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Proceedings of the 10th International Working Conference on Stored Product Protection

27 June to 2 July 2010, Estoril, Portugal

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Preface

The International Working Conference on Stored Product Protection (IWCSPP) is the most influential international conference in the field of stored-product protection. Held every four years in different countries around the world, it is now being held in Estoril, Portugal, at the Estoril Congress Centre, from 27 June to 2 July 2010, under the auspices of the Instituto de Investigação Científica Tropical and the Instituto Superior de Agronomia in Lisbon, Portugal. The idea of the conference was first discussed at an Entomological Society of America meeting in 1970, and the first conference was held in Savannah (USA) in 1974. Since then, there have been eight others: Nigeria, the USA, Israel, France, Australia, China, Great Britain, Brazil, and now the 10th IWCSPP will be held in Portugal. We expect over 300 participants from more than 40 countries, with the largest number of attendees from Portugal, Australia, China, and the USA.

Edouard Saoum, Director of the FAO, said in 1991, "We live in a paradoxical world, in which a large proportion of agricultural production is lost between the farm and the consumer, while millions of people suffer from hunger and malnutrition. These losses can be largely attributed to depredation by pests, spoilage by microorganisms, and spillage and wastage of food during handling, storage, transportation and processing."

The aim of the 10th IWCSPP is to exchange information on storage from a wide range of agricultural and economic settings, ranging from small farms to large commercial bulk storage and from tropical to temperate climates, and to present innovative solutions to storage problems that are effective, inexpensive, and environmental friendly. We hope that this conference will help to improve food availability and quality around the world.

The Proceedings of the 10th IWCSPP will be published in hard copy, CD-ROM, and online. There were 193 papers submitted, with 14 keynote addresses and 179 oral and poster presentations, as well as several workshops. The papers cover all aspects of storage and were divided into the following topics: Around the World of Stored-Product Protection; Biology, Behaviour and Detection; Engineering; Fumigation, Modified Atmospheres and Hermetic Storage; Microbiology, Mycotoxins and Food Safety; Non-Chemical Control; Residual Insecticides: Synthetic and Botanical; Integrated Pest Management; and Quarantine and Regulatory.

An Exhibition salon has attracted several international companies to present their products and field trips to grain storage and processing facilities have been organized for the last day of the conference.

The Organizing Committee wishes to thank all those who have contributed to the success of the conference: authors, presenters, and participants. This conference was made possible by the generous financial and organizational support provided by public and private organizations, and the Permanent Committee of the IWCSPP.

The Organizing Committee is also grateful to all the members of the Scientific Program Committee and Editors of the proceedings for the very hard work of selecting the themes, choosing the key-note speakers, and editing the conference papers, and to the Julius Kühn-Institut for the support and publication of the book, CD-ROM, and webpage. This conference promises to be a great success due to their invaluable contributions.

My personal thanks go to the members of the Organizing Committee for their efforts in making our conference a success, with particular consideration to Mrs. Conceição Roncon from Congressos & Incentivos, Estoril, Portugal. A special word of thanks goes to Noel White, Colin Demianyk, and Duangsamorn Suthisut in Winnipeg, Canada, they worked many long hours correcting the manuscripts that make up these proceedings, and without their help, these proceedings would not have been possible.

And last but not least, on behalf the Organising Committee, I wish to express our deep gratitude to the Chair of the Scientific Program Committee, Paul Fields, Winnipeg, Canada, for his extraordinary dedication to achieve the highest scientific quality for this conference.

Obrigado!

Maria Otilia Carvalho
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10th International Working Conference on Stored Product Protection

Estoril, Portugal
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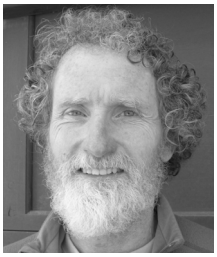

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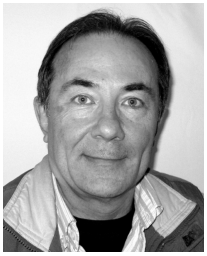

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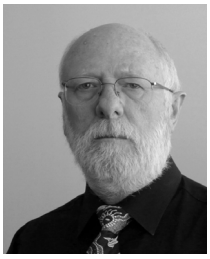

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#	Date	Location	Number of participants	Number of countries represented	Proceedings page number	Cost ($US)
1	1974	Savannah, GA, USA	214	27	705	15
2	1978	Ibadan, Nigeria	69	20	455	15
3	1983	Manhattan, KS, USA	71	23	726	NA
4	1986	Tel Aviv, Israel	165	28	667	80
5	1990	Bordeaux, France	401	50	2065	100
6	1994	Canberra, Australia	400	33	1274	NA
7	1998	Beijing, China	511	48	2003	
8	2002	York, UK	400	30	1071	
9	2006	Campinas, Brazil	698	46	1359	300

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Copies of the proceedings are still available, except for the 3rd and 6th Proceedings. See above for cost including shipping. All available proceedings (1, 2, 4, 5, and 9) may be purchased for $400 for the set.

For the Proceedings of the 7th IWCSPP held in Beijing, China, 1998, contact:
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Proceedings 9, Campinas, Brazil, 2006, 1 volume, 1359 pages:
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Section: Around the World of Stored-Product Protection

Research on stored product protection in Australia: a review of past, present and future directions

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Abstract

Since its beginning, research into the protection of stored grain in Australia has been driven by market access, in particular, the need to provide insect free grain to overseas customers

Research began in 1917 when a bumper harvest coincided with disruption of shipping due the First World War and resulted in the unprecedented accumulation of wheat stocks, which were subject to catastrophic attack by 'weevil plagues'. Initial experiments were of insect life cycles and reproductive rate. This was followed by trials of heat and mechanical disinfestation and fumigation, and preventative methods such as admixture or surface treatment with lime, sand or mineral dusts, and hermetic storage. These technologies continued to be developed into the 1950s along with aeration cooling. In addition, ground-breaking research on mathematical descriptions of grain beetle life tables was published.

The admixture of malathion to bulk grain in the 1960s allowed, for the first time, the export of insect-free grain. However, in less than ten years, resistance in target pests had become so serious that alternatives were desperately required. In response, research began on the development of new grain protectants and alternatives such as controlled atmospheres, manipulation of grain temperature, new ways of using phosphine and the development of new fumigants.

Since the mid-1990s, pest management has become dependent on use of phosphine and maintaining susceptibility to this chemical has become a priority. The greatest problem now facing the Australian grain industry is resistance to phosphine fumigant in target insect pests. Our current short-term priority is the control of phosphine resistance outbreaks, while our more strategic research is aimed at gaining a fundamental understanding of fumigant behaviour in grain storages, the movement and colonisation of grain by insect pests and the mechanisms of selection in insect populations – information that will underpin the development of long-term resistance management.

Keywords: research industry collaboration, phosphine resistance, non-chemical technology, insect ecology, controlled atmosphere

1. Introduction

1.1. Preamble

Good morning ladies and gentlemen. I wish to thank the organising committee for this invitation to speak at this the 10th International Working Conference on Stored Product Protection. The topic of my presentation is: "Research on stored product protection in Australia: a review of past, present and future directions".

Australia has a rich history of research achievement in stored products protection and I could easily fill my allotted time describing it to you. However, I think the purpose of a conference like this is to learn from each other and have the opportunity to discuss the latest research developments and for that reason, I think it will be more interesting for you to hear about the research that is happening now and about our future directions than it would be for me to cover old ground. In addition, a "Brief history of entomological problems of wheat storage in Australia" written by Jan van Graver and Bob Winks (van Graver and Winks, 1996) has already been published in the 6th proceedings of this conference series. That paper provides an excellent summary of the problems encountered in Australia and the role that

research played in solving those problems. On the other hand, I am not going to ignore previous research. I will present some highlights of past research and briefly describe the environmental and economic characteristics that have set the direction of research undertaken in Australia.

1.2. Background

Research into the protection of stored products in Australia has largely been concerned with the protection of whole cereal grains, particularly wheat, stored in bulk. Wheat is our major crop and our major export grain. On average, about 60% of total grain production, including wheat, is exported and Australia is one of the world's largest wheat exporters as well as being a major barley supplier. To compete successfully in the international marketplace, exporters must provide as high a quality product as possible. A major aspect of quality is absence of live insect contamination. In recent years, the domestic market has grown in importance mainly due to the increasing demand for animal feed with this sector requiring the same level of quality as the export market.

Wheat production in Australia faces a number of problems not shared by its international competitors. Our soils are infertile and even with the advantages of modern agriculture, yields are relatively low at around 1-2 tonne/hectare. In addition, rainfall is highly variable and unreliable, a situation now more unstable because of the vicissitudes accompanying global warming. These factors produce a crop that can vary dramatically in both quantity and quality from year to year. Grain in Australia is grown in a warm temperate to sub-tropical environment. Wheat and barley are sown in winter, grown in spring and harvested at the beginning of summer. This means that the temperature of the grain is about 30^{o}C when it is brought into storage and it is then stored through the hottest part of the year. In some regions, harvest coincides with very low relative humilities but in others it occurs in stormy weather when humidity is high. These conditions favour the growth of insect pest populations and result in high pest pressure on the crop from the very beginning of the storage period.

The seriousness of the threat of loss of markets and the high cost of remedial action if insect infestations are detected in foreign ports has motivated the grain industry from the beginning of export marketing to seek help from scientists to protect its grain from insect infestation. In addition, the size and importance of the industry to the Australian economy has ensured that governments have facilitated its development wherever possible.

Close collaboration between farmers, grain handlers, government agencies and researchers is a characteristic of stored products research in Australia. This partnership has involved a substantial investment in research by industry. This investment, and government policy supporting industry development, has set the direction of research to focus on the development of practical, cost-effective solutions to industry problems.

2. Past

2.1. The beginning

Stored products research commenced in Australia in August 1917 with the formation of the Wheat Weevil Committee in South Australia (Winterbottom, 1922). This committee was established in response to the catastrophic 'weevil plague' that appeared in April 1917 and which was seriously threatened the wheat producing areas of New South Wales, Victoria and South Australia. The Wheat Weevil Committee consisted of state government scientists and representatives of the farming, milling and grain storage sectors.

In 1915, Australia produced a record wheat harvest of 179,065,703 bushels (4,873,332 t) almost double any previous harvest. Until this time, surpluses were shipped overseas as quickly as possible and because of this policy there was no provision for the long term storage of grain. However, due to the outbreak of World War I, shipping had been severely curtailed and storage of the backlog of grain became a serious problem. Moreover, the next two years also produced bumper harvests resulting in an unprecedented accumulation of wheat stocks. At the end of 1916, half the crop from the 1915 harvest was still in storage, at end of 1917 the whole of the 1916 crop was still in stock, at the end of 1918 the whole of the 1917 crop and half of the 1916 harvest was still held and a similar situation occurred in 1919. This problem was most severe in the State of South Australia.

At that time, all wheat in Australia was stored in bags and there were no silos for the storage of bulk grain. Wheat was received from farmers at railway stations or ports in bags which were built into huge stacks. In most locations, the stacks were built in the open and a roof of galvanised iron was laid on top of the bags and in some cases the sides of the bag stack were protected from the weather with curtains of hessian. The Wheat Weevil Committee recognised that storage of grain in bulk would greatly improve insect control, however, that option was not available and they focused on the urgent problem of controlling the 'weevil plague'.

The Committee undertook experiments and trials on basic insect biology but the emphasis was on testing and developing a range of disinfestation treatments. This research is discussed in detail by Winterbottom (1922) and included (using modern terminology) heat disinfestation, fumigants (hydrocyanic acid and carbon bisulphide), hermetic seal/controlled atmosphere, admixture of lime and fine sand (unsuccessful and successful, respectively), and mechanical cleaning treatments. Of these, the Committee recommended use of controlled atmosphere for long term storage and heat disinfestation plus cleaning before export supported by thorough grain hygiene practices.

Although a large number of insect species were collected from infested bag-stacks, the major pest was identified as Calandra (Sitophilus) oryzae (L.). This species was observed to be the initial coloniser with infestations of Rhyzopertha dominica (F.) occurring once S. oryzae populations had become established. Laboratory experiments demonstrated that S. oryzae in wheat held in sealed containers were killed and that the likely reason was carbon dioxide poisoning. To trial this technique, a gas mixture of 10-15% (nominally 20%) CO2 with the balance mainly N2, generated by burning coke, was applied for 3 days to bag-stacks encased in malthoid (bitumen impregnated felt sheets). The trials were successful and the method was used on 3.7 million bags to the end of 1919. In a large percentage of bag stacks it was found that it was unnecessary to add gas to the storage as there was enough CO2 generated naturally to control insects if the bag-stack was sealed. A number of sterilisation plants, equipped with steam and direct heat disinfestation equipment, were built close to ports following laboratory experiments and pilot trials demonstrating the efficacy of this technique. These plants were used to clean and disinfest 63 million bushels of wheat before export.

Normal shipping resumed in 1919 and grain held in storage could be exported. The 1919-20 harvest was small and the grain storage emergency was over. However, the response to the weevil plague set the characteristics of stored products research in Australia: close collaboration between government, industry and researchers with an emphasis on practical, cost-effective solutions.

2.2. The concept of 'grain protection'

Until about 1960, insect infestation was managed by turning grain to equalise temperature, the rigorous application of hygiene procedures and the use of fumigants to eliminate insect infestations when detected. Aeration cooling had also been introduced, with a significant proportion of central storages being fitted with this technology. By this time, the grain industry had converted almost completely from bag to bulk handling. In 1960, the concept of 'grain protection' was introduced whereby a residual chemical was applied to the grain whether infestations had been detected or not (Bailey,1978). Experience revealed that the best time to apply the 'protectant' chemical was when grain was received from the farm into country storages (Van Graver and Winks, 1996). Maldison was the most successful protectant chemical and by the mid 1960s its use was widespread producing a dramatic fall in the incidence of insect infestation (Bailey, 1978).

However, 'the golden age of malathion' could not last and resistance was detected in several pest species, firstly in *Tribolium castaneum* (Herbst) in 1968 (Champ and Campbell-Brown, 1970), and then in S. oryzae in about 1970 and in R. dominica in 1972. By the mid 1970s, resistance had become a serious challenge to the effective control of insect pests in many regions in Australia.

The grain industry was able to respond quickly to the emergence of malathion resistance, developing an Integrated Plan for Pest Control in 1971, revised in 1974 (Anonymous, 1974). The industry committee consisted of representatives from exporters, the Australian government, researchers, and the respective state storage and handling organisations. The widespread emergence of commercially significant resistance stimulated the search for effective alternative protectants and renewed interest in non-chemical alternatives including expansion of the use of aeration, and research into controlled atmosphere and

thermal disinfestation techniques. The development of alternative protectants was seen at the time as the short term, stop gap solution while fumigation and non-chemical technologies were regarded as the way forward for insect pest management in the long term (Bailey, 1978).

2.3. Development of protectants and establishment of the National Working Party on Grain Protectants (now Protection)

The Working Party on Grain Protectants (WPGP) was established in 1973 with the sole responsibility of developing new protectants (Murray, 2003). However, it changed its name to the National Working Party on Grain Protection (NWPGP) about ten years ago as its work broadened to include fumigation and other chemical and non-chemical strategies. The Working Party was formed because of a failure of the private sector to provide alternative protectants. The world market for these is quite small and few manufacturers are interested in this area. Core representation on the Working Party has included the bulk handling companies, marketing organisations, Flour Millers Council and researchers. In the past 20 years, these have been joined by a range of stakeholders including the farmer research funding organisation (Grains Research and Development Corporation), down-stream industry groups representing the meat, dairy and pork industries, stockfeed manufacturers, brewers and maltsters, pulse and oilseed grain organisations, the Australian Pesticide and Veterinary Medicines Authority, industry standards groups, and most recently, due to the de-regulation of export marketing, a range of exporting companies.

Candidate pesticides usually begin life as pesticides developed for other uses. Crucially, appropriate toxicology and animal transfer data must be available before these materials are considered for development. The role of the Working Party participants is to generate the entomological and residue data required for Australian conditions, particularly efficacy against known chemical resistance. In collaboration with the chemical company concerned, each candidate progresses through several stages including laboratory assessment, pilot-scale field trials and full-scale industrial evaluation which includes milling trials to determine residue distribution in processed fractions and finished products. Working Party representatives liaise with food standards and regulatory authorities to ensure that all trials meet legal requirements. When a candidate meets industry requirements, it is the responsibility of the manufacturer/seller to provide all other data required by the regulatory authority for registration. It is industry policy that new grain protectants or fumigants will not be used commercially until they have Australian and international (Codex Alimentarius Commission) maximum residue limits. In addition, exporters must consider customer requirements before approving the use of new compounds on their commodity.

The following gain protectants have been developed, with most being registered in Australia, through the NWPGP program: Spinosad, Chlorpyrifos-methyl, Pirimiphos-methyl, Fenitrothion, Dichlorvos, Methacrifos, Bioresmethrin, Deltamethrin, Phenothrin, Cypermethrin, Permethrin, Methoprene, Pyrethrins, Piperonyl butoxide, Fenvalerate and Carbaryl, and many other chemicals have been evaluated.

Because of its wide industry representation, the modern role of the NWPGP has been expanded to include entomology and resistance management, insect infestation trends, grain protectants, fumigants, physical controls, pesticide residue violations, market requirements, application technology, extension, and relevant national and international regulatory requirements.

2.4. Non-chemical technologies

Aeration – Research demonstrated that aeration technology could be beneficial many regions of Australia (Bailey, 1968; Sutherland, 1968) and during the 1960s and 1970s the technology was deployed to about 30% of central storages in eastern Australia. However, these systems have been mostly dismantled as the technology was difficult to integrate with use of protectants in the first instance and then fumigation with phosphine. The cost of power also made the technology uncompetitive with chemical treatments. Aeration, however, is now seeing a renaissance for use on-farm. It was believed that this technology would be restricted to southern areas of Australia but the development of effective control systems that track ambient conditions and turn fans on only when the coolest and driest air is available, has extended its potential.

Controlled atmosphere - Extensive trials have demonstrated the feasibility of using nitrogen or carbon dioxide to protect and disinfest grain (Ripp, 1983). Except for two special cases, the treatment of organic

grain with carbon dioxide and the use of nitrogen on a large scale to treat grain at the Newcastle export terminal, this technology has not generally been adopted. The principal limitation is the cost of obtaining or producing the controlled atmosphere. However, cheaper pressure swing absorbance technology to extract nitrogen gas from air has now become available making this technology much more cost effective. Trials with the new equipment will commence in 2010-11.

Heat disinfestation - Extensive experimental work was undertaken to determine the temperature and exposure times required to kill insects in grain and at the same time leave grain quality unaffected (Banks, 1988). A large 'spouted bed' heat disinfestation system was trialled at a sub-terminal in southern Australia. Continuous spouted bed disinfestation systems have also been developed for use on-farm (Qaisrani and Beckett, 2003). Despite successfully disinfesting grain, the technology has not been adopted because of the capital and running costs of the equipment.

2.5. Fumigation with phosphine

Concerns over resistance to grain protectants and the preference of markets for residue free grain motivated a move away from the use of grain protectants to widespread use of fumigants, notably phosphine. This occurred first in Western Australia (WA) where a program of sealing all storages was commenced in the early 1980s using specifications already developed for sealing storages for use with gas technologies (Banks and Annis, 1980). This program enabled WA to fumigate grain with phosphine cheaply and effectively so that all from WA is residue free. Sealing programs developed more slowly in eastern Australia. Many grain storages, particularly vertical silos, were quite old and could not be economically sealed. During the 1980s, an innovative method of applying phosphine was developed called Siroflo® that allowed these 'leaky' storages to be fumigated (Winks and Russell, 1997). Siroflo is the application of phosphine gas, under controlled release from a cylinder. The gas is applied to the bottom of a silo under a slight pressure which forces it to move up the silo. The gas is applied at quite low concentrations for relatively long periods of time. This technology enabled grain handlers to decrease their reliance on protectants in eastern Australia.

2.6. New fumigants

The phase out of methyl bromide under the Montreal Protocol agreement motivated a search for a cost-effective alternative fumigant. Carbonyl sulphide was developed (Desmarchelier,1994) and is now under consideration for registration in Australia. This gas is effective against a range of insect pests but it requires a longer time for complete mortality of target pests than methyl bromide, however, it is quicker to act than phosphine. Ethyl formate as a mixture in CO2 has been registered for use on grain in Australia but it has seen only limited use. Sulfuryl fluoride is currently under evaluation and development for Australian conditions.

2.7. Overseas research

A feature of Australian research has been the collaboration of many of our scientists in international projects, particularly in Asia. These have mostly been managed through ACIAR, the Australian Centre for International Agricultural Research, and have involved development of grain protectants, phosphine resistance, controlled atmosphere fumigation and grain drying.

3. Present

Most research into grain protection in Australia is now managed through the Cooperative Research Centre for National Plant Biosecurity (CRCNPB, www.crcplantbiosecurity.co.au). In 2006, the major industry funders of research in Australia, the Grains Research and Development Corporation, representing farmers; and our largest grain companies: CBH, ABB Grain (now Viterra) and GrainCorp formed a consortium to submit a bid to the Australian government for financial support to join the CRCNPB. (The CRC program is an Australian government initiative to encourage end-user driven research partnerships between publicly funded researchers and end-users to address clearly articulated, major challenges that require medium to long-term collaborative efforts. Awards are made on a competitive basis). The bid was based on the threat to the Australian industry of the phosphine resistance problem. The bid was successful and a new CRCNPB research program, Post Harvest Integrity, commenced on 1 July 2007. In addition to extra funding, the advantages for the industry of joining the CRCNPB include centralised professional management of research and access to many potential research

providers, not just those specialising in stored products. The CRCNPB also has a strong commitment to the delivery and adoption of its research outputs.

Since the mid-1990s, pest management has become dependent on the use of phosphine. This fumigant provides the industry with a cost-effective, multi-commodity, residue-free treatment, compatible with grain handling logistics that is accepted by markets. Although a number of alternative chemical and non-chemical grain treatments have been developed and have and will have limited and specialised applications, for the majority of situations, none can match the combined attributes of phosphine.

Strong resistance to phosphine was first detected in 1997 in *R. dominica*, and then in *T. castaneum*, *Oryzaephilus surinamensis* (L.) in 2000, in *Cryptolestes ferrugineus* (Stephens) in 2007 and most recently in *S. oryzae* in 2009. Frequencies of Strong resistance generally remain low and we believe that this is because the resistance is mediated by two incompletely recessive genes which both need to be homozygous in an individual insect before Strong resistance is expressed. This lag has provided the industry with a window of opportunity to react in a strategic way to resistance development and undertake a research program that will provide the information needed to manage this resistance.

The greatest problem now facing the Australian grain industry is resistance to phosphine and prolonging the effective life of this fumigant is our current research priority. The project portfolio reflects the urgent necessity of being able to control of phosphine resistance outbreaks now, balanced by the need to generate information that will provide the scientific basis for the development of a long-term resistance management strategy.

3.1. Combating resistance outbreaks

The evolution of Strong resistance in *C. ferrugineus* is our greatest challenge since this resistance is several times greater than in any other species. Our response provides an example of active resistance management. In less than 12 months, and in close collaboration with industry partner GrainCorp, the project team produced a rapid resistance diagnosis test, researched and trialled new phosphine fumigation protocols and developed a successful eradication plan based on the use of protectants and scorched earth sanitation procedures.

