(2) Baited cone traps: baited with webbings, cone traps on the surface, caught more wasps in oats compared to such unbaited traps on the surface (t-Test, t = -8.721, DG=4, P <0.001) (Fig. 4). In wheat, baited traps caught on the surface a larger number of wasps, however, this difference was not significant (t-Test, t = 2.286, DG=4, P = 0.084).

Baited traps placed in 5 cm depth in wheat caugth more wasps compared to unbaited traps (t-Test, t = -3.530, DG=4, P = 0.024). In oats, no difference in wasps caught in traps placed in 5 cm depth was detected whether the traps were baited with webbings or not (t-Test, t = -1.444, DG=4, P = 0.222). More female wasps were caught in total with baits (t-Test, t = -2.119, DG=22, P = 0.046), but the presence of webbings as bait did not increased the number of males traped (Mann-Whitney Rank Sum Test, T = 125.5, n=12, P = 0.163).

Fig. 3 Records of adult *Plodia interpunctella* (red bars) in a well-isolated grain store in Southern Germany in 2015. Left axis: surface temperature [°C], right axis: no. of moths. Blue line: mean temperature, green line: maximum temperature.
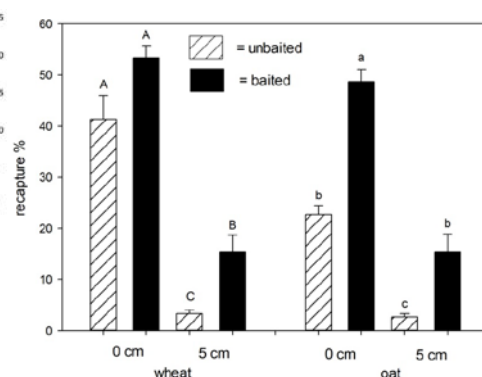
Fig. 4 Recapture of *Habrobracon hebetor* with the help of cone traps in percent, with or without 5 g webbings of *Ephestia kuehniella* as bait in wheat or oats. 50 *H. hebetor* (males and females) released. Different caps indicate significantly different recapture, major caps for wheat, small caps for oats (p<0.05).

Discussion

Under field conditions, pupation of moth larvae and the start of flight of the adult Indian meal moths in spring are mainly depending on temperature in the store. While in a non-isolated store the progeny of the second generation overwintered as larvae, in an isolated cooler store only one generation was observed anually, These observation under practical conditions is well explainable by the developmental time known from laboratory data (Mohandass et al., 2007).

The attractiveness of moth webbings in the cone traps placed in grain on the females confirms olfactometer laboratory trials showing kairomonal activity of webbings produced by different species of pyralid Lepidoptera for *H. hebetor* (Strand et al. 1989). Moreover, foraging H. hebetor were shown to enter into bulk grain in previous studies (Schöller, 2000). In the present study, female *H. hebetor* were shown to exploit signals from moth webbings in bulk grain, too. Consequently, parasitisation of Indian meal moth larvae can be expected below the grain surface, too. This behaviour of *H. hebetor* can also be used to monitor the foraging behaviour of the wasps under practical conditions of storage. In wheat, more *H. hebetor* were traped compared to oats. This might be due to the three-dimensional structure of the bulk grain.

Both male and female *H. hebetor* were caught with the cone traps. The capture of females in unbaited traps indicates this trap type is able to record passivly the movement activity oft the parasitoids. Males could potentially be attracted by already caught females, however, in our trials, a signicantly higher number of females in the baited traps did not result in a significant increase in the number of males caught.

The results on monitoring showed the possibility to record data on the phenology oft he Indian meal moth and *H. hebetor* under practical field conditions. The abiotic conditions in differnt grain stores are subject to wide variation, consequently more field trials are needed in order to develop recommendations for biological control of the Indian meal moth.

Acknowledgement

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A preliminary study of growth and development of *Cheyletus malaccensis* (Oudemans) under different humidity conditions

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Abstract

Cheyletus malaccensis (Oudemans) is a species of predatory mite, which is widely distributed in grain storage, and is a potential natural enemy of stored-product pests. Based on the typical temperatures and humidities that occur in granaries, the growth and development of *C. malaccensis* was studied at 24°C with different relative humidities (RH 65±2%, 75±2%, 85±2% and 95±2%). During this study, *C. malaccensis* was fed on *Acarus siro* (Linnaeus), a very important stored grain pest to investigate its potential to control this pest and production of this natural enemy in the laboratory. The results showed that *C. malaccensis* has five developmental stages, egg, larva, protonymph, deutonymph and adult. The deutonymph stage is absent in males. For females, the developmental time from egg to adult was shortest at 85±2 % RH and averaged 16.3 days; developmental time was longest at 65±2 % RH and averaged 18.6 days. The male mites in the 95±2% RH trials had the shortest developmental time which averaged 12.6 days; it was longest at 65±2% RH where it averaged 14.7 days. At 95±2

% RH, the male adult lived 83.5 d and its longevity from egg to adult was 95.8 d. Humidity had a significant effect on how long the adults lived and the duration of all developmental stages. At 85±2 % RH, the maximum average number of eggs per female, oviposition period and daily fecundity were 493.0, 46.2 d, and 10.3, respectively. This study provides basic biological parameters for *C. malaccensis*, a potential biological control agent for mite pests infesting stored grain.

Key words: *Cheyletus malaccensis*; development, reproduction, biological control
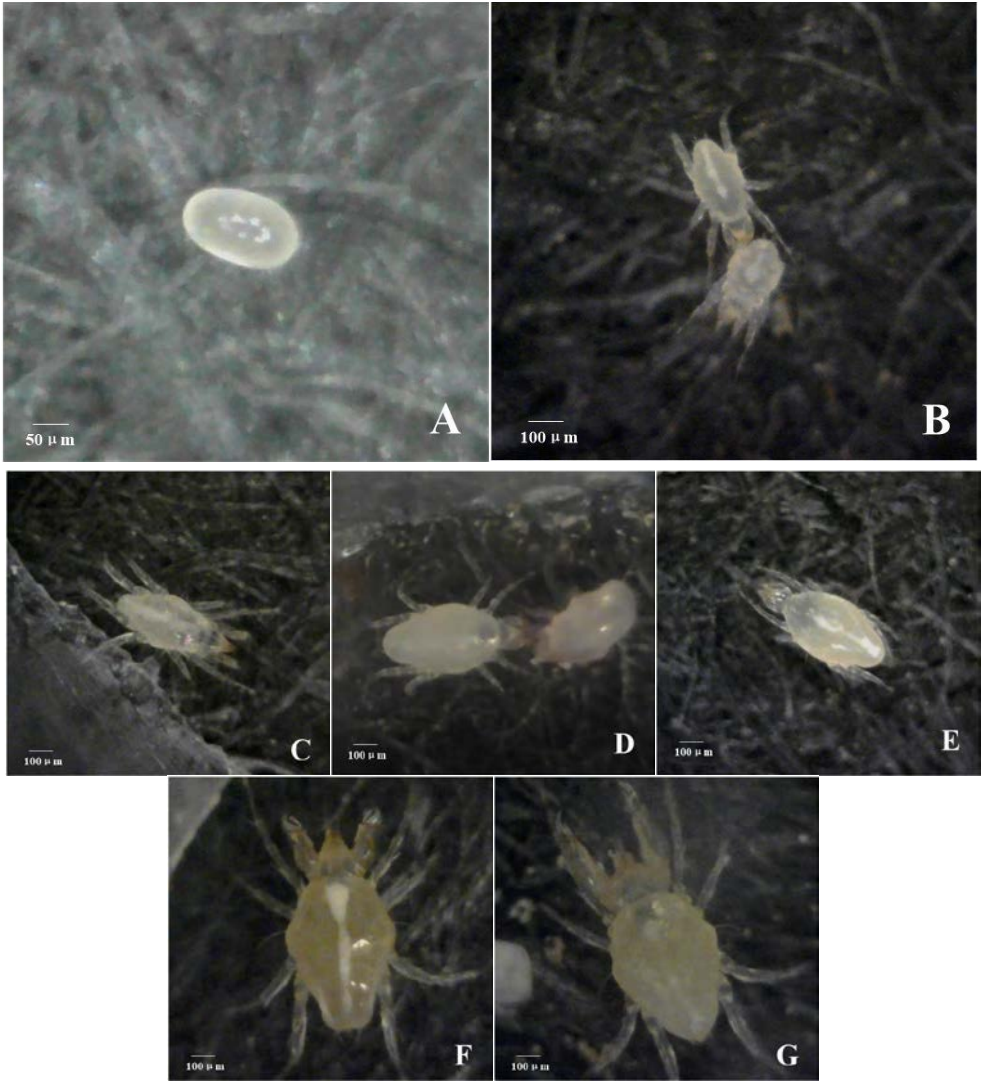
Fig.1 The development stages of *C. malaccensis* , A: Egg B: Larva C: Protonymph D: Deutonymph E: Hypopus F: Female G: Male

Table 1 The developmental duration of *C. malaccensis* **at different relative humidity conditions**