3.2. Resistance monitoring and management

It is impossible to respond to control failures and resistance development without information and Australia has had the benefit of a national resistance monitoring program for some years. This program has been re-invigorated with a review of statistical procedures and related sampling and testing methods. In 2010, this project is being expanded to focus further on the interaction of biological, environmental and pest management factors to understand how these lead to the development of resistance.

The extensive data collected during previous resistance monitoring is being analysed in an attempt to link management and other factors to the development of resistance. Insects collected in recent monitoring have been frozen and resistance gene typing of these insects will be undertaken using molecular techniques. In addition, neutral marker technology will be used to address the key question of how important gene flow is in the development of resistance, i.e. is each outbreak of resistance selected individually or is resistance due to insect movement from one site to another?

In collaboration with industry, detailed trials are underway at a large central storage complex and on-farm to evaluate the contribution of our key resistance management recommendations, avoidance of under-dosing, minimising the number to fumigations and implementation of thorough hygiene practices.

Mathematical modelling of resistance to phosphine is under development. So far, the model has demonstrated that a 2-gene resistance is necessary for realistic portrayal of observed data. A two-gene model gives substantially different predictions of resistance development in fumigated populations than a single-gene model. This was the first step in developing mathematical modelling to provide simulation capacity to examine the influence of various ecological, genetic and fumigation factors on the emergence of resistance.

3.3. Resistance genetics

An important initiative is the development of a molecular resistance diagnostic. This will provide the ‘break-through’ tool needed to undertake more fundamental analysis of the processes involved in the

development of resistance. Availability of a resistance diagnostic will also greatly enhance resistance monitoring programs and ecological research. The aim is to identify the resistance genes so that they can be used universally as markers for resistance. At time of writing, two major areas containing resistance genes have been identified on each of the genomes of the major pest species *R. dominica* and *T. castaneum*. We now have a candidate list of about 100 genes for each species. A genome for *T. castaneum* is under development elsewhere but as there is no genome for *R. dominica*, a transcriptome library (based on expressed RNA sequences) is being developed. Fine scale mapping of genes that the two species have in common is now being undertaken based on the assumption that resistance is conserved between the two species. We are also undertaking functional annotation of gene expression under phosphine exposure to identify genes that respond. It is anticipated that the diagnostic for at least one species will be available by the end of 2010.

Once the genes for resistance are identified they will be validated by comparing resistance diagnoses of insects collected during resistance surveys with traditional bioassay methods used to in resistance monitoring programs. In addition, identification of the genes will allow insight into the toxic action of phosphine. Genomic sequencing of *R. dominca* will be continued to produce a partial gene assembly in regions of the identified resistance genes and undertake a functional characterization of those genes. This may lead to the discovery of weaknesses that could be exploited to manage resistance.

3.4. The grain – fumigant interface, modelling gas flow in storages

Rapid, even application of fumigant to all parts of a grain store is fundamental to effective pest management and avoidance of under-dosing and the risk of selection for resistance. Surprisingly little is known of the behaviour of fumigants in grain storages, however. The physical and chemical interaction of fumigants with grain (sorption) is the key factor influencing behaviour of these gases, which in turn affects their movement in silos and their efficacy against insects.

Sorption phenomena are being modelled from the unique perspective of the grain as a carrier of sorption surfaces. This, and the determination of a dispersion coefficient which allows the definition of key parameters, are crucial information facilitating the development of more complex, predictive 3-dimensional flow modelling now being undertaken. Another factor investigated was the phenomenon of interrupted doses of phosphine caused by the diurnal flow of gas inside silos. Recent field trials reveal that interrupted dosing is a feature of fumigation even in fan-forced silos. Research showed that phosphine toxicity was cumulative and despite periods of low exposure, insects did not have time to recover from intoxication.

In other current work, project collaborators are measuring phosphine gas flow in industry storages and using this information to develop three dimensional models to predict fumigant movement under a wide range of conditions. The project team is using novel advanced solution techniques to quantify the fluid dynamics of gas movement in porous media. The model will simultaneously account for the varying multiple natural forces that drive gas flow in sealed and ‘leaky’ storages. This information will be used to improve fumigant application.

3.5. Storage Integrity

To be effective, fumigation with phosphine or any other gas must be undertaken in well-sealed storages so that the gas has enough time to penetrate the grain and exert a toxic effect on target pests. A corner-stone resistance management tactic is the avoidance of under-dosing, which allows the survival of heterozygotes. This means that storages must be able to hold gas at the appropriate concentration and time period and importantly, the gas must be as evenly distributed as possible to eliminate pockets of under-dosing.

Most grain storages in Australia were not designed for fumigation and have been retro-sealed to do this. However, in many cases the existing technology is failing to maintain adequate sealing standards. The aim of this project is to research better sealing technologies and techniques and to develop industry standards for the construction of new storages and the renovation of existing storages to a standard required to maintain effective fumigations. These need to be not only suitable for phosphine fumigation but also must meet the likely future requirements of a range of alternatives to phosphine.

Researchers are also investigating the safety and effectiveness of passive and active phosphine application systems for farm storages.

3.6. Insect pest ecology

Effective resistance management strategies rely on an understanding of the ecology of insect pests. Research has been initiated on the ecology of two major pest species *R. dominica* and *T. castaneum* and the project team is focusing on answering a number of key questions:

- What are the ecological processes responsible for the patterns of insect abundance in the rural and natural environments?
- What are the rates of insect movement in the natural environment and colonisation of grain storages?
- What is the impact of grain handling and transport on insect populations and selection for resistance?
- What are the key habitats and sources of infestation by stored grain beetles?
- What is the relative importance of various refuges where insects are not under selection for resistance?

In the first part of this work, a range of methodologies have been developed or evaluated including field trapping techniques using baits and pheromones, trap liquids for preserving DNA, neutral DNA markers for relatedness studies, and laboratory apparatus for studying emigration from small grain bulks.

Trapping studies in farming regions in southern Queensland and southern NSW have revealed that *R. dominica* are widely distributed away from grain storages while *T. castaneum* are aggregated around silos. Further work shows that R. dominica females have mated before leaving silos, that both sexes typically live for 3 months at 25°C, and that females captured in this way are capable of producing several hundred adult progeny during this time without further mating. In addition, resistance testing showed that there is no difference in resistance gene frequencies between insects caught near silos and those collected in paddocks. Preliminary population genetics analysis of *T. castaneum* trapped at a Queensland grain depot during 2009 suggests that the population structure at this site was stable with no temporal variation.

3.7. Alternatives to phosphine

Nitrogen - Controlled atmosphere technologies, although well developed in Australia, have seen only limited adoption since the Second World War. However, the availability of substantially cheaper pressure swing absorbance (PSA) equipment that can cost-effectively supply a large amount of nitrogen gas provides the opportunity to re-visit this technology. Laboratory experiments are currently underway to determine optimal use of N2 enriched atmosphere in relation to low O2 and CO2 concentrations. The objective is to determine the most cost-effective concentration regime and exposure periods for control of insects. In addition, a problem with N2 treatments is that air will de-sorb from grain for a period after the N2 has been added, potentially compromising its efficacy. Laboratory studies will be undertaken on various grain types to determine air desorption rates in N2 atmospheres. Exposure to N2 atmospheres is known to affect quality of some specialised grain types such as malting barley and oilseeds. Laboratory experiments will be undertaken to delineate the limitations of N2 atmospheres in relation to these grain types.

Field trials of laboratory-developed protocols will be undertaken using a commercially available PSA N2 generator. Further development of the generator will be undertaken to increase its efficiency in collaboration with the manufacturer. In addition, research will be undertaken with the aim of shortening fumigation time and targeting regions in storages that may not receive adequate concentrations of N2. The feasibility of augmenting the technology by strategic addition of other gases will be explored. This technology cannot replace phosphine but can be used at strategic points to provide an alternative to phosphine.

Sulfuryl fluoride, the availability of an alternative fumigant to eliminate phosphine-resistant populations would greatly enhance management of this resistance. DOW Agri-Science has registered sulfuryl fluoride as a grain fumigant in Australia. However, there is little information on the use of this material to fumigate large grain storages, particularly sheds and plastic covered bunkers. Research has commenced to understand the concentration-time efficacy profiles of this chemical against major insect

pests of grain at lower concentrations and longer time periods. These data will be used to develop fumigation protocols for use against resistant insect populations.

3.8. Grain knowledge networks

New technologies to manage phosphine resistance will only be as effective as the ability and willingness of individuals within the grains industry to change behaviour and practices (theirs and others') as necessary to implement them. Recommended strategies to manage resistance often compete with other standard practices and market imperatives within the grains industry, and it is perhaps not surprising that, despite some industry investment in, and progress made by, awareness and extension activities in previous years, actual behaviour change in this area has been slow.

This project aims to develop an effective change management strategy for the grains industry to improve its phosphine resistance management outcomes. This project is using a multi-disciplinary approach (based on social science, economic and market research) to find opportunities to achieve practice change and to develop a national change management program to improve phosphine resistance outcomes. Applying change management to the issue of phosphine resistance, it follows, will involve achieving a high level of understanding of individuals' knowledge, practices and motivations in order to implement and test transitional strategies to drive practice change across the industry. This project will combine a better understanding of the grain industry's knowledge network with improved knowledge transfer strategies in the area of phosphine resistance to build a strong evidence-based framework for a national phosphine resistance management strategy post-2010.

3.9. Bio-sensor based detection of insects

There would be many advantages in being able to detect insects and quantify insect infestation in bulk grain without basing population estimates on sampling. However, an effective system has remained elusive. Insect detection technology has included measurement of carbon dioxide, adult trapping, sensing insect noise, measurement of pheromones, sensing specific volatile chemicals, and measuring colour changes of samples. In-situ detection using electronic instrumentation has not been successful due to the characteristic of bulk grain to rapidly attenuate electric, magnetic, acoustic, or thermal energy as it passes through the grain (within 100 mm). Techniques to detect the immature stages of species that exist with grain kernels that are most likely to survive fumigation treatments (eggs, pupae) have not been achieved.

The aim of this proejct is to utilize olfactory receptors from *Tribolium casteneum* to identify biologically active molecules, such as pheromones, that are released by insects during colonization and infestation of grain. The first phase of the project is to isolate genes for olfactory receptors that are capable of detecting the unique olfactory signatures of stored-grain insect pests. The second phase is to express the olfactory receptors in cells and develop assays aimed at determining interaction of the beetle receptor and their unique chemical ligands. The olfactory receptor genes of *T. castaneum* that express detection capability for specific volatile chemicals exhibited by stored product insects (e.g. pheromones) will be used as the genome of *Tribolium* is known and functional genes identified.

3.10. Diagnostics for market access sensitive organisms

Australia is free of two major pest organisms important in the international trade of grain: Khapra beetle, *Trogoderma granarium* Everts, and Karnal Bunt, *Tilletia indica*, and it is important that effective tools are available for Australia to continue to claim area freedom from these pests

The aim of work with *T. indica* is to develop an internationally recognised, highly sensitive, detection system for that will enable direct diagnosis from a few spores and which does not involve time-consuming and labour intensive germination of spores required under the current standard method. The method has been developed and is now undergoing validation.

With increasing amounts of international trade, it is likely that an incursion of *T. granarium* will occur at some time in Australia. Therefore, it is important that rapid and accurate diagnostic methods are available for this insect so that eradication processes can be implemented. Trogoderma is a large genus and there are several Australian species that can be superficially mistaken for *T. granarium*, although they are not pests of stored products. This project is comparing Australian species with international material to develop reliable morphological and molecular diagnostics as well as internationally recognised expertise in identification of these insects.

3.11. Sampling strategies

There is a range of testing and sampling activities being carried out on grain whenever it is delivered, transported and prepared at port for export. The aim of this project is to capture and convert these data to maximise the power of detection and to develop flexible and statistically robust systems to calibrate and improve sampling strategies. The first part of the proejct has been to develop a statistical model of insect infestation as grain moves from farm to export terminal. Research now under way includes evaluation of current supply chain sampling with a view to identifying at-risk elements to improved sampling strategies to maximise detection and analyses.

If any reader would like more information about any of these projects, please contact me and I will forward your enquiry to the appropriate project leader.

4. Future

From its beginning in August 1917, stored products research has responded to essentially the same environmental and economic drivers as it does now. Research has been needed to develop cost-effective techniques and technologies that will ensure that our grain production, whether it is destined for international or domestic markets, is maintained at the highest quality standard, in particular, free from insect contamination.

The greatest changes that have occurred have been in the marketing of grain. Since the Second World War, the storage, handling and transport of grain and both domestic and export marketing had been heavily regulated. However, in 1989 restrictions on storage, handling and transport and domestic marketing of grain were lifted. The major outcome of this change was to diversify this sector and in particular, provide an opportunity for farmers to market grain themselves. It also caused the state owned grain storage and handling organisations to begin the process of consolidation and privatisation. In 2008, the ‘single desk’ assigned to the Australian Wheat Board for export marketing of wheat was abolished and more than 20 export licences have now been granted for the export of bulk wheat. The export of wheat in containers had been de-regulated several years earlier. Australia is now an open market for grain. We are yet to fully see the outcome of these changes.

Despite the dramatic economic changes that have occurred the biological and environmental fundamentals have not changed and the partnership between industry, government and research has continued and will do so for the foreseeable future. The current partnership is managed by the Cooperative Research Centre for National Plant Biosecurity and this will continue for another decade.

The high pressure on the system from insect infestation will always remain and for this reason industry will require research to develop cost-effective solutions to meet market demands for insect free grain. Deregulation has encouraged diversification and innovation in production and marketing and this presents many new challenges and opportunities to researchers. Although use of phosphine will continue to be the dominant disinfestation tool, diversification of grain handling and marketing provides the opportunity for the diversification of insect control tools for specialised uses and industry sectors. The threat and reality of insect resistance to phosphine is also a significant driver. It is likely that alternative fumigants including sulfuryl fluoride, carbonyl sulphide (if registered) and ethyl formate, and controlled atmosphere technology will see greater use. As mentioned previously, farmers are investing in aeration cooling and are often early adopters of new technology as it becomes available. Research into the ecology and population dynamics of grain pests is giving us insights that are invaluable in managing these insects and continuing this research will be a priority for the future. Probably the most innovative technologies and research tools will result from molecular and genomic research on the insects themselves. The enhanced insect detection systems and population dynamics tools mentioned here are only two of the potential array of innovations of the future.

In conclusion, the serious threat that insect infestation poses to market access for Australian grain will ensure a continuation of the partnership between researchers and the grain industry in the development of cost-effective solutions in grain protection.

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Stored products research in Europe – a very personal perspective!

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Abstract

The general consensus is that there are about 50 countries in Europe. There are no discrete boundaries and some countries may be both in Europe and another continent. In this short presentation, I want to dip into the history of stored products research over the past 50 years or so in this diverse geographical and political area. It is curious how the emphasis of the research has changed but perhaps more often been cyclical with areas being topical and novel, falling out of fashion, and then being "rediscovered" often with scant recognition for what has gone before. The trap of pretending that there was no research prior to the advent of electronic publication of journals and especially of abstracting services has reinforced the opinion that is apparent in much literature of the past 10-20 years that nothing before about 1970 actually happened. It may also be related to the geographical locations where research is undertaken and the accessibility, often determined by the language, of the research to a global community. The current levels of research activity are driven and limited by funding, political factors and perceived necessity. These are debatable subjects at best. So, where are the primary targets for research in the future or have the problems really been solved? This presentation will attempt to sample and illustrate how the nature of research may follow advances in methodology, how it has perhaps been a consequence of failure to appreciate what has gone before, and is likely to continue changing with demand. How and where will this be achieved?

Keywords: Food security, Insecticides, Biological control, Insect pests, Fungal infestations.

1. Introduction

Europe is an amazingly diverse continent considering its size and position. It has played a key role in human history although it is not the source of the human species, which is Africa, nor the source of agricultural life, predominantly Asia (although there are reasonable claims for the present day Israel to have been an early site of wild grain consumption), Central America and the Andean slopes. It has a lesser latitudinal range than South America or even Australasia, extending from about 35° N to about 70° N (save for a few islands such as Svalbard or Franz Joseph Land at about 80° N) and includes less than 7% of the world's land area. It is only a little larger than Australasia, half the size of South America and less than 25% the area of Asia. Its climate is temperate, much colder in the north than the south, but not as extreme as found for example in N America. Its boundaries are vague; Turkey is in both Europe and Asia, and some other Middle Eastern countries (Israel or Syria for example) as well as Russia, are sometimes included in Europe or Asia or both! Most authorities include about 50 countries in Europe, but the precise number depends on what you believe or accept!

This relatively modest area includes as many countries as Africa or Asia and at least twice as many as North America. They have their own governments and, until quite recently, their own currencies. Most have their own language, and I, like many of my fellow UK citizens, speak only one European language other than English – and that badly.I am relieved and slightly ashamed that I stand here in Portugal speaking English that most of you can comprehend; half the people in Europe can speak English although only for about 12% of the population is it a native language (European Commission, 2009). I will return to this subject later.

Europe does have a large population of around 750 million people (United Nations, 2009). To feed this population from its small land area is a major challenge especially as large northern tracts are relatively unproductive and certainly unsuitable for cereal or pulse production. It has used its relative wealth to protect its food and develop food security measures as its population has grown. Several European countries have also governed, alternative words could justifiably be used, various parts of the globe far

away. One might start with the Romans and Greeks in classical times but more recently the Austrians, and then the Germans, French, Dutch and British. One of the more desirable consequences has been the willingness of many of the latter group to invest expertise and funding in support of agriculture, including storage systems, in parts of their former empires so that work on stored products has not solely been based or targeted at domestic problems.

The consequences of these situations are that research has taken place in different countries with different cultures and, despite their geographical proximity, with relatively limited exchange of information. One of the benefits of the European Union has been the sharing of expertise, development of broadly based projects, and access to funding opportunities. To some extent this may be at the expense of targeted funding within the individual countries but it has brought countries with limited resources into the picture so that research can be funded from a central source or on a collaborative basis.

In the following pages, I want to briefly and imperfectly review some of the changes which have taken place, postulate what has brought them about, and consider the consequences in terms of the changing pattern of research activity in Europe. I acknowledge that it is an entirely personal view, it may well have a somewhat "anglocentric" stance and I have drawn freely on my association with the Journal of Stored Products Research as a source of information. I am aware that the data are imperfect but, as I shall explain, attempting to impose pseudo-rigour is not helpful.

2. Sources of information

Many of the statements made in this paper are based upon my own experience of meetings, papers, reviews and invaluable personal contacts with people from Europe and, of course, much further afield as well. I began research, not actually associated with stored products, some 43 years ago so I have watched and participated in the changes in scientific research and communication through a dramatic and prolonged period.

To try and get some sort of quantitative basis for my arguments I have utilised publications in the "Journal of Stored Products Research (JSPR)" at three periods of time, 1967-68, 1987-88 and 2007-2008. This opens the door to bias since it was first published in the UK by Pergamon in September 1965 although the very first Advisory Board had representatives from 12 other countries, including 6 from Europe. I acknowledge that the editor and her Associates were all working at the Pest Infestation Laboratory in Slough, UK, but by 1967 the journal was already pretty well established. Using abstracting services is not helpful as coverage of journals in the 1960's was extremely patchy and using journals published in one country and not in English adds at least a similar problem to using JSPR. The Journal of economic Entomology has consistently published papers of high quality back to 1908 but most, at least in the early days, were from North American authors and therefore not relevant to a commentary on activity in Europe.

An alternative source of information would be international symposia but they are often dominated by attendees from the country or geographical region in which they are located or, if not dominated, then at least the local scientific community is unusually well represented (e.g. Zuxun et al., 1998; Credland et al., 2003). This is, of course, a very good reason for moving conferences, such as that opening today, to different locations in different continents. It does however mean that consideration of attendance at such meetings provides no more objective data than from publications.

I therefore acknowledge a problem but there is no simple solution which would provide an overview of activity in Europe prior to about 1970 – and herein lies a different problem addressed later in this paper. So, accepting the limitations of the data source, what can be concluded about the history and development of stored products research in Europe?

3. Subjects of research over the years

It is curious how topics appear to have risen and fallen in popularity or importance as measured by the proportion of papers published on them.

In 1967 and 1968, publications were dominated by papers dealing with the rather basic biology of pest insects; mites and fungi were rather rarely mentioned or studied in Europe, there being only a single paper published from a European author on mites in JSPR during this period (Ždárková, 1967) and not one paper on mycological or microbiological problems (Fig. 1). Fumigants including phosphine, nicotine

and sulphuryl fluoride and residual insecticides, notably malathion and fenitrothion, were the subjects of several papers.

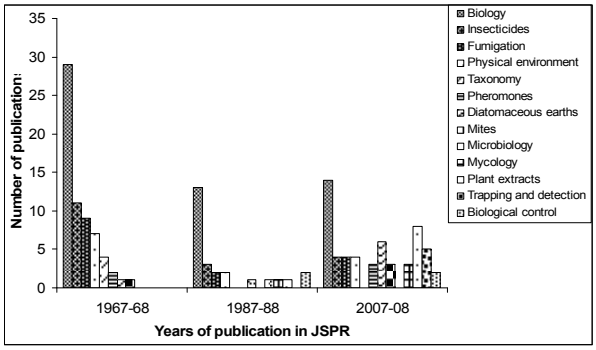

Figure 1 The broad areas into which publications in the Journal of Stored Products Research fell during three separate two-year periods of publication, 1967-68, 1987-88 and 2007-08.

Twenty years later, 1987-88, among the 28 papers published in JSPR by authors based in Europe, the dominance of papers dealing with pest biology remained. Only two papers dealt with fumigation and four with insecticides. One paper was published on fungal toxins (Bacha et al., 1988) and one on microbiology (Lacey, 1988).

The situation changed dramatically in the following 20 years although in 2007-2008 there were still more papers dealing with biology than any other (general) subject. The key change was an altogether more diverse range of interests that were included with subjects not previously represented appearing. Of course occasional papers on these subjects had appeared previously but not in the years sampled. More papers dealing with plant extracts (other than nicotine or pyrethrum) than either fumigants or insecticides were published, and several papers on diatomaceous dusts raised their profile significantly. Papers dealing with trapping and the modelling of conditions in stored commodities were also more common than previously. Four papers were published on mites and another four on fungi associated with stored products, not all with their toxin production.

What has produced this changing picture which is not, at a superficial level, greatly different from that which appears to have occurred elsewhere (although fellow contributors may correct this impression)? Partly the answer may be an autocorrelation with the increasing geographical diversity of the authors (see below) so that problems encountered in and techniques applicable to non-English speaking countries begin to make an appearance. However for reasons explained later there being no simple method of data collection which would enable this to be excluded, perception might prevail. I would suggest that the primary reason for the changing picture is that prior to the 1970's or even the 1980's there were significant gaps in our knowledge of even the most important pests of stored products whether from temperate or tropical regions. Until knowledge of the biology of these pests was available, control was rather generic in nature and, of course, the hazards associated with the use of some insecticides were not widely accepted. The Environmental Defense Fund was established in the US in 1967, two years after publication of the first issue of JSPR in September 1965, and the general use of DDT was banned in the US on 31 December 1972. Bans have followed in the UK in 1984; the Stockholm convention banned all use except for vector control in 2004. Its use had, of course diminished dramatically to be replaced largely by organophosphates such as malathion and fenitrothion, and more recently by pyrethroids.

The apparent effectiveness of insecticides for grain protection limited the demand for research on the biology of the pests but restrictions on the use of some compounds was an incentive to find out more about them. Hence the growth in biological studies and the continued interest as more focussed and selective control agents have been employed. The wide application of synthetic pyrethroids such as deltamethrin (often in K-Obiol) and permethrin which have markedly differential effects on different pests of stored products (e.g. Arthur, 1997) are one such case.

At the same time limitations on the use of grain protectants and especially the withdrawal of methyl bromide were introduced. Production and use of methyl bromide was banned by the Montreal Protocol of 1992 and European Union member governments agreed to phase out use of methyl bromide by 2005. However some countries sought 'critical use' exemptions and the use of the pesticide therefore continued. Exemptions were granted initially but there were none in 2009 in the EU so its use has been successfully phased out. This has left phosphine as the fumigant of choice and research has again concentrated on its differential effects on pest species (e.g. Pimentel et al., 2007) but awareness of resistance problems in many areas (e.g. Mills, 2001; Benhalima et al., 2004) has been growing steadily. In the UK the only organophosphates that can now be applied to grain are pirimiphos-methyl and chlorpyrifos-methyl. Diatomaceous earths have been used as alternatives but their effectiveness varies with many factors. Hence the reliance on physical controls such as temperature regulation have become of paramount importance and modified atmospheres are perceived as perhaps the next generation of broad control measures. In general terms, the same pattern is applicable in Europe as a whole; insecticides are rather rarely used, especially on food commodities and research is concerned with optimising the effect of alternatives.

An additional aspect has been the level of activity under aid programmes. There have been extensive research programmes looking at aspects of stored-product, primarily food, protection in developing countries including valuable work associated with East and West Africa, parts of South America but rather less towards the Far East or southern Asia. These programmes flourished in the late 1980s but appear to be diminishing in the current economic climate and their short-term future appears somewhat bleak. EU funding has provided some protection against the plight of individual countries but even here there appears to be increasing pressure on the funding available. The consequence has been a recent retrenchment with more research on important but local problems, collaborative studies with other parts of the world, especially North America, and perhaps a greater tendency to undertake research which is relatively cheap to undertake involving minimal technological input. Even the Consultative Group on International Agricultural Research (CGIAR) and its institutes (outside Europe) are showing aspects of financial constraint (CGIAR, 2008). One consequence of this change has been the growth of interest in plant derivatives other than pyrethrum and nicotine which have long histories of usage. Collaborative work with scientists in developing countries has resulted in a proliferation of papers on plant extracts, many actually from the plant family Labiatae (e.g. Keita et al., 2001; Stella Nerio et al., 2010). Although touted as potentially valuable and effective, most have not had their toxicological effects determined and scant regard has sometimes been paid to their application in areas other than where they are grown. Bluntly whilst some may in the future have local importance, they are, in my opinion, unlikely to be adopted in many developed countries for several reasons – unproven toxicity, the effects of residual product, cost, effectiveness in a several 1000 ton grain lot, the importance of minimising (often to zero) insect remains, etc.

4. Where has research been based in Europe?

Using the same source of information as before there has been a dramatic change in the sources of published papers (Fig. 2). It should be remembered that JSPR was published in the UK and was predominantly in English although I note that a paper in French on parasites and predators of bruchids (de Luca, 1965) appeared in the very first issue. More than half the papers in 1967-68 were from scientists in England and almost all the rest from outside Europe. The only non-UK European papers were from Israel, Czechoslovakia, Germany and Norway (still the only paper published in the journal from a Norwegian source (Sømme, 1968). By 1987-88 at which time the journal was well established and with a truly international advisory board and Regional Editors in the UK, Australia and the US, there was little change in the minimal European representation outside the UK but there were now twice as many papers from outside Europe as from the UK. The big change came over the next 20 years when coverage suddenly exploded in Europe; 16 different European countries, not unfortunately including Portugal, were represented and their papers far outnumbered those from the UK although collectively Europe is greatly outnumbered by papers from other parts of the world.

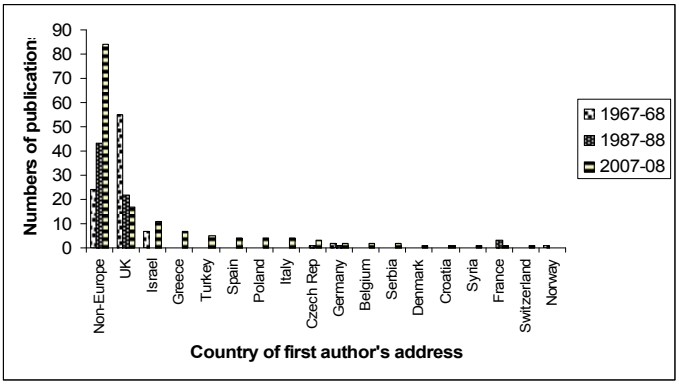

Figure 2 The geographical sources of manuscripts in the Journal of Stored Products Research fell during three separate two-year periods of publication, 1967-68, 1987-88 and 2007-08. Sources are based on the address of the senior (first author) when the work was undertaken.

One is therefore led to ask what has produced the recent explosion of work across Europe, but overall the demise of Europe as a contributor to stored products research on a global front? I would suggest that the answer lies on two fronts –language and money!

5. The importance of language

I now wish to bring down the wrath of the conference onto my head! I believe that it is impossible to produce genuinely objective data to support the argument that I propose here because of the number of confounding variables, so I stress that the views here are my own. It is my belief that English has become the language of science in the late 20th century. We all take rapid travel, electronic communications, the internet, email, etc. for granted so the world of science has been driven to move to a universal language – which may in the 21st century become a form of Chinese, so those of you younger than me, be prepared! In the early and mid-20th century publications on many subjects including stored-product protection were written in native languages. The distribution of journals in paper format was expensive and, of course, subscriptions of libraries and research institutions tended to be based on the local language with, perhaps, one or two subscriptions to journals in foreign languages, frequently English, French or German. The consequence was that relatively important work was "hidden" or unavailable, rather parallel to the lack of awareness of Mendel's seminal studies on inheritance which were also lost for 40 years at the end of the 19th century. Furthermore, abstracting and indexing services were entirely paper based so that finding published material on any subject was extremely time consuming and often required some knowledge of at least one foreign language, usually English for non-native speakers. Progressively, as transport and communication became easier so conferences, such as the 1st International Working Conference on Stored Product Entomology (IWCSPE) held in Savannah, USA in 1974, the 2nd in Ibadan, Nigeria (1978), the 3rd in Manhattan, Kansas, USA (1983), and the 4th International Working Conference on Stored Product Protection (IWCSPP) in Tel Aviv, Israel (1986) became increasingly important as venues for information exchange and it was invariably the case that papers were presented in English. Only when the 5th IWCSPP was held in Bordeaux in 1990 were a few papers delivered in French; this typifies the problem of a small continent like Europe having a richness of entrenched languages. However my general argument is that as communication improved so the shift towards a common language accelerated and because of the major influence of North America, Australasia and the British Empire in the 19th and early 20th centuries, the common language was English, with French, German and a little Spanish also occurring. Outside Europe, in the Far East, oriental languages, in particular Mandarin, Cantonese and Japanese were used and an almost separate scientific culture was established.

Overlying this progressive shift was a dramatic revolution in the transmission of information. Until the late 1960's virtually all written information had to be transmitted in conventional post; manuscripts were submitted to journals as multiple hard copies. The first notable change was the introduction of facsimile (fax) machines for document transmission; although relatively cheap, fax machines only became widely

available in the 1960's and it still took 6 minutes to transmit a single sheet of A4 in 1968 and 3 minutes in 1976! In turn the fax machine was replaced by email which really became widespread from the early 1980's. The internet which became more widely available in developed countries in the 1990's has revolutionised publication and abstracting services. Many journals now only accept manuscript submission online by using the internet. Many of us take it for granted that we can download papers from a very wide range of journals published across the globe, though most by only a few publishers, straight into our own offices. Subscriptions to paper copies continue to decline and some journals are now only published electronically. If we want to know about work published on any subject, we search international databases such as the ISI Web of Knowledge in the UK, Scopus, Current Contents and Current Abstracts, PubMed, etc. So suddenly the whole world of scientific knowledge is potentially available at your desk top. This in turn puts even more pressure on dissemination not being limited by language so virtually all mainstream journals now publish almost exclusively in English and many offer translation services for those unable to write in English effectively.

6. Money talks

It is very hard to get authentic data on investment into research in any particular area which does not have boundaries which are clearly demarcated. Furthermore, many countries contribute substantial sums to central pools such as the CGIAR so expenditure is not limited to that directly invested by the governments or agencies in their own countries.

For example, in the UK support for research on stored products has shrunk dramatically. The Natural Resources Institute was the last vestige of a succession of bodies including the Tropical Stored Products Centre and Tropical Products Institute which undertook wide ranging research in many areas. It was privatized and then downsized dramatically in 2001 leaving a valuable but relatively tiny shadow of the activities 10 and more years previously. Research at The Pest Infestation Laboratory (PIL) initially focused on protecting the security of foodstuffs held under wartime conditions. It was established on the Slough site in 1940 as the UK government realized the problems encountered when they started to create food stockpiles for the War: medium-term storage of British grains was one thing, long-term storage of foods imported from the tropical and subtropical climes of the British colonies was another. So, from the start, PIL entomologists were dealing with problems on imported food stocks as much as those on home-grown cereals (Haines, pers. comm.). After its initial establishment by the Department of Scientific and Industrial Research it was transferred through various agencies to and of the Ministry of Agriculture, Fisheries and Food (MAFF), becoming ultimately the Central Science Laboratory, and an Executive Agency of MAFF in 1992, relocating to York in 1996 and most recently becoming an Executive Agency of the Department for Environment, Food and Rural Affairs (DEFRA). Last year it became an integral part of the Food and Environment Research Agency (FERA), itself an Executive Agency of DEFRA. Although food security falls within their current remit, investigation of problems associated with stored durable commodities scarcely features in their current programmes (FERA, 2009). Almost all their research is now concerned with the pests of growing crops.

Therefore the two largest bodies traditionally working on stored products in the UK have shrunk to minimal size. If one took out their contributions to stored-product research in the UK over the past 70 years, whether dealing with national or international problems, there would be precious little left. If one adds to that the recently announced cuts of over £900 million from the budget for UK universities, the plight of stored products research is self-evident.

Conversely, despite the prevailing global economic conditions and particular problems in parts of Europe (such as Greece), France, Germany and the US are investing in higher education and research (Spencer, 2010) so there is the potential for more research activity elsewhere. The Institut National de la Recherche Agronomique (INRA), and the Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ), for example, support substantial research as do comparable agencies in other European countries. Furthermore the EU research framework programmes are well supported and FP7 expenditure is projected to increase year on year with a total budget of 5200m€, although only a small portion of this is directed towards research with any stored products element. It does, however, have the capacity to spread research activity within the EU and collaborating countries.

The overall picture is of a relative decline in UK involvement, compared with the past, but prospects of growth more widely although we can be sure that funding will always be challenging.

7. Where will or should money be spent?

At the moment, in 2010, most of Europe, certainly those parts where research takes place at a significant level, is perfectly capable of dealing with the safe storage of most of its food. Yes, there are particular problems which are being dealt with at a local level such as the storage of cheese in Spain (Sánchez-Ramos and Castañera, 2009 or hazelnuts in Turkey (Kibar and Ozturk, 2009) but the major food materials are not subject to insoluble problems. Consequently, most people do not see or foresee a problem worth worrying about. However, it is entirely predictable that phosphine resistance will continue to spread, that resistance to the remaining chemical protectants will spread, and sooner or later, there will be another major problem if not crisis in food security. It will not arise instantly but grow insidiously causing an increase in price as availability of high quality food declines. Politicians and funding bodies, at least in the UK, are usually more responsive to immediate than future problems so obtaining funding to solve future problems is hard.

I hold the view that the introduction of any foreign material to bulk stores of grain in Europe and probably other developed countries will be barred in the foreseeable future. I do not think this is a view that I hold alone. Therefore we need to identify tools to see us through the immediate future and longer term objectives.

I would argue that we are right to be looking at alternatives to current fumigants and chemicals now. Alternative fumigants such as sulfuryl fluoride have a long history and are not new; its effects on insect eggs, admittedly of different species, is studied regularly but intermittently (e.g. Outram 1967, Bell and Savvidou, 1999, Baltaci et al., 2009). Much the same can be said for other compounds and one must question whether such a long "gestation period" might indicate very limited potential.

Diatomaceous earths are currently valuable but I would predict a growth in resistance although there are relatively few published reports of it at present. Variable tolerance among Tribolium confusum du Val populations was established 9 years ago (Rigaux et al., 2001) and Vayias et al. (2008) demonstrated resistance development in T. confusum in the laboratory over relatively few generations. Nevertheless, the Woodstream Corporation still advertises in 2010 that "Insects cannot develop resistance, no build-up of chemical immunity" to its Safer Brand product called Concern® which contains "diatomaceous earth (85%) - all natural". Fields (1998) provided a more considered appraisal of their advantages and disadvantages in the 7th of this series of conferences in Beijing.

Any chemical additive, natural or synthetic, has problems and I would concur with the view that physical methods, perhaps allied with varietal control, will inevitably become the methods of choice in Europe in the future. This is probably the explanation for the increasing number of studies of modified atmospheres, vacuums and even irradiation as methods of control, allied of course to temperature and moisture content regulation.

Detection, monitoring and trapping are integral parts of effective control by any means and increasingly effective methods are being developed. This must continue alongside modeling of population and even individual insect movements in stores which are integral to monitoring efficiency. However I would argue that further biological studies of the familiar pests of most major crops stored in Europe are probably of limited practical value although they may be interesting in their own right. There is probably little to be discovered that will contribute to the increased efficiency of control in the immediate future.

There is a different argument in the context of storage in developing countries. High technology storage structures and the equipment for regulating physical conditions are generally expensive. There are exceptions such as the use of solar heat and relatively inexpensive storage means (Murdock and Shade, 1991) but they are unlikely to be solutions in many situations. In my view, the best hope is that European contributions will work alongside or educate scientists from developing countries. This is not intended to be patronising at all but to try and ensure that efforts are directed into sensible paths; simply watching them repeat the errors of creating problems with additives would be an inappropriate contribution. Hence, widespread support for the use of plant extracts without an appreciation of the source, toxicity, side effects, persistence, cumulative or synergistic effects, etc. is not really helpful and could easily lead to misdirected effort. Collaboration and education would appear to be the most productive ways forward in the short term rather than trying to export technology.

It would be remiss not to say something about the impact of threatened climate change. I do not think it is important for my purpose today to debate the causes but I do think we should be aware of the overall rise in temperatures in Europe and disruption of weather patterns we have regarded as normal. These changes will impact on the successful cultivation of crops, the variety which is grown in any one area and the conditions in which the products enter stores. They may also impact on the pest species we consider most important but there have been shifts in the past. The introduction of combine harvesters and consequent damage to wheat grains led to a reduction in the incidence of *Sitophilus granarius* (L.) in the UK and its replacement by *Cryptolestes ferrugineus* (Stephens) and *Oryzaephilus surinamensis* (L.). *Typhaea stercorea* (L.) and Ahasverus advena (Waltl) have become more common and in general species of tropical or very warm temperate origin have become more common at the expense of species regarded as typical of cooler northern areas (Armitage, 2004). Since storage conditions are generally controlled, I do not see any major rapid changes which might be needed in the near future as a direct consequence of climate change, but a watching brief is essential. However a recent extended review of the impact of climate change on food security worldwide provides a wider perspective (CGIAR, 2009).

8. Changes in methodology

Research is a somewhat cyclical activity in that new methods provide a means of approaching old problems in a new way. Previously insoluble problems are sometimes amenable to solution as methodologies develop. Both situations are applicable in the context of stored-product research in Europe.

I have said that I consider that relatively little research on the basic biology of pests of commodities stored in Europe is now justified. I stand by this but acknowledge that the identification and classification of pests, including potential pests, will be informed by the use of molecular methods. At present only the genera *Tribolium* and *Callosobruchus* have been investigated in any depth although there are individual papers concerning other genera such as Acanthoscelides and Bruchidius (Kergoat et al., 2005). I would suggest that the concentration on *Tribolium* and *Callosobruchus* has more to do with their use in other fields of biology, as model organisms, than their role as stored-products pests. There are no papers on *Oryzaephilus* or *Cryptolestes* despite their importance in Europe and virtually nothing on Sitophilus beyond an interesting paper by Hidayat et al. (1996) in which molecular data were shown to confirm some morphological distinguishing characters of S. oryzae (L.) and S. zeamais Motschulsky (but not in Europe). The complete genome of *Tribolium castaneum* (Herbst) has been published but whether any of the impressive body of information which that represents will solve the world's food security problems, I doubt. Nevertheless the fact that *T. castaneum* is a pest will be used to justify subsequent research and the title of the Nature paper (*Tribolium* Genome Sequencing Consortium, 2008, "The genome of the model beetle and pest *Tribolium castaneum*") with the genome sequence is evidence for my view! Let me say, however, that I am sure that the work will have major impacts elsewhere and I do not denigrate the effort or importance of the genome in other areas of scientific research.

Very much more likely to be of importance is the use of molecular data for identifying past or present infestations at very low levels in grain bulks. Enzyme-linked immunosorbent assays (ELISA) have been tried for many years and crop up intermittently. Brader et al. (2002) demonstrated the power of the method over a number of alternatives and it had a recent revival in the work of Atui et al. (2007) but it has never been taken from proof of concept to practical application. Efforts to utilise DNA technology to date have concentrated on Tribolium not only because of its pest importance but also because of its importance as a model insect and the availability of Genbank sequences (Nowaczyk et al., 2009). The potential sensitivity of such tools is already known (Balasubramanian et al., 2007) but there are many hurdles to overcome before they are commercially important. Nevertheless, it is pretty safe to predict that complete automation and rapid multiple sampling will be possible before long.

An entirely different but equally probable application of molecular developments is in the world of proteomics or metabolomics. Techniques employing mass spectrometry and large arrays to identify the sources of proteins or other metabolites and thereby identify their source has been demonstrated in the context of some foods (e.g. Mora et al., 2009; Ocana et al., 2009) and their application to the identification of pests or pathogens such as fungi in stored food must have considerable potential which does not yet appear to have been explored. Only two papers have apparently considered this approach

thus far (Park et al., 2008; Campbell, 2008) although the fundamental techniques are well established in European research laboratories.

Another area for development which has been explored in the recent past is means of detecting the presence of insects or pathogens either by their volatile chemical signature (electronic noses, e.g. Magan and Evans, 2000) by the sound (e.g. Hagstrum et al., 1990) which they make when feeding (acoustic detection) or by imaging methods (optical or spectroscopic detection, e.g. Perez-Mendoza et al., 2003; Fornal et al., 2007). At different times the feasibility of each has been shown but their practical application currently remains elusive; as Bengston said in 2005, "use of these techniques remains largely experimental"! However rapid advances in powerful techniques including nanotechnology may yet see one or more of these methods emerge into the mainstream. Europe has contributed and will doubtless continue to do so in the development and application of diverse novel methods.

Yet another area where there must be room for further development is in the composition of the crops themselves. This is not the place to embark on a debate about genetic manipulation or engineering and, indeed, the means of generating new cultivars is not the issue. However, growing understanding of the biochemistry, especially the gut enzyme complements of some pests allied with some form of resistance mechanism in the crop or its product to be stored will provide an adjunct to physical control methods. However, I have said before in these meetings (Credland and Appleby, 2003) that individual resistance mechanisms in a crop are likely to be no more valuable in the long term than chemicals or fumigants with a single mode of action and I stand by this assertion. Multiple mechanisms of action are far more likely to have a role into the future but are likely to be much more difficult to both understand and engineer. Europe has a long and distinguished history in the understanding of such methodologies (e.g. Hilder et al., 1987; Gatehouse et al., 1992)

I do not plan to say anything further about the sort of progress that might be made in the context of stored products and food security in developing countries. I am sure that my colleagues have or will comment on how they see both the need for collaboration with colleagues in Europe, and the areas of research which might benefit from European inputs.

9. Neglected issues

I think it only right to say something of issues I have largely ignored, and explain why. There is no doubt that mould has been a problem on stored products in the past and still is in some instances. However the fundamental control of the problem is generally well understood and physical conditions of storage are the key. Similarly, rodent infestations can have major impacts but the newer second generation anticoagulants are effective although, as with insecticides, resistance is likely to arise and proliferate. Good management of the stores allied with selective usage is probably adequate for the immediate future.

I have said nothing about biological control. It is a subject of interest to me and it clearly has a role in the management of field pests, but does it have a place in stored product pest control in Europe if contaminants of food are to be minimised? The basic concept of introducing a pathogen, whether microbial or fungal, or an animal, is anathema to most consumers despite what scientists may think. Predators or parasitoids of insect pests are other insects so, although I think they may have a valuable role in developing countries with more acute problems, and despite the protestations that I am sure I will attract, I do not see conventional biological control as playing a significant part in stored-product pest control in Europe.

There is an area which certainly will be important but which I feel unable to say anything very sensible about, but I do not want it to be overlooked. In Australia and North America, in particular, very important work has looked at the structure of stores, their design, location, orientation and so on. This work leads to other on modelling of air flow, temperature and moisture gradients, for example. With the growth in stores in Europe which will be needed to house the food for its growing population, I trust that we shall not repeat all the work undertaken elsewhere. The values of variables in the equations may be different but I am not convinced that redeveloping the models is justified.

The biggest problem that I think we face in Europe is probably the training and education of store managers. With a move to less on-farm storage and the continued growth of cooperative and commercial stores, we may need few real specialists but it is absolutely essential that growers and importers are

aware of basic requirements and know how to recognize, minimise, or better, exclude problems. But, where are the courses to train experts in Europe? It is extremely difficult to find any and I am not aware of any in the UK that provide anything like a comprehensive coverage.

So what of the future and what do I think is likely to happen?

10. To the future

I have worked in British universities and associated with scientists from many countries for 40 years, and the most important lesson to learn from that is that if you hang in there the world changes faster than you do. I have seen governments come and go, wars come and go, financial crises come and go, and often, very sadly, people come and go. I know that trying to predict what will happen tomorrow at home, never mind in Europe over the next few decades, is virtually impossible.

However, I am confident that at some time in the fairly near future the world is going to need more scientists with expertise in stored products than are currently available. I am convinced that problems of one kind or another await us all. The world's population is rapidly approaching 7 billion; it may have actually reached this figure as we speak. Europe has about 750 million inhabitants, rising steadily but declining as a proportion of the world's population because of the greater rate of increase elsewhere. There are now closer ties between many of the countries of Europe than have perhaps ever existed. Exchange of information and skills, enhanced communication both within the continent and with others further afield should leave us well placed to deal with future problems. In my opinion it is certain that we shall need to find new strategies to deal with storage problems to feed the growing number of people and that existing measures are doomed to failure in the foreseeable future. At that time, despite warnings we can give now, funding will suddenly be made available to try and conjure solutions to problems that we can predict today. I hope that we have trained enough successors to take on this task which I consider essential to avert the sort of problems that have led to conflict in the past. I urge you all, wherever you are from, to keep up your excellent work and enjoy events such as that starting here today because I think this exchange of information, education of each other, is the key to our future.