Relative humidity (%)	Egg(d)	Larva				Protonymph				Deutonymph		Life history(d)		Adult(d)		Development duration of all stages	
		Mov. (d)		Qui. (d)		Mov. (d)		Qui. (d)		Mov. (d)	Qui. (d)						
	Female Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Female	Female	Male	Female	Male	Female	Male
65±2	2.9±0.262.6±0.18	4.2±0.31	5.0±0.37	0.9±0.07	1.6±0.18	4.0±0.38	4.0±0.38	1.1±0.14	1.6±0.38	3.8±0.46	1.6±0.24	18.6±0.48	14.7±0.69	51.8±10.83	47.8±2.83*	70.2±10.79	61.8±2.98*

75±2	3.0±0.00	2.4±0.15	3.3±0.33	4.6±0.47	1.0±0.00	1.4±0.21	3.7±0.33	4.0±0.22	1.0±0.00	1.1±0.09	3.3±0.67	2.0±1.00	17.3±0.33	13.9±0.73	57.0±21.59	40.8±5.69* 74.3±21.26	54.7±5.50*
85±2	2.0±0.38	2.4±0.29	3.7±0.57	5.3±0.92	1.1±0.14	1.3±0.18	3.3±0.36	3.1±0.51	1.0±0.00	1.0±0.00	3.4±0.43	1.7±0.42	16.3±1.27	13.1±1.10	53.8±7.07	71.0±5.80* 70.0±6.72	83.5±6.00*
95±2	2.9±0.14 2.1±0.1	4.3±0.29	3.6±0.29	1.1±0.14	1.1±0.14	3.7±0.18	4.3±0.57	1.7±0.57	1.4±0.20	3.1±0.34	1.3±0.18	18.1±0.46	12.6±0.48	50.5±8.59	83.5±7.53* 68.7±8.39	95.8±7.61*	

Note: The data in the table are means±SE. *Mean significantly different ($P<0.05$).

Table 2 The developmental duration of stages and oviposition between *C. malaccensis* and *C. eruditus*

Prey	Predator	Temperature (°C)	Relative Humidity (%)	Sex	Egg	Larva Mov.	Larva Qui.	Protonymph Mov.	Protonymph Qui.	Deutonymph Mov.	Deutonymph Qui.	Life history	No. of eggs per female	Oviposition period	Author
T. putrescentiae	C. malaccensis	24-25	75		4	3.5		3.5		5.5		19.5	73	6	Zhaopeng Shen
	C. malaccensis	25	80±5	Female	4.3	7.5		6.9		6.1		24.8			Palyvos、Emmanouel
				MaleVirgin	3.9	7.4		6.7				18.0	47.6±6.9	15.3±0.6	
				Fertilized	4.3	7.8		7.2				19.3	88.6±10.1	17.5±0.5	
	C. eruditus	24	80		3.34	3.85	1.66	3.32	1.71	2.79	1.65	18.32			Bin Xia
L. destructor	C. malaccensis	18-22				5-6	3	3-4	2	3-4	3		77-107	14-16	Yanxuan Zhang
	C. eruditus	25	76		3.3	3.5		4.5		4.1			132.8	25.3	Barker
A. ovatus	C.malaccensis	25±0.1	75±2	Female	3.3	5.2	1.2	4.5	1.4	3.5	1.6	20-23		10.1±0.3	Saleh.M
				Male	3.3	5.2	1.3	4.4	1.6			15-17			
D. gallinae	C. malaccensis	25	80±5		4.74	5.24		4.38		3.96		18.38			Maicon Toldi、Faleiro
A. siro	C. eruditus	24	75		5.0	7.8		7.2		6.3					Peihuan He

Table 3 The oviposition of **C. malaccensis** parthenogenetic at different relative humidity conditions

Relative humidity （%）	65±2	75±2	85±2	95±2
No. of eggs per female	418.0±91.90	427.3±178.44	493.0±104.52	348.2±101.06
Oviposition period	45.4±10.57	44.7±18.10	46.2±8.21	34.0±7.39
No. of eggs laid by each female per day	9.5±0.33	9.4±0.99	10.3±1.20	9.0±1.70
Max. no. of eggs laid by each female per day	23.6±1.81	19.3±1.86	20.0±1.00	21.2±3.30
Pre-oviposition	3.2±1.09	2.3±1.15	3.0±1.00	1.3±1.50
Post-oviposition	3.6±2.40	2.7±2.67	7.0±2.67	7.2±1.29

Note: The data in the table are means±SE.