Acknowledgements

I am greatly indebted to Professor Chris Haines for providing invaluable information on the history of stored products research in the UK and to Emily Throgmorton for her comments on a draft of the manuscript. I must also thank the many unmentioned people who have put up with my inadequacies and contributed more than they could imagine to my knowledge of stored products research.

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Stored product protection in Africa: Past, present and future

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Abstract

More than 55% of Africans earn their livelihood from agriculture, which is also the key to economic development of the continent. The agriculture is largely traditional and grains constitute the bulk of food production. Sorghum, maize, rice, wheat and millet for cereals and cowpeas, dry beans, groundnut, chickpea and bambara groundnut for pulses, are most common in Africa. Because agricultural production is seasonal while the demands for agricultural commodities are more evenly spread throughout the year, grain storage becomes a particularly important agricultural activity. Grain storage is done on-farm, peasant farmers' residences (family granaries), community stores and large warehouses. Since most of the grains produced in Africa are destined for human consumption, storage in family granaries predominates. Unfortunately, the technology and management of family granaries and other storage structures are seriously wanting. These predispose the grains to serious attacks from biotic constraints such as insects, rodents, birds and micro-organisms. The rate of insect proliferation in these storage structures could be alarmingly high, especially with the warm climate in tropical Africa. Annual grain losses of up to 50% in cereals and 100% in pulses have been reported, although average losses stand at roughly 20%. Major insects that attack cereals and pulses include grain weevils, grain borers, grain beetles and grain moths. Pest prevention, early detection and pest control would greatly reduce grain losses during storage. Control methods comprise physical, chemical and phytochemical measures with emphasis on the use of traditional botanical pesticides. This paper discusses the major cereals and pulses stored in Africa, the different storage structures, storage losses, constraints, control measures, and the relationship between storage structures and pest infestation. It also attempts to highlight peculiarities to the African storage environment and research trends over the years, and suggests recommendations for improving grain storage in the continent.

Keywords: Stored products, Grains, Pests, Protection, Africa

1. Introduction

Over half of Africans earn their livelihood from agriculture, which is also the most important enterprise and key to economic development of the continent. Paradoxically, tropical African countries are among the world leaders in food insecurity (Pantenius, 1987; Ngamo and Hance, 2007). Almost 33% of the African population, some 200 million people, is malnourished. Food security could be achieved by increasing agricultural productivity and reducing pre- and post-harvest crop losses.

Agriculture in Africa is largely traditional and grains constitute the bulk of food production. Many cereals and pulses are grown in the continent but maize, sorghum, rice, wheat and millet for the former and cowpea, groundnut, common bean, soybean, chickpea, bambara groundnut, pigeon pea, and green gram for the latter, are most common. However, the dominant crops vary from one country to the other. Agricultural production is seasonal while demands for agricultural commodities are more evenly spread throughout the year. In this circumstance, crop storage becomes particularly important.

Storage is a way or process by which agricultural products or produce are kept for future use, it is an interim and repeated phase during transit of agricultural produce from producers to processors and its products from processors to consumers (Thamaga-Chitja et al., 2004). Grains need to be stored from one harvest to the next in order to maintain its constant supply all year round and to preserve its quality until required for use. For small scale farmers in Africa, the main purpose of storage is to ensure household food supplies (reserves) and seed for planting (Adetunji, 2007). The stored crop is gradually released to the market during off-season periods, which also stabilizes seasonal prices (Adejumo and Raji, 2007). In

the dry Sahelian countries in the northwest of Africa, crop storage is a matter of subsistence and survival (Mikolo et al., 2007).

Three techniques of storage involving different structures have been identified in Africa (Adesuyi et al., 1980; Udoh et al., 2000), namely: traditional/local grain storage at the farm and domestic level which includes local cribs and rhombus, platforms, open fields, roofs and fire places; improved/semi modern grain storage techniques at farm and domestic level which are ventilated cribs, improved rhombus and brick bins; and modern centralized storage at the commercial level involving silos and warehouses. Since farming is mostly done by subsistence farmers, the first two storage techniques predominate. Stored grains may suffer from serious attacks from pests (insects, fungi, rodents and birds), especially when not protected and in the presence of poor store hygiene.

In the tropical countries, Hill (1975) listed 407 insect species of major and 788 of minor importance occurring in 48 major groups on stored products. Fleurat-Lessard (1988) stated that all grain and seed insect species belong to two principal orders: Coleoptera and Lepidoptera, with some minor species belonging to the order Psocoptera. Traditionally, the grain weevils, *Sitophilus* spp. (Coleoptera: Curculionidae) and the Angoumois grain moth, Sitotroga cerealella (Olivier) (Lepidoptera: Gelechiidae) on cereals and three genera of bruchids, Acanthoscelides, Zabrotes and Callosobruchus spp. on pulses are the most important pests of stored grain in Africa (Abate et al., 2000). In addition to direct destruction of grains through feeding and reproduction, insects' presence has direct influence on grains causing an increase in grain temperature and moisture contents which leads to an increase in respiration and consequently loss in quantity and quality of the grain (Odogola, 1994). Grain losses caused by insect pests in Africa are quite high and vary from country to country and from region to region. However, annual grain losses of over 50% (Abraham and Firdissa, 1991) in cereals and up to 100% (Boeke, 2002) in pulses have been reported, although the average stands at 20% (Youdeowi and Service, 1986; Philips and Throne, 2010). In general, the damage caused by insects is much higher than those caused by other agents like rodents and micro-organisms. Fungi are the major microorganisms causing spoilage in stored grains and seeds, resulting in significant losses to farmers, traders and food and feed manufacturers (Twiddy, 1994). The major grain storage fungi are Aspergillus, Fusarium and Penicillium spp. Rodent species that damage stored products when they are searching for food water or better leaving environments vary from region to region and from country to country. The three common species across Africa are the black rat, Rattus rattus, Fischer de Waldheim, brown rat, Rattus norvegicus, (Berkenhout) and common mouse, Mus musculus L. Pest control are mainly traditional and also the use of synthetic chemicals.

This review presents the major cereals and pulses stored in Africa, the different storage structures, storage losses, constraints, control measures, and the relationship between storage structures and pest infestation. It also attempts to highlight peculiarities to the African storage environment and research trends over the years, and suggests recommendations for improving grain storage in the continent.

2. Fundamentals of the African storage environment

2.1. Stored commodities

Generally, over 70% of grains harvested in Africa are stored for human consumption or for marketing (Mallamaire, 1965; Talabi, 1989). In the Sudan and Guinea Savanna of Nigeria 40-85% of grains harvested are stored (Ivbijaro, 1989). The commodities stored and their relative quantities are generally related to their production statistics - the higher the quantity produced, the more grains of that commodity stored. Familiarity with the map of the agro-ecological zones of Africa is important at this point since crop production patterns are related to the different zones (Fig. 1).

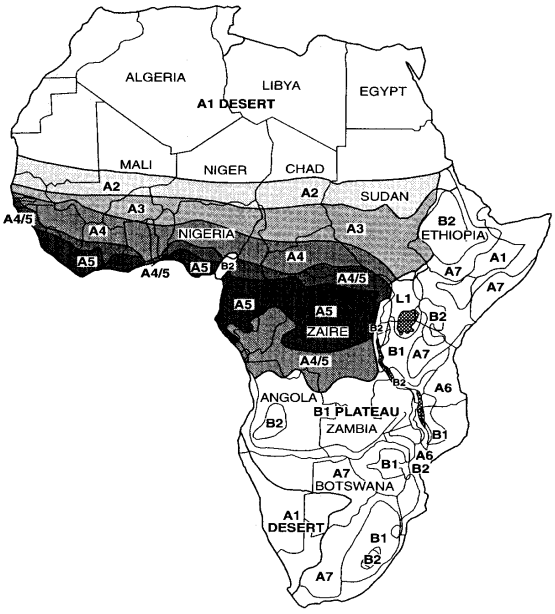


Figure 1 Major agro-ecological zones of sub-Saharan Africa (from Geddes, 1990): 1.) The Sahel (A2): characterized by erratic rainfall of 250 to 500 mm per annum, more than 8 months of dry season, and altitudes of less than 900 m above sea level. 2.) The Sudan savannah (A3): rainfall 500 to 900 mm, dry season 8 months, altitude less than 900 m above sea level. 3.) The Guinea savannah (A4): rainfall 900 to 1500 mm (mostly unimodal), dryseason of 5 to 7 months, altitude less than 900 m above sea level. 4.) The forest–savannah transition (A4/5): rainfall 1300 to 1800 mm (unimodal or bimodal), dry season of 4 months, altitude more than 900 m above sealevel. 5.) The forest (A5): rainfall 1500 to 4000 mm, virtually no dry season, altitude less than 900 m above sea level. 6.) The east coast (A6): rainfall 750 to 1500 mm (bimodal in some countries), altitude less than 900 m above sea level. 7.) The semi-arid east and south (A7): rainfall 250 to 750 mm, more than 8 months of dry season, altitude less than 1500 m above sea level. 8.) The plateaux (B7): rainfall 750 to 1500 mm (mostly unimodal), dry season 5 to 8 months, altitude 900 to 1500 m above sea level. 9.) The Uganda and Lake Victoria shore (L1): rainfall 1000 to 1500 mm (bimodal), altitude 1135 to 1300 m above sea level. 10.) The mountain (B2): rainfall 750 to 1800 mm (unimodal or bimodal), altitude more than 1500 m above sea level.

Cereals like maize, sorghum, rice, wheat, millet are important in many African countries, generally featuring among the first five cereals and first 15 crops in terms of production (Table 1) (FAOSTAT, 2007). Barley production is high in the Southern (3rd cereal and 17th crop) and Northern African countries (4th cereal and 12th crop). Oats appears to be common only in Lesotho (4th cereal and 9th crop) and teff in Ethiopia and Eritrea. Maize is the leading cereal crop across Africa, while millet and/or sorghum dominate in many countries in the drier areas of the continent like Burundi, Eritrea and Ethiopia in the east, Cameroon and Chad in the middle, Sudan in the north, and Nigeria, Niger, Ghana, Benin, Gambia, Burkina Faso, Senegal, Mali and Guinea in the West. In the dryer parts of Southern Africa (in Angola, Namibia, Zimbabwe, Botswana, Zambia and South Africa), millet is traditionally grown as a staple and surpluses are hardly traded (Smith, 1991). The more developed and sophisticated countries in Africa, characterized by arid or semi-arid sub-tropical warm climate like South Africa (2nd cereal and 4th crop) in the south and most of the countries in the north (Algeria (1st cereal and 1st crop), Egypt (1st cereal and 3rd crop), Libya (1st cereal and 8th crop), Morocco (1st cereal and 2nd crop), Tunisia (1st cereal and 1st crop), wheat is a dominant cereal crop (FAOSTAT, 2007). Wheat production is also high in Ethiopia (1st cereal and 4th crop), Kenya (2nd cereal and 15th crop), Sudan (2nd cereal and 3rd crop) and Zambia (2nd cereal and 5th crop).

Table 1 The top four cereal crops in Africa

Area	Crop	Production	Ranking[a]
World	maize	788112128	1 (2)
Africa		47229918	1 (3)
Eastern Africa		18578052	1 (3)
Middle Africa		3012277	1 (2)
Northern Africa		6412794	3 (8)
Southern Africa		7275806	1 (2)
Western Africa		11950989	3 (5)
World	sorghum	minor	minor
Africa		25134711	2 (5)
Eastern Africa		4505970	3 (10)
Middle Africa		1127801	2 (10)
Northern Africa		5857965	3 (8)
Southern Africa		215712	5 (18)
Western Africa		13427263	2 (4)
World	rice	657413530	2 (3)
Africa		20883913	3 (7)
Eastern Africa		4965075	2 (9)
Middle Africa		536359	5 (15)
Northern Africa		6933280	2 (5)
Southern Africa		minor	minor
Western Africa		8445829	4 (6)
World	wheat	611101664	3 (4)
Africa		18590367	4 (8)
Eastern Africa		3774128	4 (12)
Middle Africa		minor	minor
Northern Africa		13630393	1 (2)
Southern Africa		1922026	2(4)
Western Africa		1922026	2(4)

Compiled from FAOSTAT 2007; [a] Figure without bracket is rank among cereal crops and figure within brackets is rank among all crops

Cowpea, groundnut, common bean, soybean, chickpea, bambara groundnut, pigeon pea and green gram are the more common pulses grown in Africa. Nigeria (2,800,000 Mt), Niger (1,001,139 Mt), Burkina Faso (253,190 Mt), Cameroon (95,000 Mt) and Kenya (83,251 Mt) are respectively the 1st, 2nd, 3rd, 5th and 6th producer of cowpea in the world (FAOSTAT, 2007). Tanzania (7th in the world), Uganda (8th), Kenya (9th), Rwanda (13th) and Ethiopia (15th) for dry bean, Ethiopia (1st), Chad (2nd), Tunisia (7th), South Africa (9th) and Tanzania (11th) for chickpea, Nigeria (3rd), Sudan (8th), Ghana (10th), Congo DR (11th) and Senegal (12th) for groundnut, Malawi (3rd), Kenya (4th), Uganda (5th), Tanzania (6th) (1st cereal and 2nd crop), and Congo DR (9th) for pigeon pea, and Nigeria (13th) and South Africa (19th) for soybean are the highest producing countries in Africa.

2.2. Length of storage

Grain storage periods generally range between 3 and 12 mo across Africa. The length of storage depends on the agro-ecological zone, ethnic group, the quantity of commodity stored, the storage condition, the crop variety stored, etc. (Hell et al., 2000; Ngamo et al., 2007). The length of storage of grains tends to be longer in the dryer areas of Africa. Ngamo et al. (2007) reported an increase in storage length from 3-8 months in the Sudano-Guinean Agro-ecology to over 24 months in the Sudano-Sahelian zone of Cameroon. In the Northern Guinea Savanna of Benin, maize is usually stored between 3 and 8 months and in the Sudan Savanna 7-12 months (Hell et al., 2000). Storage for 5-12 months is common in the

Forest/Savanna Mosaic and the Southern Guinea Savanna of Benin. In the Forest/Savanna Mosaic, a few farmers store maize for more than 12 months. In this area, dominated by the "Mina" ethnic group, the size of maize stores is used to assess the wealth and social prestige of their owners and maize can be stored for up to 3 years (Smith, 1991).

The length of grain storage in the Sudan and Guinea Savanna of Nigeria is between 5 and 12 mo, except for soybean with usually less than 5 months storage because of its high demand (Ivbijaro, 1989). However, a maximum storage period of between 7 and 10 years for sorghum and millet in the Sudan Savanna was recently reported by Adejumo and Raji (2007). In Namibia, Keyler (1996) reported that the fear of the effect of drought made farmers store grains from 4 to 6.5 years.

2.3. Storage structures

The structure used for grain storage depends on the level of storage: On-farm, village and city or central. On farm storage involves individuals, while village storage may implicate individuals (family granary) or a group of individuals (community stores) (Fig. 2). The city and central storage facilities include large warehouses and are usually own by government agencies or non-governmental organizations (national or international). They are usually built with expertise from the developed world. Since most grains in Africa are produced by rural farmers, storage at the farm/village level will be emphasized in this paper. It is also at this level that traditional structures typical to Africa could be better discerned.

Open Platform (Cameroon)

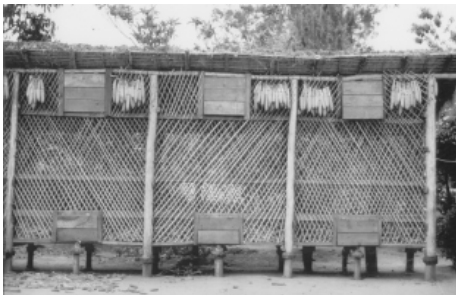
Woven basket (Cameroon)

Woven Basket (Burkina Faso)

Pots (Cameroon)

Mud Rhombu (Cameroon)

Mud Rhombu (Nigeria)



Maize Crib, Bamboo and straw roof (Madagascar)

Community store (Cameroon)

Figure 2 Common storage structures in Africa.

Many publications have reported on traditional storage structures in Africa (Gilman and Boxall, 1974; Youdeowei and Serive, 1986; FAO, 1994; Adejumo and Raji, 2007). These storage facilities are made of local materials (plant materials and soil) and constructed by the villagers themselves. Some structures are used for temporary storage (mostly intended for the drying of the crop), while others are for long-term storage (FAO, 1994). Temporary storage methods are grouped into aerial storage (maize cobs or sorghum and millet panicles are sometimes tied in bundles, which are then suspended from tree branches, posts, or tight lines on or inside the house), storage on the ground, or on drying floors and open timber platforms. Long-term storage methods include (i) storage baskets (cribs or thatched rhombus) made exclusively of plant materials, (ii) calabashes, gourds, earthenware pots, etc., (iii) jars, (iv) solid wall bins (mud rhombus), and (v) underground storage.

In humid countries, where grain cannot be dried adequately prior to storage and needs to be kept well ventilated during the storage period, traditional granaries (cribs) are usually constructed entirely out of locally available plant materials: timber, reeds, bamboo, etc (Fig. 2). The small capacity containers (calabashes, gourds, earthenware pots, etc.) are most commonly used for storing seed and pulse grains, such as cowpeas. If the grain is dry (less than 12% moisture content) there is usually no problem with this kind of storage. Jars are generally kept in dwellings; they serve equally for storing seeds and legumes.

A solid wall bin or mud rhombus is a specially built structure from a mixture of clay and dry straw (Adejumo and Raji, 2007). It consists of a bin resting on large stones, timber or earth. Such grain stores are usually associated with dry climatic conditions, under which it is possible to reduce the moisture content of the harvested grain to a satisfactory level simply by sun-drying it. Solid wall bins are therefore traditional in the Sahel region of Africa, and in southern African countries bordering on the Kalahari Desert (FAO, 1994). Its shape could be spherical, circular or cylindrical (Adejumo and Raji, 2007).

Underground pit storage is practiced in the Sahelian countries and southern Africa, and is used in dry regions where the water table (low) does not endanger the contents. Conceived for long term storage, pits vary in capacity (from a few hundred kg to 200 t) (FAO, 1994). Their traditional form varies from region to region: they are usually cylindrical, spherical or amphoric in shape, but other types are known (Gilman and Boxall, 1974). The entrance to the pit may be closed either by heaping earth or sand onto a timber cover, or by a stone sealed with mud.

As with the length of storage, storage structures also vary with the agro-ecological zone, ethnic group, the quantity of commodity stored, the storage condition, etc. Description of the storage structures and habits in Benin and Namibia highlights some of these variations. Storage structures in the south of Benin (Southern Guinea Savanna) which has a bimodal rainfall pattern differ from the stores used in northern Benin (Northern Guinea Savanna and Sudan Savanna), where the rainfall is unimodal (Fiagan, 1994). In the south, stores are constructed out of plant materials, whereas in northern Benin a high percentage of stores are built of clay (Fiagan, 1994). Fiagan (1994) observed that the storage of maize in an intermediary structure may lead to the contamination of maize with pests and pathogens. Many farmers use two stores during the storage season, with the initial store built in the field (on-farm). Field stores are

taken down in the dry season from February to April when bush fires and theft, because of depleting food stocks, might endanger the stored maize.

In Namibia, northern communal farmers store threshed grain either in granaries in the homestead, or inside the home in different types of containers (bags, baskets, drums, etc.). Traditional pearl millet storage in the Caprivi and Kavango regions, involves a variety of storage containers and structures, including granaries made of earth blocks or poles and mud, raised on a low platform and roofed with thatch. In the North Central Regions of Namibia, innovative grain storage containers are being made by farmers: small concrete "silos" (similar to a water reservoir) and thick baskets with lids made of Makalani palm leaves, Hyphaene petersiana Klotzsch ex Mart. Just as the husband and wife or wives have their own fields, they also have their own pearl millet storage places. The wife's or wives' pearl millet supplies are used first and only then that of the husband (Eirola and Bradley, 1990). Households of the North Central Regions own one or more granaries according to the size of their fields and quantity of pearl millet produced. When a family has only one granary, it is filled with the last harvest. If grain from the preceding year remains, it is placed on top of the new grain or placed in another container. But if a family has several granaries, the different harvests are kept separate.

2.4. Storage problems

All stocks constitute an entity made of the grain to be stored on one hand, and the environment where they evolve on the other hand, and where they are subjected to different attacks causing enormous losses. All of these losses are linked to two principal factors, which may be abiotic (granary architecture, humidity and temperature) or biotic (micro-organisms, rodents, birds and insects) (Scotti, 1978).

2.4.1. Abiotic factors

2.4.1.1. Storage structure architecture and management

The typical African traditional storage structures expose the grain to insect attack and favourable climatic conditions for their proliferation and those of micro-organisms and rodents. One of their major weaknesses is the presence of a single orifice for loading and removing grains, which also serves as an entry port for pests (FAO, 1994; Ngamo, 2000; Adejumo and Raji, 2007). The structures are generally not hermetically sealed giving room for pests to make their way into the structures. When constructed of plant materials, rodents easily destroy the structures and favour other sources of infestation (CIRAD, 2002).

Many authors have contended that a major cause of losses in traditional granaries is the lack of hygiene (Bell, 1996; Ngamo, 2000; Hoogland and Holen, 2001). At the time of filling the storage structure with newly harvested grain, the residues of old grain are not always completely removed, and these serve as a source of infestation for new grain. These impurities can attract pests from the exterior. Danho et al. (2003) showed that infested grain is attractive to pest insects, particularly to females for oviposition. Farmers in most areas of Eritrea keep old and new harvested grains in the same vicinity, which causes an easy migration or infestation of the new grains from the old grains (Haile, 2006).

2.4.1.2. Influence of climate

Humidity is the principal climatic element which acts in the storage system. Traditional cribs for example give room for limited air circulation, and when grain is not very dry there is an increase in grain moisture content in the structure (CIRAD, 2002). Biological activity occurs only when moisture is present. Therefore, moisture content of the product itself, as well as the moisture content of the surrounding air, is important for safe storage (Hayma, 2003). Stored products, as well as the organisms attacking stored products are living things: they breathe. During respiration ("breathing"), oxygen is used up and carbon dioxide, water and heat are produced. The rate of respiration, and thus the amount of carbon dioxide, water and heat that are produced is strongly dependent on the temperature and the moisture content of the product. Higher temperature and moisture content values of grains favours insect and fungus development and a decline in the germination capacity of the grains (Hayma, 2003).

2.4.2. Biotic factors

Living organisms like insects, rodents, birds (on-farm storage) and micro-organisms are serious constraints to the traditional storage systems of Africa (Ngamo, 2000; Nukenine et al., 2002; Haile, 2006). Amongst these living organisms, insects are responsible for the greatest storage losses in cereals and pulses.

The common insect pests reported in stored cereals and pulses are given in Table 2. However, traditionally the grain weevils (*Sitophilus* spp.) and the Angoumois grain moth (*Sitotroga cerealella*) on cereals and three genera of bruchids (*Acanthoscelides*, *Zabrotes* and *Callosobruchus*) on pulses are the most important pests of stored grain in Africa (Abate et al., 2000). *Callosobruchus chinensis* L. is the most important pest of chickpea in Eritrea (Haile, 2006). Wheat and sorghum in storage were attacked by *S. cerealella*, (Sitophilus spp.), confused flour beetles, *Tribolium confusum* Jacquelin du Val, sawtoothed grain beetles, *Oryzaephilus surinamensis* (L.) and mites (Haile, 2006). The most significant pearl millet pest in Namibia is reported to be *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (NRI, 1997). These moth infestations result in masses of grain held together by webbing (silk) produced by the larvae as they move through the grain seeking a pupation site. Many individual grains have their embryos removed by the feeding larvae. In order to use the grain, they have to be rubbed and sieved to remove the webbing, or alternatively the masses of clumped grain are fed to chickens (NRI, 1997).

Table 2 Common insect pests of stored cereals and pulses in Africa according to Mallamaire (1965), CAB International (1999), Mikolo et al. (2007) and Ngamo and Hance (2007)

No	Scientific name	Order	Family	Commodity	Country
1	*Lasioderma serricorne* F.	Coleoptera	Anobiidae	Some pulses and rice	Mostly East and West Africa
2	*Stegobium paniceum* L.	Coleoptera	Anobiidae	Some cereals and pulses	Mostly North Africa
3	*Araecerus fasciculatus* DeGeer	Coleoptera	Anthribidae	Some cereals and pulses	Mostly West African
4	*Prostephanus truncatus* Horn	Coleoptera	Bostrichidae	Maize and some pulses	Mostly West Africa
5	*Rhyzopertha dominica* F.	Coleoptera	Bostrichidae	Mainly cereals and some pulses	Mostly North and West Africa
6	*Acanthoscelides obtectus* Say	Coleoptera	Bruchidae	Beans, cowpeas	Mostly South and East Africa
7	*Bruchidius atrolineatus* (Pic)	Coleoptera	Bruchidae	Beans, cowpeas	East and West Africa
8	*Callosobruchus chinensis* L.	Coleoptera	Bruchidae	Primarily pulses	Mostly East Africa
9	*Callosobruchus maculatus* L.	Coleoptera	Bruchidae	Mainly cowpeas and some beans	Mostly East and West Africa
10	*Callosobruchus rhodesianus* (Pic)	Coleoptera	Bruchidae	Pulses	Cameroon, Kenya and Zimbabawe
11	*Callosobruchus subinotatus* (Pic)	Coleoptera	Bruchidae		Cameroon
12	*Caryedon gonagra* (F.)	Coleoptera	Bruchidae	Pulses	East and West Africa
13	*Sitophilus granarius* L.	Coleoptera	Curculionidae	Many cereals	Mostly North Africa
14	*Sitophilus oryzae* L.	Coleoptera	Curculionidae	Mainly cereals and some pulses	Across Africa
15	*Sitophilus zeamais* Motschulsky	Coleoptera	Curculionidae	Mainly cereals and some pulses	Across Africa
16	*Trogoderma granarius* Everts	Coleoptera	Dermestidae	Mainly cereals and some pulses	Across Africa
17	*Carpophilus dimidiatus* L.	Coleoptera	Nitidulidae	Groundnut, Maize, Rice	Mostly East and West Africa
18	*Tenebroides mauritanicus* L.	Coleoptera	Trogossitidae	Mainly cereals and some pulses	East, West and South Africa
19	*Oryzaephilus mercator* Fauvel	Coleoptera	Silvanidae	Groundnut, Rice	Mostly East Africa
20	*Oryzaephilus surinamensis* L.	Coleoptera	Silvanidae	Mainly cereals and some pulses	Mostly south Africa

No	Scientific name	Order	Family	Commodity	Country
21	*Zabrotes subfasciatus* (Boheman)	Coleoptera	Bruchidae	Beans, cowpea	West Africa
22	*Tribolium castaneum* Herbst	Coleoptera	Tenebrionidae	Cereals and Pulses	Across Africa
23	*Tribolium confusum* Jaquelin du Val	Coleoptera	Tenebrionidae	Cereals and Groundnut	Mostly East Africa
24	*Sitotroga cerealella* (Olivier)	Lepidoptera	Gelechidiae	Cereals	Across Africa
25	*Corcyra cephalonica* Stainton	Lepidoptera	Pyralidae	Primarily cereals, secondarily pulses	Mostly West Africa
26	*Ephestia cautella* (Walker)	Lepidoptera	Pyralidae	Mainly cereals and some pulses	Across Africa
27	*Ephestia elutella* (Hübner)	Lepidoptera	Pyralidae	Cereals	Mostly North Africa
28	*Ephestia kuehniella* Keller	Lepidoptera	Pyralidae	Many cereals	Across Africa
29	*Myelois ceratoniae* Zeller	Lepidoptera	Pyralidae	Rice, flour, pulses	Mostly North Africa
30	*Plodia interpunctella* (Hübner)	Lepidoptera	Pyralidae	Many cereals and groundnut	Mostly south Africa

2.5. Damage and losses

Average grain weight loss for cereals and pulses in Africa stands at 20% (Youdeowei and Service, 1986; Philips and Throne, 2010). However, the ranges for grain damage and losses across Africa are very broad. Grain damage and losses could result from the attacks of insects, micro-organisms, rodents and birds. Mainly damage from insects is considered in this section. In Kenya, De Lima (1979) reported the main causes of damage and weight loss in maize to be insect pests (4.5%) and rodents (1.5%). Farmers in the Adamawa Region of Cameroon attributed 50%, 47% and 3% stored maize damage to insects, rodents and micro-organisms, respectively (Nukenine et al., 2002). Although grain losses could include non-storage losses (harvesting and drying, threshing and shelling, winnowing and transport) and storage losses, only the storage losses in traditional systems are given consideration here. As explained earlier, traditional storage structures in Africa expose grains to serious insect infestations. Additionally, all small scale African farmers rely on sun drying to ensure that their crop is sufficiently dry for storage. If weather conditions are too cloudy, humid or even wet then the crop will not be dried sufficiently and losses will be high. This bad weather is frequent in wetter regions of Africa and could partly explain the higher grain damage level in the continent compared to those of the developed world. Tadesse and Eticha (1999) cited many studies on stored maize damage and losses in Ethiopia. Damage ranged between 11 and 100% and weight loss between 2.9 and 20% for storage periods of 2-12 months. In Eritrea, The germination loss due to the attack of storage pests on cereals and pulse grains ranges from 3-37 and 4-88 %, respectively (Haile et al., 2003). The weight loss for these grains also ranges from 4.4-14 and 9-29% for cereals and pulses respectively. During the usual 5-12 month storage period of grains in the Sudan and Guinea Savanna of Nigeria insect damage ranged from 40-60% for unthreshed sorghum and cowpea, to 36-55% for wheat grains (Ivbijaro, 1989). On-farm physical losses in grain weight were crudely estimated to range from 10% after one storage year to more than 30% over longer storage periods in Namibia (NRI, 1997). With the introduction of Prostephanus truncates (Horn) (Coleptera: Bostrichidae) average dry weight losses of farm-stored maize in Togo were estimated to have risen from 7 to 30%, for a storage period of 6 months (Pantenius, 1987; Richter et al., 2007). In Kenya, weight loss of stored maize increased from 4.5 to 30%, 20 years after the introduction of P. truncatus in the country. Delobel (1988) reported 60% groundnut damage caused by C. serratus after 10 months of storage in some traditional granaries in Congo Brazzaville. In this country a single granary containing cereals or pulses can be infested by up to 10 insect pests leading to 100% grain damage (Mikolo et al., 2007).

2.6. Stored product protection measures

Farmers in Africa predominantly use traditional methods in the management of stored product insects from time immemorial. Some farmers are also attracted to the use of synthetic insecticides. Nonetheless, many farmers apply no protection measures in their storage structures. Approximately 50% of farmers in Benin do not do anything to counter storage problems (Hell et al., 2000). All the farmers interviewed in Northern Ethiopia apply one or more management practice to stored maize, whereas 23% in the South of the country applied none (Tadesse and Eticha, 1999). In the Ngaoundere area of Cameroon, 47% of farmers were unable to protect maize stocks (Nukenine et al., 2002).

2.6.1. Traditional methods

The use of traditional stored product protection methods is very popular among small-scale farmers in Africa. The methods are numerous, diverse and widespread in the continent, with regional and country particularities. In Ethiopia alone, Tadesse and Eticha (1999) reported 25 traditional management practices for stored maize. Eighty-eight percent of the farmers in the North Central Regions of Namibia use traditional methods like ash or leaves in the protection of stored pearl millet (Keyler, 1996). Farmers in Uganda use banana juice, pepper, Mexican marigold, Tagetes minuta L. and eucalyptus leaves for bruchid control in storage (Giga et al., 1992). Belmain and Stevenson (2001) presented a list of 16 plants commonly used by farmers in northern Ghana for stored product protection. The leaves, flowers, seeds or roots in whole, decoction, powder extract forms are admixed or layered with the grains. Tapondjou et al. (2000), Nukenine et al. (2003) and Ngamo et al. (2007) reported over 20 insecticidal plant species in Cameroon with most of them being employed in storage protection by rural farmers.

Animal wastes such as goat and cow urine or dung are also used in the management of storage pests. For example, farmers in parts of Tanzania and the Sahel stored beans in sacks soaked and dried in goat urine which provided protection against storage pests (Gahukar, 1988).

2.6.2. Synthetic chemicals

The use of chemical insecticides in the form of sprays, fumigant or dusts against grain pests is common in large scale farms. Due to their rapid action, small-scale farmers are also attracted to these chemicals and those who have access to them are beginning to reduce the use of, or even abandon plant materials, which are lower in insecticidal efficacy. In some parts of Ethiopia (Tadesse and Eticha, 2000), Benin (Hell et al., 2000), Cameroon (Nukenine et al., 2002) and Eritrea (Haile, 2006), 70, 50, 23, and 12% of the farmers, respectively, treated their grains with synthetic chemicals. The usual chemicals recommended for stored product protection are employed, but also insecticides meant for the treatment of field crops like cotton or those internationally banned like DDT, are used by farmers in countries like Cameroon, Benin, Eritrea, etc. (Haile, 2006).

3. Trends in stored product protection

Owing to a lack of access to literature (electronic database and hard copies) in Cameroon, basically only the abstracts of all the articles published in the Journal of Stored Products Research from 1965 to 2009 have been consulted. This may be far from being exhaustive, but the journal is the single source of the most comprehensive published literature on stored product protection in the world. However, whenever possible reference is made to other publication sources.

3.1. Country of researchers

From Table 3, most research on stored product protection in Africa is published by researchers from Nigeria, Kenya, Cameroon and Benin. Benin is a small country but has a research centre of the International Institute of Tropical Agriculture, leading to their presence among the top African countries publishing on stored product protection. Africans also publish more articles in the Journal of Stored Product Research than Asians, although overall, Asians are likely to have much more research in the area than Africans. This is because Asian countries as compared to their African counterparts have far more scientific journals.

Table 3 Number of research papers concerning stored product protection for insects, micro-organisms and rodents in Africa, published in the Journal of Stored Products Research from 1965 to 2009.

Country	Papers in Journal of Stored Product Research
Nigeria	25
Kenya	9
Cameroon	9
Benin	7
Ghana	4
Senegal	4
Togo	4
Zimbabwe	3
Egypt	2

Country	Papers in Journal of Stored Product Research
Ethiopia	2
Tanzania	2
Burkina Faso	2
Morocco	1
Rwanda	1
Uganda	1
Zambia	1
Others (country not mentioned)	3
Africa	81
Asia	63
World	533

3.2. Commodity

More research on stored product protection seems to have been done on cereals than pulses in Africa (Table 4). Most of the research works were done after the year 1980. Maize for cereals and cowpea for pulses have been at the forefront of research. The last decade compared to the previous ones has been a flourishing period for maize protection research.

Table 4 Number of research works concerning stored product protection for different commodities in Africa, published in the Journal of Stored Products Research from 1965 to 2009

Types	commodity	Period (range)	Grouped years	# articles
cereal	maize	1970 - 2009	-	27
			1965 - 1979	2
			1980 - 1999	9
			2000 - 2009	16
	wheat	1969 - 2000	-	7
			1965 - 1979	3
			1980 - 1999	1
			2000 - 2009	3
	sorghum	1967 - 1980	-	4
			1965 - 1979	2
			1980 - 1999	2
	rice	2007	-	1
			2000 -2009	1
pulses	cowpea	1969 - 2009	-	22
			1965 - 1979	7
			1980 - 1999	13
			2000 - 2009	2
	bean	1970 - 2009	-	7
			1965 - 1979	1
			1980 - 1999	2
			2000 - 2009	4
	groundnut	1978 - 2006	-	4
			1965 - 1979	1
			1980 - 1999	0
			2000 - 2009	3
	Bambara groundnut	2001 -2003	-	
			2000 - 2009	3

3.3. Storage structures

Originally storage structures in the continent where made of only plant materials and mud. This trend is changing as a few farmers have replaced or are replacing mud rhombus with metal silos and plant material-woven cribs with those built of timber and corrugated iron roof. Most research in the late 1960s to the 1970s was focused on assessment of the prototypes of storage structures (Gilman and Boxall, 1974). Later research to date, have focused on improving traditional granaries for better durability, air-tightness, etc. (Adetunji, 2007).

3.4. Storage pests

Callosubruchus maculatus (F.) and Sitophilus zeamais Motschulsky which respectively attack the most important pulse (cowpea) and cereal (maize) in Africa are leading in research works. There are more research in Africa on *S. zeamais* than *S. oryzae*, but this trend is reversed when the entire world is considered (Haines, 2000). This is because *S. zeamais* is more of a pan-tropical species while S. oryzae is more cosmopolitan. *Tribolium* spp., *P. truncatus*, *Acanthoscelides obtectus* (Say) are also serious insects in the African stored product research landscape. Research on these insects has been on the rise from the 1960s to date. More research is focused on control measures rather than pest biology, infestation and ecology.

3.5. Control methods

Botanical insecticides, natural chemical products based on powders, extracts or purified substances of plant origin and physical control methods like manipulation of the temperature and humidity of the storage environment plus grain drying, are topping research on control measures for food storage in Africa (Table 5). Modest research has been carried out for synthetic chemicals, biological control and grain resistance to pests. Pest biology and inert dust have attracted very limited research in the continent.

Table 5 Periods when research on different control methods were reported in the Journal of Stored Products Research from 1965 to 2009.

Method	Period (range)	Grouped years	# articles
Inert dust	2000 - 2008	-	
		2000 - 2009	2
botanicals	1978 - 2009	-	26
		1965 - 1979	1
		1980 - 1999	6
		2000 - 2009	19
biological control	1997 - 2007	-	9
		1980 - 1999	2
		2000 - 2009	7
biology	1967 - 2006	-	3
		1965 - 1979	1
		1980 - 1999	0
		2000 - 2009	2
synthetic chemicals	1969 - 2009	-	12
		1965 - 1979	7
		1980 - 1999	3
		2000 - 2009	2
physical methods	1969 - 2009	-	26
		1965 - 1979	2
		1980 - 1999	10
		2000 - 2009	14
varietal resistance	2000 - 2003	-	7
		2000 - 2009	7

Inert dust: DE and Ash; Botanicals: whole leaves, powders, fractions, essentials oils, oils, solvent extracts; Biological control: parasitoids, predators, entomopathogens; Synthetic chemicals: lindane (1965, 1969), DDT (1967), malathion (OP) (1968, 1980), pyrethrin (P) (1969,1970, 1982), dichlorvos (1969), diazon (1969), methyl bromide (1970), permethrin (1982,1991), Fenitrothion (1975, 1980), tetrachlorvinphos, (1975, 1980), pirimiphos-methyl (1980), fenthion (1975), iodophenphos (1975), jodfendos (1980), deltamethrin (1991) phosphine (2004), allyl acetate (2004) Physical: temperature, humidity, drying (grain moisture)

Haines (2000) reported that, in the 1960s there was much published research on synthetic pesticides, but in the late 1990s there have been very few studies of synthetic insecticide efficacy. This trend is similar to what obtains in Africa (Table 5). From the late 1990s to date, instead storage pest control research has focused on alternatives, notably botanicals. Within the past five decades, 75% of research on botanical storage pest control in Africa was carried out during the last decade. The recent popularity in botanical research benefited from the well known demerits of synthetic insecticides (Haines, 2000). Botanical research in Africa is predominated by efficacy studies, with little works on mammalian toxicity and commercialization prospects as well as the bioactivity of individual phytochemicals.

Although still wanting, research on biological control involving arthropod predators and parasites in storage pests in Africa has been significant only within the last two decades (Table 5). Compared to the rest of the world, research in this area is not significant (Haines, 2000): The limited research in this area from the continent is dominated by works at international organizations, especially the International Institute of Tropical Agriculture, GTZ and FAO. There is also a paucity of research on inert dust in Africa, especially diatomaceous earth, compared to the rest of the world. These few studies were carried out during the last decade (Mvumi and Stathers, 2003; Demissie et al., 2008)

4. Concluding remarks

The majority of farmers in Africa store grains in traditional granaries which are flawed by structural and functional inadequacies, calling for an improvement of these structures. The process must take into consideration the technologies of the farmers. The farmers will readily accept a concept or technology that builds up or improves that which they are used to rather than one which imposes a totally new idea. For example, the mud rhombus could be replaced by cribs made of brick or concrete blocks and woven baskets by metal bins. Farmers, who already use improved granaries and experienced less pest damage in storage, should be encouraged to convince their friends to do same. The grouping of farmers into cooperatives and the construction of flawless community warehouses should be given priority.

African scientists have been less interested on research concerning the biology and ecology of storage pests, even though less is known about these pantropical species. Such research works are indispensable for the development of sound IPM strategies for such systems.

There is still the use of banned synthetic pesticides by some farmers in different African countries. This practice should be abandoned. As part of their duties, phytosanitory workers should monitor pesticide use in grain storage structures and sensitize the farmers of the dangers involved, for the abandonment to be effective. Most synthetic insecticides are produced in the temperate world where efficacy tests are done. African researchers should engage in field trials of such chemicals under African condition, especially as the effective dose may be lower under tropical conditions, compared to temperate conditions, thus reducing the overall quantity of each pesticide in Africa.

Many scientists in Africa are more concerned with research on determining the insecticidal efficacies of botanicals, while making unverified assumptions about their effects on operators and consumers. Such generalizations are clearly fallacious since many botanicals in crude and pure forms (e.g. opium, nicotine, and curare) have pharmacological, hallucinogenic or acute toxicity effects on humans and other organisms.

Despite the wealth of research on botanicals in Africa, practically no commercial product has emanated from the continent in the past three decades. Instead of broadening the spectrum of the tested plant species, future research should focus on a few plants and insist on their propulsion through the production chain.

More research needs to be focused on diatomaceous earths, both local and imported as these products may prove useful in stored product protection in the dryer areas of Africa.

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Overview of North American stored product research

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Abstract

Major locations for stored product research in North America are in Winnipeg, Manitoba, Canada, and Manhattan, Kansas, USA. Recent personnel changes and research areas are reviewed. One of the pressing research areas in the U.S. is reducing the need for fumigations in flour mills and evaluating alternative treatments. Long-term studies are beginning to show efficacy of better IPM practices, including use of aerosol treatments, for reducing the need for fumigation. Heat treatment as an alternative to fumigation continues to be refined through research. Models have been developed for optimizing heat treatments and fumigations. Studies at grain elevators are optimizing insect management at these large storage facilities, including better sampling methods and computer programs that aid in decision making. Recent studies are beginning to look at insect populations in feed mills and their association with microbes. A number of studies have investigated the biology and control of psocids, which are emerging pests of stored products in the U.S. There have been major research efforts in both Canada and the U.S. to develop better sampling and detection methods, including thermal imaging, automated digital x-rays, molecular methods, and development of better attractants and improved interpretation of trap catches. The red flour beetle, *Tribolium castaneum*, is the first agricultural pest to have its genome sequenced, and mining the genome has produced vast knowledge of various physiological processes that might be exploited for control of this and other pests. Expected future research trends are on aerosol treatments of structural facilities, improved methods for detecting internal insect pests, psocid biology, improved attractants, improved interpretation of trap catches for making pest management decisions, and application of genomic technologies for insect control.

1. Locations for stored product research

The main center for stored product research in Canada is in Winnipeg, Manitoba. Entomologists Paul Fields and Noel White are at the Agriculture and Agri-Food Canada Cereal Research Centre, and biosystems engineers Digvir Jayas and Jitendra Paliwal are at the University of Manitoba. Most of the storage research is at the Canadian Wheat Board Centre for Grain Storage Research on the University of Manitoba campus.

The main center for stored product research in the United States is in Manhattan, Kansas. The Stored Product Insect Research Unit (previously the Biological Research Unit) at the Center for Grain and Animal Health Research (previously the Grain Marketing and Production Research Center), which is part of the United States Department of Agriculture's Agricultural Research Service (USDA-ARS), has seven scientists working on basic and applied aspects of stored-product entomology. These are Frank Arthur, Dick Beeman, Jim Campbell, Paul Flinn, Jeff Lord, Brenda Oppert, and Jim Throne. During the past four years, Tom Phillips moved from Oklahoma State University, where he had faculty responsibility for stored-product entomology, to be Head of the Department of Entomology at Kansas State University, which has a long history of training students in stored-product entomology; George Opit moved from a postdoctoral position at the USDA-ARS Stored Product Insect Research Unit to take Tom Phillips' position at Oklahoma State University; and Dirk Maier moved from Purdue University to be Head of the Department of Grain Science and Industry at Kansas State University. Bhadriraju Subramanyan continues his work on stored-product entomology in the Department of Grain Science and Industry at Kansas State University. Other North American university faculty with major assignments in stored products are Linda Mason at the Purdue University Department of Entomology, Charles Woloshuk at the Purdue University Department of Botany and Plant Pathology, and Carol Jones at the Oklahoma State University Department of Biosystems Engineering. A number of other state, federal, and university researchers in North America have partial appointments in stored products.

2. Research highlights and trends in North America during the last four years

2.1. Reduced use of methyl bromide for structural fumigations

Use of methyl bromide for structural fumigations has decreased steadily in North America since its phase-out was proposed. Most facilities have reduced fumigations from three calendar-based fumigations per year to one or fewer per year. This has been achieved through better pest management practices, such as improved monitoring and sanitation, and through use of aerosols or spot treatments with heat or insecticides. Heat is also used for insect control in some facilities, instead of fumigation. Sulfuryl fluoride is also being used for fumigation of some facilities, rather than using methyl bromide.

Efforts have been made to model structural fumigations to try to improve efficacy. Chayaprasert et al. (2008, 2009) developed a model for structural fumigations, and they used it to investigate fumigation leakage rates from flour mills under different weather conditions. They showed that leakage rates varied within the same facility at different times of the year, indicating that leakage rate should be calculated from sealing factors for the particular facility and using historical local weather conditions for the actual fumigation period. Their results showed that leakage rates for methyl bromide and sulfuryl fluoride did not differ when simulations were run under the same weather conditions for a facility, when the fumigants were applied at the same dosages. Cryer (2008) also found that predicted leakage rates for the two fumigants were similar.