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Evaluation of the potential value of the F₁H and F₂H Diatomaceous earth formulations as grain protectants against *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae)

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Abstract

An insecticidal efficacy of two **newly developed** grain protectant formulations were assessed against lesser grain borer *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) on wheat and corn after 6 months period of. Tested formulations, marked as F₁H and F₂H, based on inert dust, laurel leaves, lavender essential oil, corn oil, silica gel (both F₁H and F₂H) and pyrethrin (only F₂H) were tested at six doses (from 100 ppm to 600 ppm) depending on formulation and type of grain. The appropriate weights of each formulation, were added seperately to plastic containers containing 10 kg of wheat or corn. An initial population of 200 adults of *R. dominica* were added into each container and left under natural environmental conditions for up to 6 months. A commercial diatomaceous earth (DE) insecticide, Celatom® Mn 51, was used for the comparison of the results, in addition to the untreated control. After six months, both formulations showed higher insecticidal effect than DE Mn 51 in corn and in wheat. Furthermore, the initial population of *R. dominica*, introduced in wheat was suppressed almost completely, with only 0.7%-5.3% live adults found, depending on formulations and dose. The order of efficacy was F₁H>F₂H>DE Mn 51. Similar suppression of the initial population was recorded in corn, where F₂H was slightly more effective than F₁H with 2.0%-10.6% and 4.1%-9.5% live adults found, respectively. At the same time, in the treatments with DE Mn 51 there were 4.7%-74.7% and 33.4%-56.1% live adults in wheat and corn, respectively.

Keywords: inert dust, botanicals, grain protectant, stored product insects, insecticidal effect

Introduction

Minimising food commodity losses, both qualitative and quantitative, during longer period of storing represents a main challenge for all economies. Stored-product insects play a significant role in postharvest losses, causing losses in grain weight, affects on baking quality and seed viability (Sánchez-Mariñez et al., 1997; Stejskal et al., 2015), which lower cereal market value. The use of synthetic insecticides is globally the most common way of controlling stored product insect pestsof the negative effects of pesticides on stored products includes: toxic residues (Fang et al., 2002), resistant strains within the insect populations (Chaudhry, 2000; Boyer et al., 2012), adverse effect on human health and environment (Fields and White, 2002). Thus there is an urgent need for alternative strategies which would be sufficiently effective against insects but less toxic for the environment.

The use of inert dusts, especially diatomaceous earth (DE), suits most of those requirements. Its main advantages are low mammalian toxicity and stability (Maceljski and Korunic, 1972; Subramanyam and Roesli, 2000) and an efficient insecticidal activity without leaving hazardous residues (Korunic, 1998; Shah and Khan, 2014; Liška et al., 2015; Korunić et al., 2017.). Despite this there are several limitations which hinder wider commercial use of DE for direct mixing with grains and are described by Korunić (2016). Diatomaceous earths, inert dusts in general, have physical mode of action and therefore act more slowly than conventional contact insecticides. Depending greatly on ambient conditions, it could take a several days to control most target insect species (Korunić et al, 2016), providing enough time for oviposition. Further, there are different sensativities of insect species to DEs, varying effects of DE on insects depending upon the commodity being treated and a negative effect on bulk density (Korunic, 2016; Korunić et al, 2017). Possible solutions for minimising or avoiding those implications include incorporating DE with other methods, such as extreme temperature (Dowdy, 1999), mixture with synthetic insecticides (Athanassiou, 2006; Korunic and Rozman, 2010), mixture with entomopathogenic fungi (Batta and Kavallieratos, 2018) or with botanicals (Korunic et al, 2014; Adarkwah et al, 2017).

Most experiments with inert dusts and their mixtures are carried out in controlled conditions, and less in the real conditions where various environmental and storage conditions could impact efficacy and subsequently, storage duration of the commodity.

The objective of this study was to test insect activity of two new developed formulations based on Croatian inert dust, bay leaves, lavandin essential oil, bait, corn oil, silica gel and pyrethrin against *R. dominica* in wheat and corn grain after six months period of storage.

Materials and Methods

Test insects

A local strain of *R. dominica*, was used in the experiments. Insects were reared on clean soft whole wheat kernels under controlled conditions (28±2°C, 65±5% RH, in dark). Two hundred, unsexed adults (7-21 days old) were used for each treatment.

Commodity

Locally available commercial corn and soft wheat were used in the treatments. Commodities were sifted prior to tests in order to segregate broken kernels and other impurities. Grain moisture content and grain temperature were measured by the GAC 2100-Agri Grain analysis computer (Dickey-john). The initial measurements were 11.1 % m. c. and 23.2°C for wheat, and 10.7 % m. c. and 23.3°C for corn. Ten kg of clean wheat and corn grain was used for each treatment.