Although aerosol insecticide applications are being used more frequently in flour mills and other structures, there are few recent reports on their efficacy. Toews et al. (2006b) monitored insect populations in an operating flour mill during a 22-month period. They found that insects were almost always captured inside the mill in the first trapping period after fumigation or aerosol application with dichlorvos+pyrethrin, but it wasn't clear if the insects survived treatment or immigrated from outside. Arthur and Campbell (2008) showed that survival of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae), increased when food was provided after treatment with a pyrethrin-CO2 aerosol, emphasizing the importance of sanitation in facility pest management programs. This aerosol treatment seemed to have no long-term effect on resident insect populations in the study warehouse. Arthur (2008) investigated efficacy of synergized pyrethrin and methoprene aerosols for control of the red flour beetle, *Tribolium castaneum* (Herbst), and *T. confusum* in a commercial food storage facility, and in this study found no effect of presence of food on efficacy of the synergized pyrethrin aerosol. *Tribolium confusum* adults were less susceptible to the synergized pyrethrin aerosol than *T. castaneum* adults. Few treated larvae or pupae of either species survived to the adult stage. Few larvae of either species exposed to the insect growth regulator methoprene survived to become adults, and only 13% of Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), larvae embedded in food media and exposed to methoprene survived to the adult stage. Overall, results of aerosol studies show good efficacy of aerosols and good promise for the use of aerosols to reduce the need for structural fumigations.

Much research has been conducted on efficacy of heat for controlling insects in structures, but recent efforts have begun to synthesize these data to provide tools to users for optimizing heat treatments. Boina et al. (2008) developed a model for predicting survival of *T. confusum* during heat treatments. The model was validated with independent data from nine heat treatments in structures, and the model predicted the observed mortality well.

2.2. Insect management at grain elevators

Efforts continue to improve insect management at large, central grain storages. Arthur et al. (2006) sampled grain residues at nine elevators over a two-year period and found that 80% of the insects in grain residues were in the genera Sitophilus and Cryptolestes. Numbers of Cryptolestes in samples increased rapidly in spring, and then remained fairly constant. Numbers of Sitophilus in samples increased through warm months, and then decreased. Pest insects were present in 42% of samples, while beneficial insects were found in 5% of samples. The parasitoid *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) comprised 90% of beneficial insects found. Pest insects were most prevalent in samples taken from elevator boot pits and tunnels. The large numbers of insects in these samples indicate the need for good sanitation around grain storages.

Flinn et al. (2007) extended their previous expert system to aid in pest management decision making for farm-stored grain to be applicable to elevator storages. Users can input current grain temperatures and moistures and numbers of insects from grain samples, and the expert system uses built-in models to make predictions about future insect populations and to recommend management actions. The validity of the expert system was tested in a four-year project at commercial grain elevators. The program failed to identify unsafe storage conditions in only 2 of 399 bins in Kansas, and it correctly identified unsafe storage conditions in all 114 bins in Oklahoma. The number of bins fumigated was reduced because only bins with higher densities of insects were fumigated, rather than treating all bins on a calendar basis. The expert system is being used by a scouting company that was developed as a result of this project. That company contracts with elevators to sample their grain and provide management recommendations.

Insect populations in bins were determined during the elevator study (Flinn et al., 2010). In these bins that were 30-35 meters tall, the lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), and *T. castaneum* made up 44, 36, and 19% of the insects in the top 3.7 meters of grain and 84, 8, and 8% from 3.8 to 12.2 meters, respectively. Few insects were found below 12 meters, so this suggested that sampling the center of the top 12 meters of grain provided the best estimate of insect populations in the bins. Sampling was done with a vacuum sampler. *Cryptolestes ferrugineus* was prevalent near the start of the storage period in June, *C. ferrugineus* and *R. dominica* were found in approximately equal numbers in autumn, and then *R. dominica* was prevalent in winter. Infestation in the bins began at the top and moved down throughout the storage season.

2.3. Insect sampling and detection

The Insector® electronic probe trap is a commercial system for monitoring insects in stored grain by automatically counting the insects falling into traps and identifying the insects falling into the traps to species based on size. Flinn et al. (2009) tested the system in bins of wheat in Kansas to determine accuracy of counts and species identification. They developed models that could be used to convert trap catch to density (insects/kg of grain), and they adapted the Stored Grain Advisor expert system to input trap catches and make management recommendations. Management decisions made using the expert system were similar to those made based on grain samples. However, the accuracy of the species identifications depended on the species involved. The identifications were accurate for C. ferrugineus, but the system could not accurately differentiate *R. dominica* and *T. castaneum* because of their similarity in size. In the same study, Opit et al. (2009a) found that the Insector® system worked well for electronically counting the psocids *Liposcelis decolor* (Pearman) (Psocoptera: Liposcelididae) and *L. entomophila* (Enderlein), although the system can't differentiate psocid species.

Manickavasagan et al. (2006) tested the use of thermal imaging of a small (1.5-m diameter by 1.5-m high) experimental bin of barley to detect hot spots, but found that the method was not accurate. Manickavasagan et al. (2008) tested the use of thermal imaging to detect all stages *C. ferrugineus* inside wheat kernels, and found around 80% accuracy in determining whether or not a kernel was infested.

There have been expanded efforts to use molecular methods to detect insects in samples of grain. Atui et al. (2007) tested the myosin ELISA method originally developed by Quinn et al. (1992) because there were concerns by industry that the myosin breaks down over time if the insects are dead, resulting in lower predictions of insect density than actually present. They found that the myosin level was reduced 58% in the first two weeks after R. dominica died, but that there was no further degradation after that time. Balasubramanian et al. (2007) showed that DNA fingerprinting could be used to detect fragments of *T. castaneum* or *T. confusum* in flour at concentrations of 1% (2 mg of insect parts in 200 mg flour), but not at lower concentrations.

Pearson and Brabec (2007) developed an electronically conductive roller mill that measures electrical conductance as a grain sample is crushed, and can detect fourth instar and older *R. dominica* or the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), in the grain with 83% accuracy based on detection of water in the insects. The accuracy of the method is similar to that for other rapid detection methods, but the method has the advantage that it can process one kilogram of wheat in one minute. Toews et al. (2006a) investigated the use of computed tomography (multiple x-rays taken across a sample) to detect S. oryzae pupae in wheat kernels, and they found 87-94% accuracy of the method for detecting infested kernels, depending on infestation level. Given cost and processing time involved to

detect this relatively large insect stage, the method does not look promising for use by industry at this point. Singh et al. (2009) used near-infrared (NIR) hyperspectral imaging for the detection of wheat kernels damaged by *C. ferrugineus*, *R. dominica*, *S. oryzae*, and *T. castaneum*, and found 85-100% accuracy of the method. Narvankar et al. (2009) used x-rays and automated analysis software to detect infection of wheat kernels by the storage fungi Aspergillus niger, *A. glaucus* group, and *Penicillium* spp., and they were able to differentiate healthy and infested kernels with 93-99% accuracy. Haff and Pearson (2007) used x-rays and automated analysis software to detect granary weevil, *Sitophilus granarius* (L.), larvae in wheat kernels with about 85% accuracy, which was similar to accuracy obtained when the x-rays were examined by lab personnel.

2.4. Insects in feed mills

Little work has been done on insects in feed mills, but insects can be a major problem in feed mills and can potentially transmit diseases in the feed. Larson et al. (2008b) surveyed eight feed mills for a year in the midwestern U.S., and they found that the largest number of insects (38,933) was found in the mill interior, followed by the mill exterior (3776), the load-out area (1068), and the receiving area (620). In the mill, 49% of the insects were *T. castaneum*; and 90% of the insects in the mill exterior, 67% in load-out area, and 38% in the receiving area were the warehouse beetle, *Trogoderma variabile* (Ballion) (Coleoptera: Dermestidae). Species presence depended on the crop type processed and was influenced by the degree of pest management and sanitation practiced. Also, lures for *T. castaneum*, *T. variabile*, and the cigarette beetle, *Lasioderma serricorne* F. (Coleoptera: Anobiidae), were placed in the traps, which presumably influenced the species captured.

Larson et al. (2008a) surveyed enterococci bacteria associated with stored-product insects in feed mills in the U.S. These bacteria are usually not a direct disease threat to humans, but they are considered as reservoirs of antibiotic resistance. A number of enterococci bacteria were found associated with the beetles from the feed mills, and intermediate to complete resistance to the antibiotics chloramphenicol, ciprofloxacin, erythromycin, neomycin, streptomycin, tetracycline, and vancomycin was found.

2.4. Psocids as emerging pests of stored products

Psocids became pests of increasing concern in Australia and China in the 1990's, but were not of concern in the U.S. until the 2000's. In a two-year study of psocids in bins of wheat stored in Kansas, Opit et al. (2009b) found only *L. entomophila* in 2005 and only *L. decolor* in 2006. Psocid density increased quickly after the grain was put into storage in July, and peaked in October before dropping to almost zero in December as temperatures decreased. But, some live psocids were found throughout the winter. Cardboard refuges placed on the surface of the grain or on the hatch and Insector® probe traps were good ways to sample psocids (Opit et al., 2009a). Placing cardboard refuges on the hatch of the bin is probably the simplest and least expensive way to sample psocids, and catches correlated well with numbers of psocids found in grain samples. The numbers of psocids caught using the various methods varied greatly; for example, in 2005 there were 77,502 psocids in the Insector® probe traps, 33,615 in surface refuges, 3395 in hatch refuges, and 547 in trier samples.

The biology of many psocid species has been poorly studied, so there have been efforts to expand our knowledge of biology of psocid species found in the U.S. Opit and Throne (2008b) characterized the population growth and development of the psocid *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae) at constant temperatures. *Lepinotus reticulatus* did not survive at constant relative humidities of 32 to 55%, but populations increased at 75% relative humidity at 22.5 to 32.5°C. Populations declined at 35°C. Population growth of the psocid *Liposcelis brunnea* Motschulsky was similar to that for *L. reticulatus*, except that *L. brunnea* populations could develop from 55 to 75% relative humidity and developed more rapidly at 65% than 75% relative humidity (Opit and Throne 2009).

Opit and Throne (2008a) compared suitability of six grains for development of *L. reticulatus* and *L. entomophila*. Numbers of *L. reticulatus* produced were greatest on oats (175 progeny produced by five females in 32 days) followed by rice (134), barley (120), milo (115), wheat (115), and maize (18). Much larger numbers of *L. entomophila* were produced: wheat (502), barley (490), milo (275), rice (172), oats (154), and maize (146). This may help explain the relative prevalence of *L. entomophila* worldwide. In a similar study, Athanassiou et al. (2010) showed that milo was the most suitable crop for development of Liposcelis bostrychophila Badonnel, *L. decolor*, *L. paeta* Pearman, and *L. entomophila*, followed by

wheat and rice. Maize was the least suitable for all species. They also showed that all four species could develop on whole grain, although progeny production was maximized when some cracked grain was present. In addition, they showed that progeny production was greater on durum wheat than on hard red winter, hard red spring, soft white winter, soft white spring, soft club, soft red winter, or hard white wheats.

Most control technologies for stored-product insects have been developed for beetles, and psocids don't always respond the same as beetles to these control technologies. Recent studies have investigated control technologies that are in common use in the U.S. for control of psocids. Guedes et al. (2008b) showed that *L. entomophila* and *L. reticulatus* are susceptible to heat, although it took about twice as long to kill *L. entomophila*. They investigated the production of heat-inducible proteins when these two species of psocids were exposed to high temperatures, and they found heat-inducible proteins only in *L. entomophila*. This may help explain the more widespread distribution of this species worldwide because these heat-inducible proteins may have a protective function when the insect is stressed.

Athanassiou et al. (2009a) tested all insecticides registered for stored wheat, rice, and maize in the U.S. for effectiveness against psocids, and found that the organophosphate class of insecticides was most effective for psocid control. Effectiveness of methoprene, spinosad, and pyrethrin varied with the grain on which they were tested and with psocid species and stage (egg, nymph, or adult). Efficacy of three diatomaceous earth formulations for control of psocids varied greatly, with adult mortality sometimes exceeding 90%, but progeny production was never reduced more than 39% indicating that diatomaceous earth alone would not be suitable for psocid control (Athanassiou et al. 2009b). Guedes et al. (2008a) tested surface insecticides that are used for structural treatments for control of psocids, and they found that the insecticides ß-cyfluthrin and chlorfenapyr, but not pyrethrins, were effective for control of *L. bostrychophila* and *L. entomophila*. *Liposcelis bostrychophila* was slightly more tolerant than *L. entomophila*, and behavioral studies showed that this may have been due to less movement and that *L. bostrychophila* tended to keep their abdomen raised which may have resulted in less contact with the insecticide. Interestingly, psocids were able to move at a rate of 0.5 cm per second, which is quite rapid for an insect that is only about 1 mm in length.

2.5. Attractants and trap interpretation

Monitoring insects is an important component of an integrated pest management (IPM) program, and this is routinely practiced as part of structural IPM. But much work remains to be done in this area, including improvement and development of attractants and better interpretation of trap catches for pest management decision making. Edde and Phillips (2006a) investigated the impact of male age on rate of pheromone emission in *R. dominica*, which produce an aggregation pheromone, and found that emission was about twice as high in younger males (12-weeks old) than older ones. Edde and Phillips (2006b) investigated the response of *R. dominica* adults to volatiles from different plant materials and found that response was greater to volatiles from wheat than from acorns, cowpeas, peanuts, or dried potato tubers. Males feeding on wheat also produced greater quantities of pheromone (Edde et al., 2007).

Mahroof and Phillips (2007) found that volatiles from *Capsicum* spp. were most attractive to *L. serricorne*, among a number of plant volatiles tested, and mated females exhibited the highest response to these compounds. Mahroof and Phillips (2008) found that *L. serricorne* were equally attracted to three commercial pheromone lures, but more beetles were captured when volatiles from *Capsicum* spp. were included with the lures.

Nansen et al. (2006) investigated ways to improve implementation of traps and interpretation of trap catches of *P. interpunctella*. They investigated response of males in a very simple environment where there was no food or females present except when used as lures. They found that male catches were similar when using concentrations from 1 to 2,000 µg of pheromone, captures of males decreased with distance from the release point, and the greatest numbers of insects were caught within three meters of the release site.

2.6. Genomics

Tribolium castaneum was the first agricultural pest to have its genome sequenced (*Tribolium Sequencing* Consortium, 2008). RNAi has been a useful tool in *T. castaneum* for knocking out genes to determine their functions. Much of the postgenomic work has concentrated on genes involved in cuticle breakdown and synthesis during molting because this is a very vulnerable stage for insects, making it a target for new insect control tools. A number of chitinase genes have been found with varying functions, and these are often specialized to act in a certain part of the body at a certain molt (e.g., Zhu et al., 2008). Another potentially vulnerable process in stored-product insects is water regulation because these insects live in a relatively dry environment, and a number of genes involved in osmoregulation have been identified (Park and Beeman, 2008).

3. Future trends in research

The number of stored product researchers in North America seems to have stabilized, after a decade of reductions. Recently, positions have been filled as people leave them.

Use of aerosol treatments in structural facilities continues to increase, and further studies will be required as new aerosolized insecticides and new application technologies are introduced.

Studies on insect detection technologies will continue because raw grain handlers and processors are very interested in improved detection technologies. Ideally, they would like detection systems that can detect all stages of internal insects automatically, accurately, and quickly, such as processing a 1-kg sample of grain in one minute.

Studies on psocid biology will continue, as there are many species of psocids about which we know little, and the biology of the different species appears to vary greatly. Industry personnel have expressed interest in psocid attractants to monitor and possibly mass trap psocids, and almost nothing is known about psocid attractants.

There is still a need for improved attractants for the major pests of food processing and warehouse facilities, and for better interpretation of monitoring data to aid in pest management decision making.

Although we have learned much about the genes involved in many physiological processes of *T. castaneum*, there is a need to take this technology to the next step of applying genomics for insect control.

✧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.

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The major achievements of grain storage in P. R. China

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Abstract

During the past several decades, China has made great progress in grain storage research, which greatly improved and promoted grain storage techniques and facilities. Especially in the 1990s, modern grain storage depots were constructed, in which four new technologies of grain inspection automation, machinery aeration, grain cooling, and phosphine recirculating fumigation were popularized. The main technologies to phase out methyl bromide in national depots are under-film phosphine recirculation fumigation and mixing fumigation of phosphine and carbon dioxide. In grain storage scientific research, research programs from applied to molecular fields have been implemented in institutes and universities. The 4th national survey of stored grain insects showed 270 species including 44 natural enemies were recorded. Among these stored grain insect pests the psocid, *Liposcelis bostrychophila* is generally more prevalent in stored grain with high moisture content. Hence, bionomics, ecologies, control measures, and molecular biology have being conducted since 1990s, and many achievements have been obtained. Efficacy of new grain protectants such as diatomaceous earth and spinosad against the main stored grain insects were evaluated. Researches on effective concentration of phosphine, application technique, recirculation fumigation, combination phosphine with carbon dioxide, and phosphine resistance mechanism were executed. Other fumigants tested included ethyl formate, carbon disulfide, and sulfuryl fluoride against stored grain insects were evaluated, or reevaluated. The fumigation activities and mechanisms of ethyl formate to the main stored product insects were systemically studied and the results showed ethyl formate controlled Sitophilus oryzae, *Tribolium castaneum*, *Rhyzopertha dominica* and *Liposcelis bostrychophila* effectively in a very short time, and the fumigation efficacy at relatively low temperature was better than that at relatively higher temperature. With the rapid progress of technology and scientific research, the objectives of high quality, high nutrition, high benefit, low waste, low pollution, and low cost in grain storage will be achieved, even during prolonged storage.

Keywords: Review, Grain storage, Insect pest control, Fumigants, Psocids

1. A retrospect of grain storage in China

Chinese grain storage technology has a long history. Research showed that Chinese ancestors began to store grain and other seeds at least 7000 to 10000 years ago (Li and Jin, 1999; Zhu, 2008). Fence-style depots were used to aerate and store grain in southern China 7000 years ago and underground vaults were adopted to store grain in Central China 5000 years ago. Plant-source medicines were utilized to control stored grain insect pests about 2000 years ago. China was one of the oldest cradles of low temperature, controlled atmosphere and fumigation technology (Zhu, 2008). However, few fumigants for grain storage had been used in China before the People's Republic of China was founded in 1949. Most of them were still in the process of experimentation. Since the 1950s, fumigants such as chloropicrin, carbon, bisulfide, methyl bromide, aluminum phosphide, calcium phosphide, cyanhydric acid, dichloroethane and dichlorvos have been put into production and practice one after the other (Wang and Bian, 2004).

During the past 60 years, China has made great progress in grain storage research, which highly improved and promoted the grain storage technique and facilities. The Chinese government has been paying attention to the development of grain storage science and technology. In the middle of 1950s, institutions for grain science and research were set up. In the middle of the 1960s, the Chinese Research Institute of Grain Storage was established and the grain bureaus of various provinces, cities, and autonomous regions also began to set up grain research organizations. Since the 1970s, the research has been made more thorough, especially the 'modern grain storage technique', which was listed in the key

science and technique programs for the 6th to 11th 'five-year plans', and gained great achievements (Li et al., 1999). In the 1990s, China constructed some modern grain storage depots, which featured large-scale grain bulks, high-level grain layers. In these depots, China has popularized four new technologies such as grain inspection automation, machinery aeration, grain cooling, and phosphine recirculation fumigation.

2. Four new technologies and ecological grain storage and environmentally friendly grain storage

Since 1998, with the loans from the World Bank and China's national debt, some modern grain storage depots, which included large warehouse, squat silos and vertical silos, have been constructed. Each single depot capacity ranged from 5000 to 10,000 tonnes. These depots combined modern grain storage technology to popularize four new technologies which were grain inspection automation, machinery aeration, grain cooling, and phosphine recirculation fumigation. The objectives of these four new technologies were to adjust grain physical and chemical properties, to decrease grain temperature, to control mold and insect pest infestations. These newly-constructed depots were demanded to implement "Technical Regulations for Grain and Oil Seed Storage", "Technical Orders for Grain Electronic Monitoring and Analysis System", "Technical Orders for Grain Mechanical Areation", and "Technical Orders for Grain Storage in Large Warehouses". The four new technologies have made Chinese grain storage occupy a forefront place in the world (Zhu, 2008).

Ecological grain storage means fully using and controlling the ecological conditions which are favorable to stored grain quality, such as low temperature, low oxygen, therefore, ensuring safe grain storage. Environmentally friendly grain storage means that technologies leave no residues on stored grain. The two concepts are consistent with each other (Zhu, 2008). The theoretic principle for environmentally friendly grain storage is stored grain ecology, especially the stored grain ecosystem. Integrated pest management (IPM), which focuses on controlling ecological factors in a stored grain ecosystem, is the fundamental technology of environmentally friendly grain storage (Jiang, 2002). In China, the major technology for ecological environmentally friendly grain storage is low temperature storage combined with controlled atmosphere storage in the suitable climatic zones.

Low temperature grain storage means that average temperature of a grain bulk in the depot is below 15ºC and the highest temperature is not over 20°C in China, whereas quasi-low temperature storage means that the average temperature of grain bulk is below 20ºC with highest temperature not over 25ºC. The major methods to obtain low temperature and quasi-low temperature are natural low temperature, machinery aeration, and grain cooling. Controlled atmosphere technologies include natural low-oxygen, application with deoxidizers, double-low and three-low storage, controlled atmosphere with carbon dioxide and nitrogen enriching, bin sealing materials and technologies. China has made great progress in controlled atmosphere grain storage. In 2000, the Sichuan Mianyang CO2 controlled atmosphere grain depot was completed. Since then, some other CA grain depots have been constructed (Zhu, 2008).

3. Methyl bromide alternatives

Due to destruction of the ozone layer in the atmospheric stratosphere, and an overall negative effect on living conditions for all mankind on Earth, methyl bromide (MB) has been labeled an ozone deleting substance and is restricted or banned for application by the United Nations Environment Program. The Chinese Government attached great importance to methyl bromide substitute technology research. The Chinese government approved the Copenhagen Amendment of the Montreal Protocol. Since December 31, 2006, MB has not been allowed as a fumigant by any grain depots in the Chinese grain storage industries. Since January 2007, MB was completely prohibited in Chinese grain storage industries, in order to make proper contribution for carrying out the amendment of the Montreal Protocol completely in China. By the year 2015, MB should be phased-out completely except for use in quarantine fumigation. The substitute technology of the industry has been confirmed. The situation of methyl bromide application in grain industry has been grasped. For stored grain protection, tarped phosphine recirculation fumigation and phosphine and carbon dioxide mixing fumigation were confirmed as the main alternative technology to phase out methyl bromide (Liang, 2005; Li et al., 2007).

4. Stored-grain insect fauna and Chinese grain storage ecological zone

Four national surveys of stored-grain insects were conducted from 1955 to 2005. The 1st national survey started in 1955 and ended in 1958. One hundred and nine species of insect pests and 2 species of natural enemies were found. Between 1974 and 1975, the 2nd survey was implemented and 126 insect pest species and 6 natural enemy species were found. Between 1980 and 1982, the 3rd survey found 161 insect pest species and 10 natural enemies. In the 4th survey, 270 species of stored grain insects (including 226 species of insect pests and 44 species of natural enemies) were recorded (Yan et al., 2008). In the conventional two-low (low oxygen and low pesticide storage environments) national depots of Chongqing municipality and Sichuan Province, the investigations showed that the mite Tyrophagus putrescentiae (Schrank) and the psocid Liposcelis bostrychophila Badonnel were the two dominant species (Deng et al., 1995). By means of GJ89 type of probe trap, investigation and analysis of community composition and structure of stored grain insects in newly-constructed large warehouses, which were equipped four new grain storage technologies, were carried out in Beibei State Grain Reserve Depot, Chongqing. The results showed the main pests were *T. putrescentiae*, *L. bostrychophila*, *Cryptolestes ferrugineus* (Stephens), and *Sitophilus zeamais* Motschulsky in large warehouse and the dominant species was *L. bostrychophila*. The occurrence numbers of the four kinds of stored grain insect pests were the most at 0-50 cm grain depth, but a few were common at 100-150 cm grain depth. The four indexes of population richness, dominance, diversity and evenness were higher at 0-50 cm deep than the other depth. The occurrence of stored grain insects at horizontal levels had no obvious difference (Cui et al., 2006).

During the national survey of stored grain insects, bionomics and control for the main stored-grain insect pests including *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Tribolium confusum* Jaquelin du Val, *Sitotroga cerealella* (Olivier) were researched. Southwest University (the former Southwest Agricultural University) in Chongqing also did a lot of surveys on stored grain mites. Especially for stored food mites, systematic investigations were conducted and 79 species of food mites, including 18 new species and 21 species of new Chinese records, were found. The research on ecology and control measures for T. putrescentiae showed essential oils combined with controlled atmosphere could obtain satisfactory control (Li et al., 2009). Chinese scientists also summarized a stored grain ecosystem theory to guide grain storage and depot construction. It included: reasonable partition of Chinese grain storage ecological zones; reasonable depot type selection and design in different zones, grain depot equipment in different depot types and zones, rational configuration of machinery and special safety-ensuring grain storage equipment for different depot types and zones; evaluation indices and systems on safe grain storage technology (Zhu, 2008; Li et al., 2009).

5. Psocids

In the last two decades, a series of papers about psocids have been published about their bionomics (Dong et al., 2007; Jiang et al., 2008; Wang et al., 2009), ecology (Wang et al., 2004), control measures (Wang et al., 2001), and molecular biology (Wang et al., 2006; Dong et al., 2007; Jiang et al., 2008; Tang et al., 2009) by Key Laboratory of Entomology and Pest Control of Chongqing Municipality (Southwest University, China). The main differences between *L. bostrychophila* and *Liposcelis entomophila* (Enderlein) about biochemical and toxicological characteristics of detoxification, protection, target enzymes and energy sources were clarified (Wang et al., 2004; Cheng et al., 2004; 2007). The activity of carboxylesterase (CarE) extracted from *L. entomophila* was significantly higher than that from *L. bostrychophila*, while for acid phosphatase (ACP), alkaline phosphatase (ALP) and glutathione S-transferases (GSTs) (Cheng et al., 2007), *L. bostrychophila* had more activities. To inhibition by dichlorvos, acetylcholinesterase (AChE) and CarE played the most important roles, followed by GSTs or superoxide dismutase (SOD) (Cheng et al., 2007). Besides, on the basis of GSTs (Dou et al., 2006, 2009) and AChE (Xiao et al., unpublished) purification of psocids (*L. bostrychophila*, *L. entomophila*, *Liposcelis paeta* Pearman) through the affinity chromatography method, the biochemical and toxicological characterizations of purified enzymes from different resistant strains and field populations were systematically analyzed. Compared to the susceptible strain, the specific activities of GSTs in resistant strains were significantly higher. The comparison analysis of in vitro inhibition of insecticides revealed that compared to the counterparts in resistant strains GSTs from susceptible strain were most sensitive and sensitivity difference to inhibitors existed for GSTs in resistant strains.

At molecular levels, gene cloning of detoxification and target enzymes (such as P450, AChE, CarE, etc.), further mRNA expression level and heterogeneous expression of functional genes were carried out, mainly from *L. bostrychophila*, *L. entomophila*, *Liposcelis decolor* (Pearman), and *L. paeta* (Jiang et al., 2008; Tang et al., 2009). To date, two full length cDNAs encoding AChE were all cloned from *L. bostrychophila*, *L. entomophila*, *L. decolor*, respectively, by the methods of RT-PCR and RACE. The mRNA expression levels of two ace genes from *L. bostrychophila* in different strains, development stages, and insecticide treatments were studied using Real Time PCR. The results showed that the expression levels of two ace genes in resistant strains were significantly higher than those of a susceptible strain. After treated by dichlorvos or phosphine, the expression levels of two ace genes were all significantly increased over the control. The highest expression level of two ace genes was detected at the second stadium and the lowest was at the first stadium and adult stage. The Real Time PCR determination of ace genes from other psocids has also been conducted. Furthermore, two full length cDNA encoding CarE were cloned from L. bostrychophila by the methods of RT-PCR and RACE, named Lb est1 (GenBank Accession No.: EU854151) and Lb est2 (GenBank Accession No.: EU854152). The followed Real Time PCR analysis showed that the expression level of Lb est2 in dichlorvos and phosphine strains were 1.91 and 1.42 fold- higher than that of susceptible strain, respectively. For P450, a number of genes from CYP4 and CYP6 families were cloned and their mRNA expression levels were studied by real time PCR among different developmental stages (strains or treatments by insecticides). Meanwhile, some housekeeping genes were also cloned gradually from different psocids species. These results not only fastened the acquaintance of psocid molecular toxicology and enriched the content of evolution and genetic variation, but also contributed to development of a molecular diagnostic technique for psocid resistance in the field and paved the way for designing new insecticides and developing new strategies for pest management.

6. Grain protectants

Grain protectants are applied either as a liquid or a dust to specific areas or stored grain to suppress insect populations for a period of time ranging from months to a year or more. Grain protectants have been thoroughly researched since 1950s. During 1970s and 1980s, some protectants including malathion, fenitrothoin, pirimiphos-methyl, deltamethrin and mixture of organophosphorous with deltamethrin were studied as grain protectants. In China, grain protectants which are being used at present include: malathion, fenitrothion, deltamethrin, piperonyl butoxide, mixture of malathion and deltamethrin, mixture of fenitrothion and deltamethrin, and pirimiphos-methyl.

Spinosad is a commercial biological insecticide produced by fermentation culture of actinomycete Saccharopolyspora spinosa. To evaluate the effect of spinosad in controlling stored grain pests, the touch effects of 2.5% spinosad were tested in the laboratory on three main stored grain insects *L. entomophila*, *Oryzaephilus surinamensis* (L.), and *S. oryzae*. Results indicated that spinosad is effective in pest control for the three insects, more effective for *L. entomophila* than the other two insects (Cao et al., 2007).

7. Fumigants

Fumigants are toxic gases which penetrate into stored grain. Maintaining an adequate concentration of a fumigant for enough time kills most insect pests. The most popular fumigant in China is phosphine (PH3). A lot of research has been done since the 1960s. It included: PH3 efficiency, factors affecting PH3 efficacy (pest species, pest developmental stages, temperatures, grain absorbability to PH3, warehouse sealing condition, and airflow in grain bulks), gas diffusion, PH3 toxicity to human being, mixed fumigation with CO2, operational methods of different PH3 fumigation techniques (PH3 + CA fumigation, airflow fumigation, intermittent fumigation and slow releasing fumigation), dispensing apparatus, and combustion condition and prevention. Other fumigants involved were ethyl formate, carbon disulfide, carbonyl oxysulfide, ethylene oxide, and sulfuryl fluoride against stored grain insects and they were tested, evaluated, or reevaluated.

Since the 1970s, sulfuryl fluoride has been widely researched by Chinese scholars (Xu et al., 2006) and was registered for fumigating such materials as wood, official files, books, embankments and buildings, and was already taken into application in more ranges of buildings and quarantine ministries. Due to the notable pharmacodynamic action and excellent efficacy against stored products insect pests such as *Trogoderma granarium* Everts, *S. oryzae*, *S. zeamais*, *Tribolium castaneum* (Herbst), *Sitophilus granarius* (L.), *Callosobruchus chinensis* (L.), and *Lasioderma serricorne* (F.), temporary registration

certification has been approved to take into practice in grain (Li et al., 2008). Ethyl formate (EtF) is a promising and environmental friendly fumigant, which was registered as a dry fruit fumigant in 2002 in Australia. In China, the fumigation activities and mechanisms of ethyl formate to main stored product insects were systemically studied and the results showed that ethyl formate controlled *S. oryzae*, *T. castaneum*, *R. dominica*, *L. bostrychophila* and *L. entomophila* effectively in a very short time period, and the fumigation efficacy at relatively lower temperature was better than that at relatively higher temperature (Tang et al., 2006 a b; Li et al., 2006; Deng et al., 2008).

8. Plant materials and essential oils

The bioactivities of plant materials and essential oils as fumigants, repellents, antifeedants, and insect growth inhibitory agents were researched because of pesticide residue and insect pest resistance to traditional pesticides. Fumigant toxicities of nine essential oils including *Citrus tangerina*, *C. limon*, *C. hongheensis*, *Litsea cubeba*, *Mentha spicata*, *Pinus tabulaeformis*, *Cinnamoum camphora*, *Melaleuca alternifolia* and *Eucalyptus globules* on adults of maize weevil, *S. zeamais* were investigated and the results proved that fumigation efficacies of the essential oils of *C. camphora*, *M. alternifolia*, *Citrus limon*, *M. spicata*, and *P. tabulaeformis* were better than those of the other four essential oils. Among the tested essential oils, *C. camphora* oil gave the best fumigation toxicity (Deng et al., 2004). The fumigant toxicities of the nine plant essential oils against adults of R. dominica showed that four plant essential oils of *E. globules*, *C. limonum*, *M. alternifolia* and *C. tangerina* resulted in higher adult mortality of *R. dominica* and *E. globules* oil obtained the best efficacy (Zhang et al., 2004). The population inhibition, repellency activity and contact toxicity of nine plant essential oils showed that *C. camphora* and *E. globules* were most effective (Zhang et al., 2004). The fumigation activity, contact toxicity, repellence activity, attractant activity and population inhibition effect of several prickly ash extracts against the adults of *S. zeamais*, *T. castaneum* and other stored-product pests were also systematically studied (Nie et al., 2006; 2007).

Although great progress has been achieved in China's grain storage, some new problems including farmer storage loss reduction, high moisture grain in Northern China, environmentally friendly storage and transportation need to be addressed. In the future, China should strengthen its scientific research and upgrade its grain storage technology. We believe the objectives of high quality, high nutrition, high benefit, low waste, low pollution, and low cost in grain storage will be achieved with the rapid progress of technology and scientific research in China.

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Challenges and characteristics of the South American grain and oilseed postharvest system

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Abstract

Concerning grain production, South America is divided in two main regions: 1) the Mercosur region (Argentina, Brazil, Uruguay and Paraguay) which produces more than 250 million tonnes of grains and oilseeds, and 2) the Andes Mountain region countries, which are net importers of these products. The main challenges related to grain postharvest that South America is facing are to minimize the quality and quantity losses; improve the food safety; enhance the capability for segregation and traceability of identity preserved (IP) grains; and incorporate technology to maintain the overall efficiency of the postharvest system. Among the critical points affecting the efficiency of the system are the shortage of permanent storage capacity; large storage structures which affects the segregation of IP grains; deficient transportation system (roads and railroads); poor management of integrated pest control system; and unsatisfied demand of formal and informal education in suitable grain postharvest technologies and practices. However, the region remains highly competitive in producing and delivering food for the rest of the world and it has demonstrated high capacity for incorporating cost efficient grain handling technologies. As a result, one of the main changes in the region was the appearance of the silobag system for temporary storage of dry grain and oilseeds. Each silobag can hold approximately 200 tonnes of wheat and with the available handling equipment is quite simple to load and unload. During the 2008 harvest season, more than 33 million tonnes of grain were stored in these plastic bags in Argentina (including corn, soybean, wheat, sunflower, malting barley, canola, cotton seed, rice, lentils, sorghum, beans and even fertilizers). The silobag technology is also being adopted not only in neighbor countries, but also in countries around the world such as the USA, Mexico, South Africa, Australia, Russia and Ukraine, among others.

Keywords: Logistic, Transportation, Storage Capacity, Hermetic Storage

1. Introduction

South America is one of the most productive regions of food for the world. The top six commodities produced in South America are soybean, rice, wheat, maize, dry beans and sunflower seeds. In 2007, the total production of these six commodities was higher than 250 million tonnes (FAO, 2010). The grain production in South America is mostly located in the countries conforming the economic block called "Mercosur" (Argentina, Brazil, Uruguay and Paraguay). These countries produce more than 230 million tonnes of the mentioned commodities, which represents about 92% of the total production of South America, and 10% of the world production (Table 1). The rest of the South American countries are net importers of these products, although in some of them, grain production is a fairly common practice.

Table 1 World, South America and Mercosur (Argentina, Brazil, Uruguay and Paraguay) production of soybean, rice, wheat maize, beans and sunflower seeds and percentage of the share for Mercosur production in South America and in the world (FAO, 2010).

Commodity	World	South America	Mercosur		
	(million tonnes)	(million tonnes)	(million tonnes)	% of World	% of South America
Soybeans	220	114	112	51	98
Rice, paddy	657	22	13	2	61
Wheat	611	24	22	4	94
Maize	788	85	76	10	90
Beans, dry	20	4	4	18	91
Sunflower seed	26	4	4	14	92
Total	2322	252	231	10	92

Brazil and Argentina, the two main countries in the Mercosur, contribute 56 and 39% of the total production of the common market, respectively. Both countries produce soybean in similar proportions (52 and 42% for Brazil and Argentina, respectively). Brazil is the main producer in the region for rice, maize and dry beans, while Argentina is the main producer for wheat and sunflower seeds. Paraguay and Uruguay contribute 4 and 1% of the total production of the economic block, soybean being the main product in Paraguay and rice in Uruguay (Table 2).

Table 2 Production and percentage of participation of soybean, rice, wheat, maize, beans and sunflower seeds of the four member countries of the Mercosur (Argentina, Brazil, Uruguay and Paraguay) (FAO, 2010).

Commodity	Brazil		Argentina		Paraguay		Uruguay	
	(million tonnes)	(%)	(million tonnes)	(%)	(million tonnes)	(%)	(million tonnes)	(%)
Soybean	57.9	52	47.5	42	6.0	5	0.8	1
Rice, paddy	11.1	82	1.1	8	0.1	1	1.1	9
Wheat	4.1	19	16.5	75	0.8	4	0.7	3
Maize	52.1	68	21.7	29	1.9	2	0.3	0
Beans, dry	3.2	89	0.3	9	0.1	2	-	0
Sunflower seed	-	0	3.5	94	0.2	5	0.1	1
Total	128.3	56	90.6	39	9.1	4	3. 0	1

The predicted population of the Mercosur countries and all of South America in 2010 is about 244 and 392 million people, respectively (UN, 2010). This implies that South America, and the Mercosur in particular, have one of the highest rates of food production per capita (0.64 and 0.95 tonnes, respectively, (Table 1)). Table 3 shows a list of nine grains and agricultural products derived from grain produced in South America. The sub-continent, through the Mercosur countries, export 30% of the total world trade of these commodities (about 129 million tonnes), being soybean and its related products (cake of soybean and soybean oil) the commodities in which South America participate with the highest percentage. In terms of total tonnes exported, maize and wheat also are important products (Table 3). This manuscript will focus on maize and wheat, especially in Argentina and Brazil, due to the higher importance of these grains in the Mercosur countries

Table 3 Total world trade (tonnes) and South American participation (tonnes and percentage) of the main exporting commodities of South American countries (FAO, 2010).

Commodity	World	South America	
	tonnes	tonnes	%
Wheat	132.8	10.1	8
Soybeans	74.4	39.9	54
Maize	109.7	28.2	26
Cake of Soybeans	61.4	40.4	66
Rice Milled	27.4	1.0	4
Soybean oil	12.4	9.2	74
Sunflower Cake	4.0	1.0	24
Sunflower oil	4.7	1. 0	21
Sunflower seed	3.1	0.1	4
Total	429.0	128.9	30

2. Main challenges of the postharvest system

2.1. Shortage of storage capacity

In Argentina, a report from National Institute of Agricultural Technologies (INTA PRECOP, 2008) indicated that the total permanent storage capacity in Argentina can be estimated at 73 million tonnes, of which 25% are on farm, 11% in ports, and 64% in commercial elevators, cooperatives and industry. Almost 100% of the storage capacity is for handling grain as bulk.

The 2007-2008 harvest season had an historic peak of production of more than 95 million tonnes. This implies a shortage of permanent storage capacity of about 22 million tonnes (storage capacity/production ratio: 0.77). This gap between the total production and the permanent storage capacity is partially covered with temporary storage capacity (silobags). The expectation is that Argentina will continue to

increase the production beyond the 100 million tonnes in the near future, thus, it will be a challenge for the country to catch up with the investment in permanent storage capacity.

According to the National Supply Company (CONAB), the permanent storage capacity for grains in Brazil in 2008 was 128.5 million tonnes, of which 80% are for bulk storage and 20% for bagged product. Most of the new investment in permanent storage capacity is for handling grain as bulk (Deckers, 2009). Brazil used to have a balanced ratio between storage capacity and production (storage capacity/production ratio: 1). However, in the last 15 years the production started to increase at a higher pace than the investment in new storage capacity. Today, the total grain production in Brazil is 140 million tonnes, which results in a deficit of 12% for the storage of grains, not including coffee and sugar (Deckers, 2009).

A document from the Brazilian government indicates that the expectation for Brazil is to increase the production to 180 million tonnes by 2018. This will create some pressure in the system to incorporate extra permanent storage capacity. Another characteristic of the Brazilian grain storage system is that only 15% of the permanent storage capacity is located on-farm. A significantly lower proportion, compared with other important grain producing countries (Deckers, 2009).

2.2. Postharvest losses

Even when there was not a rigorous study for South American countries, the postharvest losses were estimated in Argentina at about 10% of the total grain production (INTA PRECOP, 2008). According to the Ministry of Agriculture of Brazil in the process of grain storage the losses are around 10% of the Brazilian crop. These numbers seems to be higher than reasonable, especially for Argentina where the temperate weather should reduce the impact of pests. However, every year both countries have to face higher than acceptable losses according to the current technology available for the grain postharvest operations.

In Uruguay, the level of losses should be similar to Argentina, whereas in Paraguay and Bolivia, due to the warmer weather conditions and the lower level of technology applied, the losses should be higher.

2.3. Grain segregation capacity

The great expansion of agriculture in South America was related to the rapid increase in production of the soybean. As shown in Table 2, almost half of the grain production in Brazil and more than half in Argentina is soybean.

The soybean production does not require segregation since it is mostly commercialized as grade number 2. As a result, the postharvest system was oriented towards prioritizing the low cost-high efficient handling of few grains. The recently installed storage units have rather large storage capacity, making them not suitable for segregation of small amounts of different grains. Additionally, the amount of on-farm storage capacity is much lower than in countries that have well established segregation programs, such as Canada, Australia and USA.

2.4. Transportation and logistics

Recently, the Inter-American Development Bank made a study that concluded that in general, for Latin American and Caribbean countries, the impacts of the inefficiency of transportation costs on exports are much more significant than the protectionist surcharges in major markets. A survey, conducted by the National Confederation of Industry (CNI) of Brazil with the exporters, identified the high costs of freight transport and logistics services as the main obstacle to be overcome by companies (Deckers, 2009). A study performed by the Rosario Board of Trade reached similar conclusions for Argentina (Bernasconi, 2009).

One distinctive characteristic of South America is that most of the grain is transported by truck, although the region has one of the most extended waterways of the planet (Amazon, Paraná and Paraguay, San Francisco and Tietê-Paraná and Uruguay). It seems reasonable that in the future South American countries should increase the transportation through the waterway system, since it is substantially more efficient and less expensive compared to the highway system.

The railroad system is highly efficient in transporting grain and other goods when the distances are large. However, the total railroad system for Argentina is only 28 000 km, and 29 706 km for Brazil, almost the same size it was 80 years ago.

In South America the rural road system is not paved, creating logistic problems during harvest time due to the poor maintenance of the roads, delaying the harvest and the transportation of the grain from the farm to the elevator.

The direct consequences for the critical situation of the South American road system are the low productivity, low average speed, high fuel consumption, accelerated wear of the fleet and high rate of accidents involving deaths. Another characteristic is that the truck fleet is rather old, increasing the inefficiency of the system.

There are some distinctive characteristics regarding to the logistics in the different South American countries, especially regarding to the distance between the production regions and the ports (Table 4).

Table 4 Transportation matrix for Brazil, Argentina and the USA, and average distance from production area to the port.

Mode	**Movement of grain by mode (%)**		
	Brazil	**Argentine**	**USA**
Waterway (%)	5	2	61
Railroad (%)	28	16	23
Highway (%)	67	82	16
Average distance to port (km)	900 to 1000	250 to 300	1000

Argentina has its main exporting ports located on the Parana River, close to Rosario city, where the ocean-going vessels are loaded. Along the ports are also located the most important oil crushing complexes of the country. This area is the heart of the most productive region of Argentina (soybean and corn), so a large proportion of the production is transported only a few kilometers from the farms to the final destination (ports or processing factories).

Brazil has 30 sea ports and 10 river ports (interior), operated by private companies. However, the productive regions are located far away from most of the important ports, with the average travel distance between the production areas and the ports being large (900 to 1000 km on average) (Deckers, 2009).

Uruguay exports most of its grains through Nueva Palmira port located on the Uruguay River (245 km west of Montevideo) or the ports in the south of Brazil. The average transportation distance in Uruguay for exporting grain is relatively short. On the other hand, Paraguay and Bolivia need to transport the grain considerably further distances from the production areas to the exporting ports located on the Parana River, close to Rosario city in Argentina. There is a barge system in the Parana River, that transports the grain from the North of Argentina to the ports in Rosario area. This barge system is also used for transporting the grain (mostly soybean) from Paraguay and Bolivia. However, a significant proportion of the distance is covered by truck, increasing transportation costs.

2.5. Technology applied to postharvest operations

The technological level of the grain postharvest system in the Mercosur countries (especially in Argentina, Brazil and Uruguay) is considerably higher than in the rest of the South American countries. Brazil and Argentina have a competitive and traditional industry that manufactures equipment for grain handling, drying, storing, monitoring and processing. This industry not only satisfies the internal demand of equipment, but also exports cost competitive technology to the rest of the world. However, it can be appreciated there is a non-uniform incorporation of technology across the postharvest system. Usually, farmers have a lower level of technology in the postharvest system in Mercosur countries, when compared to farmers in more developed countries. This difference is less noticeable when comparing large grain elevators, the grain processing industry or exporting ports.

In the out-lying areas of Mercosur countries the level of technology in the grain postharvest operations is rather poor, with the exception, in some cases, of the grain-processing industry (i.e. wheat milling).

In general, the poor management regarding to pest control leads to a non-appropriate and inefficient use of pesticides, with phosphine fumigation being the main tool for insect control. IPM programs should be more widely implemented in the South American grain postharvest system in the near future.

2.6. Education of personnel

An important limitation in the South American countries is the poor education of the personnel related to grain handling operations. In most cases, the personnel have to acquire expertise at work, where the experience is transmitted from generation to generation in an informal fashion. With the exception of Argentina and Brazil, there are few possibilities for formal professional education in grain handling, storing and processing operations. In Brazil, the EMBRAPA (Brazilian Consortium of Agricultural Research) and some universities have programs for professional education in grain postharvest operations. In Argentina, the professional education is covered by INTA and APOSGRAN (Argentine Grain Postharvest Association), which recently launched a distance learning program (entirely in Spanish) for grain handling in the postharvest system, especially designed to make up for this lack of knowledge in this area in Latin American countries.