Formulations

Two powder formulations labelled as F_1H and F_2H, were based on an inert dust of Croatian origin, dried and milled bay leaves, essential oil of lavandin (Lavender x Intermedia), bait, corn oil, silica gel

and pyrethrin (only F_2H). A commercial DE insecticide, Celatom® Mn 51, was used for the comparison of the results. It belongs to a group of DE's with medium to high efficacy against stored-product insects. Formulations and DE were tested at different doses depending on the treatment. In wheat, F_1H and DE Celatom® Mn 51 were tested at 300, 400 and 500 ppm, and F_2H at 100, 150 and 200 ppm, while in corn F_1H and DE Celatom® Mn 51 were tested at 400, 500 and 600 ppm, and F_2H at 200, 250 and 300 ppm.

Bioassay

The dose rates of the tested formulations and DE were chosen based on results of preliminary laboratory test (unpublished data). Plastic containers of 15 L volume were filled with 10 kg of clean wheat or corn respectively. The appropriate weights of the formulations or Celatom DE were added into each container and mixed thoroughly with a power drill. Containers with untreated grain served as controls. After the dust had settled, 200 unsexed, 7-21 days old adults of *R. dominica* were added into each container which were then closed with perforated plastic lids. The bioassay was conducted for six months storing the containers in a wooden structure which simulated an average floor warehouse. The air temperature during the entire period of storing varied between 18.5°C and 23.0°C and relative humidity between 55.0% and 82.0%. After the end of the bioassay trial, the entire content of the plastic containers was sieved and all insects, dead and live, were counted.

Results

After six months of storing of treated corn and wheat, tested formulations F_1H and F_2H showed different efficacy against *R. dominica* depending on the cereal type and the applied dose.

In wheat (Figure 1), both formulations successfully preserved grain quality against *R. dominica* during the period of six months. Even at the lowest dose, both formulations (300 ppm of F_1H and 100 ppm of F_2H) almost completely suppressed the initial population of *R. dominica,* therefore after six months, there was no population increase with only 0.7-3.8% (depending on dose of F_1H) and 3.8-5.3% (depending on dose of F_2H) of the original live adults found. For comparison, Celatom® Mn 51 at the lowest dose was not effective, resulting in a population increase (1688.5% higher than the initial population) within 74.7% live insects found. Population increases were also observed in the control treatment in wheat where number of insects from the initial 200 individuals increased up to 11817 within 93.13% live adults.

In corn (Figure 2), both formulations also showed better efficacy then Celatom® Mn 51. According to the number of adults found at the end of the testing period, formulation F$_2$H was more effective in regard to F$_1$H. Further, even at the lowest dose (200 ppm) no population increase was observed, while F$_1$H resulted with 62% and 14.5% of the population increase (at the dose of 400 ppm and 600 ppm, respectively). However, in the treatment with DE Mn 51, the highest dose (600 ppm) was not efficient enough to control *R. dominica*, and the population increase was in the range from 64.5 to 107.5% (higher than initial population) (depending on dose) within average of 45.4% live adults found. Concerning control treatments, unlike in wheat no population increase was observed in corn. Due to high temperature and high moisture content of the grain during six months fungi developed intensively and the whole stock become glued mass, so insects could not survived in those conditions.

Fig. 1 Efficacy of the formulations F$_1$H and F$_2$H against *R. dominica* after six months of wheat storing

Fig. 2 Efficacy of the formulations F$_1$H and F$_2$H against *R. dominica* after six months of corn storing
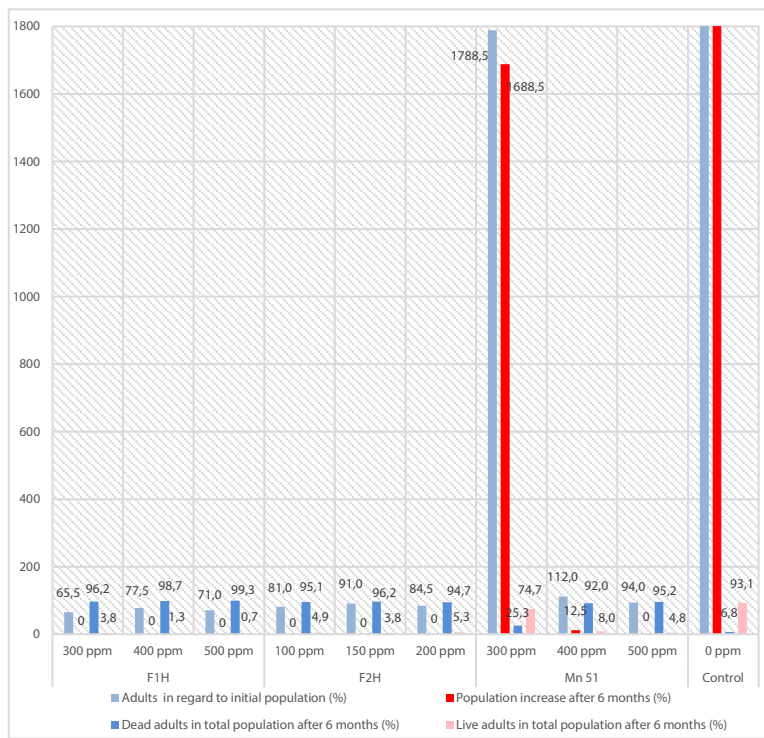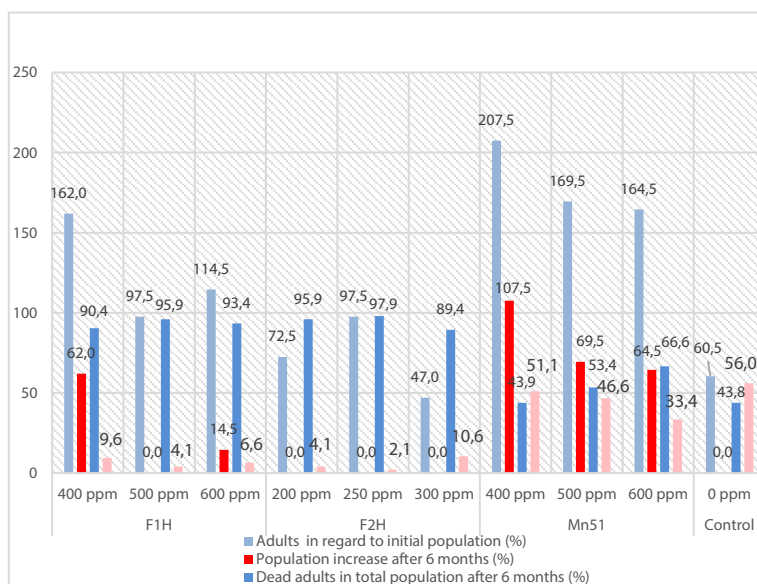
Discussion

Increased efficacy of the tested formulations against *R. dominica,* compared to DE alone was expressed because of the mixture of different active ingredients within their composition and due to their different modes of action. Besides inert dusts, the main composition of the formulations are the botanicals powdered bay leaves and lavandin essential oil. These compounds possess fast toxic activity against many coleopteran pests of stored products (Kostyukovsky et al 2002; Rajendran and Sriranjini, 2008; Nerio et al 2010; Koutsaviti et al, 2018). Comprised of volatile monoterpenoids and sesquiterpenenoids, the essential oils interfere with basic metabolic, biochemical, physiological and behavioural functions in insects possessing contact, inhalation and ingestion toxicity, antifeedant activity, developmental delay of adult emergence and fertility, different effects on oviposition and repellent activity (Obeng-Ofori 2007; Caballero-Gallardo et al, 2012; Nenaah 2014; Germinara et al, 2017). DEs and general inert dusts, with physical mode of action, are slow acting protectants (Korunic, 1998). Apparently, as a mixture of plant powders, essential oils and inert dusts, our formulations accelerate the knock down effect of the adults within the initial population of *R. dominica* which resulted in prevention of mating and reduced oviposition. Food grade bait composed within our formulation probably also accelerated insect mortality. Presumably, it attracted insects which kept them in the contact with inert dust for longer period. Consequently, insects picked up more inert dust particles on their body which led to faster desiccation (Korunić et al, 2016).

Adarkwah (2017, 2017a) reported faster activity of the mixture of DE and plant powders against three different stored product insects.While, mentioned authors conducted their trials in laboratory conditions and only during 7 days of exposure, our tested formulations secured effective control of the tested pest during the whole testing period of six months in conditions that might more realistically represent true storage conditions.

Overall two developed formulations, as a combination of inert dusts and botanicals could be promising insecticides with residual effect for protecting stored wheat and corn against insect infestation.

Acknowledgement

based on inert dusts and botanicals to replace synthetic, conventional insecticides", www.diacromixpest.eu.

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Olfactory host location and host preference of *Holepyris sylvanidis* (Hymenoptera: Bethylidae) and *Cephalonomia waterstoni* (Bethylidae), two natural enemies of *Tribolium* and *Cryptolestes* species

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Abstract

Parasitoids can suppress populations of their host and thus play a primary role in Integrated Pest Management. In the stored product environment, stimuli deriving from plant products, damaged plant products and hosts might be important for host location by the parasitoids. We studied foraging cues in *Holepyris sylvanidis* (Hymenoptera: Bethylidae), a larval parasitoid of *Tribolium* species and *Cephalonomia waterstoni* (Bethylidae), a natural enemy of the rusty grain beetle *Cryptolestes ferrugineus* (Coleoptera: Cucujidae). Our studies in a four-chamber olfactometer revealed that the host complexes of both *Tribolium* species and different living host stages attract naive *H. sylvanidis* females, whereas no reaction was observed to uninfested substrates. The olfactory response of *C. waterstoni* was found to be strongly elicited both by chemicals emitted by the dust, adult *C. ferrugineus* and *C. ferrugineus* third and fourth instar larvae. Our findings may contribute to the development of biological control strategies of *T. castaneum*, *T. confusum* and *C. ferrugineus* with parasitoids.

Keywords: natural enemies, Bethylidae, stored product pests, biological control

Introduction

The bethylid wasp *Cephalonomia waterstoni* Gahan is an external, arrhenotokous idiobiont larval ectoparasitoid. Hosts are *Cryptolestes ferrugineus* (Stephens), *C. pusillus* (Schönherr) and *C. turcicus* (Grouvelle) (Coleoptera: Cucujidae) (Finlayson, 1950a; 1950b). *C. waterstoni* is able to find hosts by recognizing residual kairomonal cues on infested substrates, similar to other parasitoids (Howard & Flinn, 1990). Hagstrum (1987) and Reichmuth et al. (2007) reported the ability of *C. waterstoni* to maintain the population of rusty grain beetles below the economic threshold.

The parasitic wasp *Holepyris sylvanidis* (Brèthes) (Hymenoptera: Bethylidae) is a larval parasitoid of *Tribolium confusum* Jacqueline du Val and *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), the economically most important stored product pests worldwide (Athanassiou et al., 2005; García et al., 2005). The host-searching behaviour of *H. sylvanidis* is influenced by the presence of host faeces, in which two compounds are thought to be responsible for the attraction: (*E*)-2-nonenal and 1-pentadecene (Fürstenau et al., 2016). The ability of *H. sylvanidis* to penetrate cracks and crevices makes it a promising natural enemy against stored-product pests. Pest larvae are often hidden under thin layers of substrate, in aeration ducts, in machines and in areas that are difficult to clean, but this wasp is able to access these critical environments.

Materials and Methods

Olfactory responses of *C. waterstoni*

The experiments were carried out in a static four-chamber olfactometer proposed by Steidle & Schöller (1997). The experiments evaluated the parasitoids response towards the following odour sources: (1) healthy grain (HGR), consisting of undamaged and uninfested wheat harvested in Germany in 2013; (2) grain infested by *Sitophilus granarius* (L.) (Coleoptera, Dryophthoridae) (IGR), which was obtained from the stock rearing of Hohenheim University; (3) a mixture of grain dust from the mass rearings of *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae), *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) and *C. ferrugineus* on durum wheat (DST), which was collected from cultures kept at 30°C and 60 % relative humidity (RH), the insects and the wheat were kept in a 100 l bin for about 6 months; (4) dust which was obtained by sieving infested durum wheat after being fed on by adult S. granarius from Hohenheim's University laboratory (DSH); (5) diet without *C. ferrugineus* (DIT), consisting of big oats, small oats and wheat in the ratio 1:1:2, this diet also contained one tea-spoon of yeast and water, respectively; (6) diet plus *C. ferrugineus* (DCR), which was like the diet without *C. ferrugineus*, but in this case 50 randomly chosen adults were added, (7) male and female *C. ferrugineus* adults (ADU), (8) mixed larvae (LRM), i.e. 50 randomly chosen larvae of *C. ferrugineus*.

Olfactory responses of *H. sylvanidis*

In the experiments we studied the parasitoid females reaction towards the following odour sources: (1) *T. castaneum* host complex (CAH) consisting of 0.5 g whole grains, 0.5 g broken grains, first to second instar larvae (n = 2), fourth instar larvae (n = 2), pupae (n = 2) and adults (n = 2); (2) *T. confusum* host complex (COH) consisting of 0.5 g whole grains, 0.5 g broken grains, first to second instar larvae (n = 2), fourth instar larvae (n = 2), pupae (n = 2) and adults (n = 2); (3) whole grains (WHG) consisting of 1 g whole grains (*Triticum durum*); (4) flour (FLR) consisting of 1 g of flour (*Triticum aestivum*); (5) *T. castaneum* first to second instar larvae (CA1-2) (n = 10); (6) *T. castaneum* fourth instar larvae (CA4) (n = 10); (7) *T. confusum* first to second instar larvae (CO1-2) (n = 10); (8) *T. confusum* fourth instar larvae (CO4) (n = 10); (9) *T. castaneum* pupae (CAP) (n = 5); (10) *T. confusum* pupae (COP) (n = 5); (11) *T. castaneum* adults (CAA) (n = 5); (12) *T. confusum* adults (COA) (n = 5).

Results

Olfactory responses of *C. waterstoni*

Table 1 Substrates tested and mean walking time upon each odour source.

Substrates	Mean Time ± SE	P-value
DCR vs DIT	189.50 (±27.70) – 96.80 (±14.00)	P<0.05
DST vs EMP	202.43 (±28.10) – 94.22 (±15.70)	P<0.05
DSH vs EMP	198.16 (±28.40) – 110.70 (±17.70)	P<0.05
DST vs HGR	272.54 (±24.80) – 77.36 (±13.70)	P<0.05
DST vs IGR	214.53 (±33.03) – 71.30 (±20.50)	P<0.05
DCR vs DST	203.50 (±33.50) – 107.75 (±17.70)	P>0.05
ADU vs 3-4 L	164.16 (±31.40) – 95.39 (±21.80)	P>0.05
LRM vs ADU	121.74 (±28.10) – 102.64 (±22.80)	P>0.05
LRM vs EMP	178.29 (±15.40) – 114.43 (±10.70)	P<0.05
3-4 L vs 1-2 L	166.50 (±25.60) – 103.90 (±22.30)	P<0.05

Olfactory responses of *H. sylvanidis*

Table 2 Substrates tested and mean walking time upon each odour source

Substrates	Mean Time ± SE	P-value
CAH vs EMP	250.16 (±81.90) – 81.9 (±61.30)	P<0.05
COH vs EMP	248.22 (±93.20) – 102.74 (±46.55)	P<0.05

CAH vs COH	282.78 (±85.49) – 118.78 (±48.57)	P<0.05
WHG vs EMP	162.00 (±52.00) – 129.00 (±44.00)	P>0.05
FLR vs EMP	131.00 (±81.00) – 163.00 (±48.00)	P>0.05
WHG vs FLR	135.00 (±55.00) – 162.00 (±57.00)	P>0.05
CA1-2 vs CA4	183.36 (±70.78) – 123.54 (±42.06)	P<0.05
CO1-2 vs CO4	228.85 (±83.21) – 97.18 (±56.40)	P<0.05
CA1-2 vs CO1-2	204.77 (±74.37) – 143.92 (±62.77)	P<0.05
CA4 vs CO4	150.79 (±69.59) – 120.35 (±66.03)	P>0.05
CAP vs COP	190.25 (±64.27) – 138.26 (±69.90)	P<0.05
CAA vs EMP	208.00 (±96.00) – 136.00 (±80.00)	P<0.05
COA vs EMP	177.10 (±78.89) – 118.79 (±46.85)	P<0.05
CAA vs COA	164. 38 (±72.04) – 152. 18 (±59.85)	P<0.05

Discussion

C. waterstoni is an arrhenotokous ecto-parasitoid ables to locate hosts recognizing their kairomonal cues left on infested substrates (Amante et al., 2017 b,c; Howard & Flinn, 1990). Among the odours tested in the host habitat experiments, dust was the most attractive. The dust contains host faeces and particles from the host's feeding substrate, i.e. plant materials. Our study showed dust from infested products plus larvae and adults were most attractive to the parasitoid *C. waterstoni*. Consequently our study suggests laboratory reared *C. waterstoni* released for biological control would be arrested in areas infested by *C. ferrugineus*, but would not stay in uninfested stored products.

The present study demonstrates that *H. sylvanidis* primarily relies on volatile chemical cues from all host stages for host location. Our results demonstrate that the host complexes of at least two *Tribolium* species, *T. castaneum* and *T. confusum*, release volatile chemical signals, which are attractive for naive *H. sylvanidis* females. In our experiments designed to identify the attractive elements of the host complex, naive wasps did not react to uninfested whole grains and flour, but to most of the host stages of both *Tribolium* species. Whether the kairomonal cues identified could be used to manipulate the behaviour of these parasitoids in order to increase the effectiveness of biological control has to be investigated in future studies (Amante et al., 2017 a, b, c).

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