In Argentina and Brazil, a few universities offer a graduate degree in grain postharvest operations, a sort of "specialization" type of title.

3. Distinctive characteristics

3.1. Expanding agricultural frontier

The Mercosur countries have undeveloped land areas for agriculture, so the agricultural frontier is moving far away from ports and traditional urban areas. The FAO statistics (FAO, 2010) shows that Argentina only has 30% of its arable land under production, Brazil 9.2%, Paraguay 10.5% and Uruguay 9.2% (Table 5). Although it is unreasonable that all the potentially arable land could be turned into production because of practical, political and ecological reasons, it is clear that the Mercosur countries can substantially increase their production in the near future by incorporating new areas for agriculture. However, the lack of infrastructure such as roads, communication, storage capacity and the large distance to the ports and urban areas makes the production and marketing cost increase compared to traditional agricultural areas. Another important limitation is the lack of personnel with experience in grain handling, since there was not a tradition of that kind of activities in the new agricultural areas.

Table 5 Total area of the country, potential arable land and percentage of potential arable land in use for the Mercosur countries (Argentina, Brazil, Uruguay and Paraguay) (FAO, 2010).

Country	Total area (1000 km²)	Potential arable land (1000 ha)	Potential arable land actually in use (%)
Argentina	2780	90571	30.0
Brazil	8563	549389	9.2
Paraguay	400	21589	10.5
Uruguay	179	14245	9.2

3.2. Storage in hermetic plastic bags: silobags

As mentioned before, Argentina has a permanent shortage in storage capacity that, according to the harvest, could be from 20 to 35 million tonnes. Due to this insufficient storage capacity, an important proportion of the Argentine grain production had to be delivered directly from the field to the regional grain elevators and from there to the terminal ports.

To overcome these unfavorable circumstances, a new storage technique has gained popularity among farmers. It consists of storing dry grain in hermetically sealed plastic bags (silobags). Each bag can hold approximately 200 tonnes of grain and with the available handling equipment is very easy to fill. Local companies also developed machineries to unload the plastic bag transferring the grain directly to the truck or wagon. The new generation of high capacity combines found in the silobag system the ideal partner, since the loading capacity of the bagging machine is basically limited to the transportation capacity between the combine and the place where the bag is filled. Another advantage of the silobags is that they can be easily incorporated into a grain identity preservation (IP) programs. Silobags can be set up in the field, right next to the crop, reducing contamination risks of the specialty grain with other commodities.

In the 2008 harvest season, more than 33 million tonnes of grain were stored in these plastic bags in Argentina (including corn, soybean, wheat, sunflower, malting barley, canola, cotton seed, rice, lentils, sorghum, beans and even fertilizers). The silobag technology is also being adopted not only in neighboring countries (Uruguay, Paraguay, Bolivia, Brazil and Chile) but also in countries around the world such as the USA, Mexico, South Africa, Australia, Russia and Ukraine among others.

The INTA has been conducting research on storage of grain and oilseed in hermetic plastic bags since the year 2000. The system has proven to work efficiently for storing quality corn, wheat, soybean and sunflower during at least six months (Bartosik et al., 2008a). Currently, research is being conducted storing rapeseed, malting barley, cotton seed, paddy rice and dry beans in hermetic plastic bags (INTA PRECOP, 2009).

A novel grain quality monitoring system was also developed for the silobags and implemented by farmers, grain elevators and the industry. The system measures the CO2 concentration inside the bag and relates it to the biological activity, allowing sorting the bags according to a storage risk factor (Bartosik et al., 2008b). This technology will help in the adoption of this novel storage system.

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Section: Biology, Behaviour and Detection

Implications of the *Tribolium* genome project for pest biology

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Abstract

The universal availability of the complete *Tribolium castaneum* genome sequence assembly and annotation (Richards et al., 2008) and concomitant development of the versatile *Tribolium* genome browser, BeetleBase (Kim et al., 2010, http://beetlebase.org/) open new realms of possibility for stored product pest control by greatly simplifying the task of connecting biology and behavior with underlying molecular mechanisms. This genome has enabled sequence similarity searches that have resulted in a flood of new discovery involving thousands of genes with important functions in digestion, osmoregulation, metamorphosis, olfaction, xenobiotic metabolism, vision, and embryonic and larval growth and development. The value of the *T. castaneum* genome sequence is greatly enhanced by the availability of a sophisticated functional genomic toolkit for laboratory studies of this insect. These tools include high-resolution physical and genetic maps, genomic and cDNA libraries, balancer chromosomes, and effective and reliable techniques for specific knockout of any target gene *via* RNA interference (RNAi). In this paper we briefly discuss just two areas of *Tribolium* biology research that are being revitalized by the availability of the genome sequence, namely olfaction and exoskeleton, or "smell and skin".

1. Pheromone biology, olfaction and genomics

Many common stored product beetles are long-lived as adults (weeks or months) and use male-produced aggregation pheromones to attract both sexes for mating and for achieving critical population densities for effective conditioning of the microhabitat (Phillips et al., 2000). Examples include species of *Tribolium*, *Tenebrio*, *Sitophilus*, *Cryptolestes*, *Oryzaephilus*, *Rhyzopertha,* and *Prostephanus*. In contrast, adult beetles with female-produced sex pheromones, including stored-product pests in the families Anobiidae, Bruchidae and Dermestidae, tend to be short-lived (days to weeks), and may require only nectar for sustenance. In *Tribolium confusum* (Duval) and *T. castaneum* (Herbst) the predominant male-produced aggregation pheromone is 4,8-dimethyldecanal (DMD; Suzuki, 1980), which has two chiral carbons whose configurations affect biological activity (Levinson and Mori, 1983; Suzuki and Mori, 1983). A 4:1 mixture of the (4R,8R) and (4R,8S) diastereomers elicits a near-optimal attractive response, showing more than ten-fold greater attractive potency than either the corresponding 1:1 blend or the pure (4R,8R) isomer (Suzuki et al., 1984). Since a 1:1 blend of (4R,8R):(4R,8S) is presumably the recipe used in commercially available Tribolure, there is potential for development of a much-improved blend that could be a powerful research tool in studies of *Tribolium* population biology.

As the name implies, this pheromone may function only for aggregation, while other semiochemical cues might be needed to evoke mounting and copulation. The latter idea was first suggested by Keville and Kannowski (1975), who found evidence that 1-pentadecene and other hydrocarbons elicit copulatory behavior in *Tribolium confusum*. It has been observed that, in contrast to the highly effective *Rhyzopertha dominica* (F.) aggregation pheromone, the synthetically-produced *Tribolium* aggregation pheromone (Tribolure) is a relatively weak attractant, to the extent that it is not useful for mass-trapping of large numbers of beetles. Tribolure-baited traps are, however, extremely useful for population monitoring. The possibility that Tribolure contains an unnatural or nonideal blend of diasteriomers, reducing its effectiveness, has already been mentioned, but the relatively low attractiveness of Tribolure-baited traps might also be a function of the behavior and ecology of *Tribolium*. In addition, it has been

suggested recently (Verheggen et al., 2007) that minor or trace components of a natural *Tribolium* aggregation pheromone blend might exist and might be critical for maximum activity. Candidate trace constituents include several benzoquinone and hydrocarbon secondary metabolites that are known to be produced in *Tribolium* spp. For example, in *T. confusum* two different 1, 4-benzoquinones and several mono-unsaturated hydrocarbons, previously detected in several *Tribolium* species (Howard, 1987; Markarian et al., 1978), were shown to be attractive in behavioral assays and elicited electroantennagram (EAG) responses in isolated antennae (Verheggen et al., 2007). These include some of the same components previously reported by Keville and Kannowski (1975) to be active in eliciting mating behavior. No one has yet reported on attempts to increase the potency of synthetic Tribolure by creating new blends that incorporate these candidate components as minor constituents, but this would seem to be worthy of investigation. It has been observed that Tribolure-baited traps become more attractive after a number of beetles have been captured, perhaps because they are releasing important minor components not included in the commercial pheromone blend (Jim Campbell, unpublished observations).

Chemical, physiological and behavioral studies have revealed much useful information about the olfactory and pheromonal biology of stored-product insects, but, as highlighted above, fundamental questions remain unanswered. The recently completed genome sequence of the red flour beetle, *T. castaneum*, opens a window to a vast, untapped reservoir of opportunity for gaining new knowledge about many aspects of the biology of this pest species, including the biology of olfaction. Establishing or confirming pheromone and secretome biosynthetic pathways (e.g. Kim et al., 2005) will be facilitated by the availability of the genome sequence. In many cases candidate olfaction genes can be readily identified based on sequence conservation, and the powerful technique of RNAi can then be used to knock down candidate genes and enable follow-up functional studies.

Annotation of the *T. castaneum* genome sequence has already revealed unexpectedly large numbers of intact olfactory receptor (OR) and gustatory receptor (GR) genes (259 and 220, respectively, Engsontia et al., 2008), in comparison to other species. For example, *Drosophila melanogaster* has only 62 and 68 OR and GR genes, respectively. In addition to the relatively very large numbers of intact OR/GR genes in the *T. castaneum* genome, there are also 79 OR pseudogenes and 76 GR pseudogenes. RNAi has been done for *TcOR1*, which is one of the 259 *OR* genes in *T. castaneum*, and is a clear ortholog of the *D. melanogaster Or83b* gene, the latter having been demonstrated to be required for function of olfactory reception in *D. melanogaster*. RNAi-mediated knockout of *TcOR1* completely eliminated the attractiveness of the synthetic aggregation pheromone DMD (Tribolure) to adult beetles (Engsontia et al., 2008). So far as we are aware, no other olfaction genes have been subjected to functional analysis in *T. castaneum*, but the success of this experiment and the large number of olfaction genes in this species hint at the wealth of functional information that could be revealed by systematic, high-throughput RNAi knockdown studies.

The primary mediators of odor detection in insects are the odorant binding proteins (OBPs) and their smaller, more highly conserved cousins, the chemosensory proteins (CSPs). According to Foret and Maleszka (2006) there are 46 *OBP* genes in the *T. castaneum* genome, which is within the normal range (20~70) as currently defined by the available insect genome sequences. In contrast, the gene count for insect *CSP*s is considerably lower, totaling less than 10 each in *A. mellifera*, *D. melanogaster* and *A. gambiae*. We could find no published data on complete *CSP* annotations in *T. castaneum*, but allusions to individual *CSP* genes can be found in Maleszka et al. (2007), Lu et al. (2007) and Vieira et al. (2007). According to our BLASTP analysis, there are 14 genes encoding highly conserved CSP proteins in *T. castaneum* (Fig. 1), as well as three additional *CSP*-like genes that encode slightly more divergent proteins.

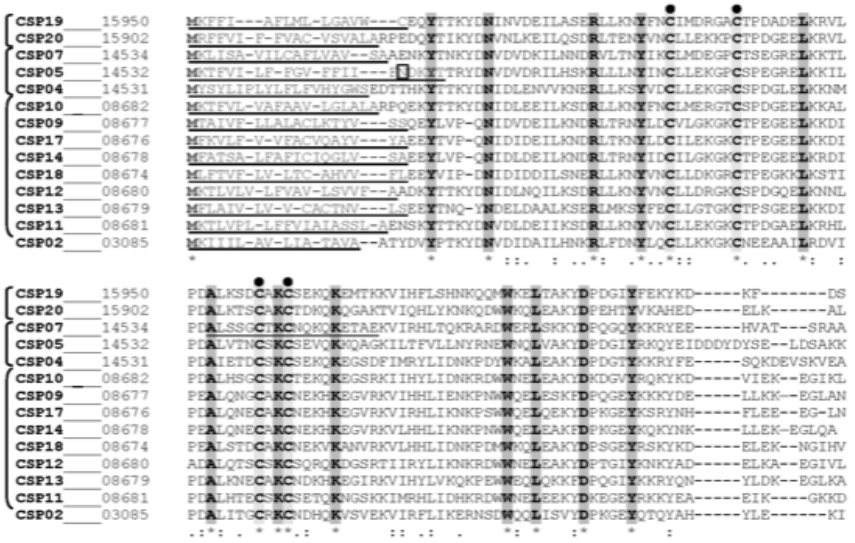

Figure 1 Alignment of Chemosensory Proteins (CSPs) in *Tribolium castaneum*. CSP proteins were detected by BLASTP analysis using a 19-residue query sequence derived from a conserved region of CSP7 (underlined) containing two of the cysteine residues involved in disulfide bridging. Multiple sequence alignment was done using T-COFFEE (Notredame et al., 2000). Fourteen of the 17 known CSP proteins are included. The remaining three (CSP06, 08 and 15) are somewhat divergent from the 14 shown. Secretion signal peptides at the amino termini of each protein, indicated in red font and by underlining, were determined with SignalP 3.0. Invariant residues are indicated by shading and bold font, and the four diagnostic cysteine residues (forming two disulfide bridges) are indicated by dots above the alignment. The curated version of CSP5 lacked a signal peptide and contained a unique, 14-residue insertion, both resulting from an apparent misannotation. We defined a previously unrecognized, 42-nt intron at the Asn (N) residue (boxed) near the terminus of the signal peptide, which reestablished a plausible signal peptide cleavage site and removed the atypical insertion. NCBI protein identities are as follows: CSP18 = NP_001039286.1, CSP17 = NP_001039284.1, CSP09= NP_001039283.1, CSP10= NP_001039278.1, CSP11= NP_001039279.1, CSP12= NP_001039280.1, CSP13= NP_001039281.1, CSP14= NP_001039282.1, CSP19= NP_001039276.1, CSP20= NP_001039274.1, CSP07= NP_001039289.1, CSP05= NP_001039287.1, CSP04= NP_001039285.1, CSP02= NP_001039277.1. The following CSPs are not shown: CSP15= NP_001039291.1, CSP08= NP_001039290.1 and CSP06= NP_001039288.1.

Eight of the 14 proteins shown in Figure 1 are encoded by a closely linked cluster of *CSP* genes located on chromosome 7. Inspection of the tiling array tracks for this chromosomal region in the *Tribolium* genome browser BeetleBase (http://beetlebase.org/index.shtml) suggests that the various *CSP*s are differentially expressed and highly regulated (Fig. 2). For example, *CSP10* expression is largely restricted to the larval and early pupal stages, whereas *CSP11* is expressed predominantly in the late pupal and adult stages. *CSP12* appears to have two peaks of expression, one in the early part of the last-instar larval stage and another in the late pupal stage. Still other *CSP* genes (*CSP9*, *13-15* and *17*) have very low or no expression throughout the last larval instar and pupal and adult stages. The latter could have vital but highly localized expression domains (individual sensillae or appendages?) preventing detection of such low-abundance transcripts in whole-animal tiling arrays.

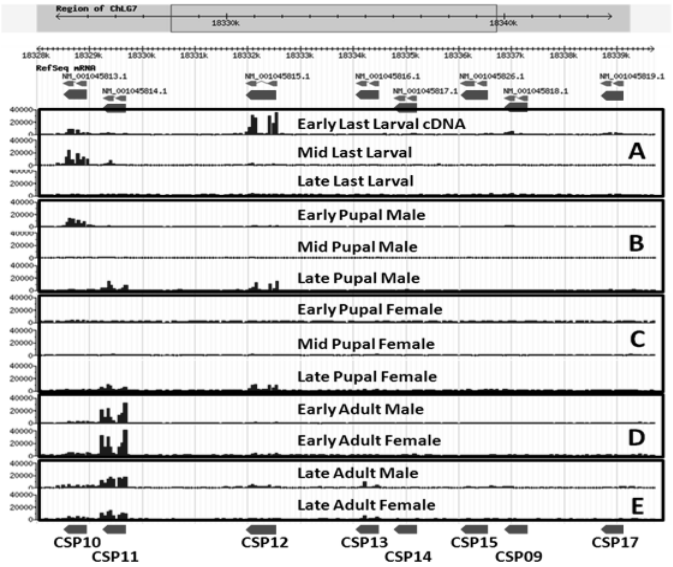

Figure 2 Tiling Array Expression Patterns of a Cluster of Eight Chemosensory Protein (CSP) Genes on Chromosome 7 of *Tribolium castaneum*. Depictions of results from Nimblegen expression tiling array hybridizations were selected in the GBROWSE genome viewer in BeetleBase. Vertical axes represent relative expression as determined by hybridization to microarrays containing contiguous 50-mer probes spanning the entire genome (excluding repetitive sequences). cDNAs used for hybridizations had been isolated from whole insects at the stages indicated, ranging from last instar larvae (panel A) through mature adults (panel E). Horizontal red bars (duplicated above and below tiling array panels) indicate positions of genes on the chromosome, and point in the direction of transcription. Computed gene structures and NCBI RefSeq names are shown just above the upper set of bars.Future work in our group will focus on description and RNAi-mediated knockdown of gene products responsible for pheromone biosynthesis, release or perception, with the general goal of understanding the basis for these functions in insect biology, and with a specific goal of knocking down one or more systems in a manner that could facilitate pest management. There is a total of "only" 63 OBP and CSP genes that are the presumed direct mediators of all chemosensory responses in the *Tribolium* repertoire. With specific gene knockdown by RNAi now a routine procedure, the previously impossible task of functionally characterizing all first-response odor detection genes now seems feasible.

2. Chitin biology and genomics

The insect exoskeleton is a noncellular biomaterial that functions both as skin and as waterproof armor that is sufficiently flexible to accommodate growth and enable mobility. This complex and fascinating physiological adaptation, together with other chitinous structures such as the digestive "peritrophic matrix" of the midgut, has contributed to the great evolutionary success of arthropods. We and others have identified more than 200 genes in the *Tribolium* genome that appear to be directly involved in the composition, biosynthesis, deposition and turnover of these structures (Tab. 1), offering a wealth of potential new biotargets for selective pest control. The proteins encoded by these genes can be divided into four major categories, including: **(**1**)** structural "cuticle proteins" that, together with the polysaccharide chitin itself, contribute most of the bulk and substance of the finished cuticle; **(**2**)** enzymes involved in chitin synthesis, modification or degradation; **(**3**)** cuticle assembly proteins involved in deposition and layering of chitin and cuticle structural proteins; and **(**4**)** cuticle maturation enzymes that control the processes of tanning, crosslinking and pigmentation to confer the final color, rigidity/elasticity and waterproofing to the finished cuticle. Many of the gene models have been validated by sequence analysis of cDNAs, and many have been functionally characterized by RNAi or other methods (e.g. Arakane et al., 2005a & b, 2008, 2009a & b, 2010; Dixit et al., 2008; Hogenkamp et al., 2008; Jasrapuria et al., 2010; Richards et al., 2008; Zhu et al., 2008;).

Table 1 *Tribolium castaneum* genes involved in assembly and metabolism of cuticle and peritrophic matrix

Category	Gene	Genbank/GLEAN accession #	dsRNA knock-down phenotypes	proposed function
Chitin synthases (CHS)	TcCHS-A-8a	AY291475	Prevents L-L, L-P and P-A molting	chitin synthesis
	TcCHS-A-8b	AY291476	Prevents adult eclosion	chitin synthesis
	TcCHS-B	AY291477	loss of PM chitin, starvation	chitin synthesis
UDP-NAG pyrophos-phorylases	TcUAP1	GU228846	not tested	chitin synthesis
	TcUAP2	GU228847	not tested	chitin synthesis
Chitinases	TcCHT2	GLEAN_09872	not tested	chitin degradation
	TcCHT4	EF125543	No visible phenotype	chitin degradation in digestive peritrophic matrix
	TcCHT5	AY675073	Affects P-A molting	chitin degradation
	TcCHT6	EFA00965	No visible phenotype	chitin degradation
	TcCHT7	DQ659247	arrested pupal wing expansion and pumping motion	chitin degradation
	TcCHT8	DQ659248	No visible phenotype	chitin degradation in digestive peritrophic matrix
	TcCHT9	DQ659249	not tested	chitin degradation in digestive peritrophic matrix
	TcCHT10	DQ659250	Prevents L-L, L-P and P-A molting	chitin degradation
	TcCHT11	GLEAN_15665	No visible phenotype	chitin degradation
	TcCHT12	GLEAN_09178	not tested	chitin degradation in digestive peritrophic matrix
	TcCHT13	DQ659252	not tested	chitin degradation in digestive peritrophic matrix
	TcCHT14	GLEAN_09628	No visible phenotype	chitin degradation in digestive peritrophic matrix
	TcCHT15	GLEAN_09629	No visible phenotype	chitin degradation in digestive peritrophic matrix
	TcCHT16	AY873915	No visible phenotype	chitin degradation in digestive peritrophic matrix
	TcCHT17	GLEAN_09625	not tested	chitin degradation in digestive peritrophic matrix
	TcCHT18	GLEAN_09630	not tested	chitin degradation in digestive peritrophic matrix
	TcCHT19	GLEAN_09175	not tested	chitin degradation in digestive peritrophic matrix
	TcCHT20	AY873913	not tested	chitin degradation in digestive peritrophic matrix
	TcCHT21	AY873916	not tested	chitin degradation in digestive peritrophic matrix
	TcCHT22	DQ659251	not tested	chitin degradation in digestive peritrophic matrix
N-Acetyl glucosamini-dases (NAG)	TcIDGF2	DQ659253	No visible phenotype	cell proliferation/remodeling
	TcIDGF4	DQ659254	Prevents P-A molting	cell proliferation/remodeling
	TcNAG1	EF592536	Prevents L-L, L-P and P-A molting	chitin degradation
	TcNAG2	EF592537	Prevents L-L, L-P and P-A molting	chitin degradation
	TcNAG3	EF592538	compromises L-L, L-P and P-A molting	chitin degradation
	TcFDL	EF592539	Prevents L-L, L-P and P-A molting	N-glycan processing/chitin catabolism
Chitin deacetylases (CDA)	TcCDA1	EU019711	Prevents L-L, L-P and P-A molting	chitin modification
	TcCDA2a	EU019712	Nonarticulation of femoral-tibial joints	chitin modification
	TcCDA2b	EU019713	Affects epidermal cuticle morphology	chitin modification
	TcCDA3	EU190485	No visible phenotype	chitin modification
	TcCDA4	EU190486	No visible phenotype	chitin modification
	TcCDA5A	EU190487	No visible phenotype	chitin modification
	TcCDA5B	EU190488	No visible phenotype	chitin modification
	TcCDA6	EU190489	No visible phenotype	chitin modification
	TcCDA7	EU190490	No visible phenotype	chitin modification
	TcCDA8	EU190491	No visible phenotype	chitin modification
	TcCDA9	EU190492	No visible phenotype	chitin modification

Category	Gene	Genbank/GLEAN accession #	dsRNA knock-down phenotypes	proposed function
Cuticle assembly genes	TcRtv	GLEAN_07384	not tested	cuticle assembly
	TcKNK1	GLEAN_10653	not tested	cuticle assembly
	TcKNK2	GLEAN_12301	not tested	cuticle assembly
	TcKNK3	GLEAN_02304	not tested	cuticle assembly
Peritrophic matrix-associated genes	TcPMP1-A	GU128096	not tested	peritrophic matrix structural protein
	TcPMP1-B	GU128097	not tested	peritrophic matrix structural protein
	TcPMP1-C	GU128098	not tested	peritrophic matrix structural protein
	TcPMP2-A	GU128099	not tested	peritrophic matrix structural protein
	TcPMP2-B	GU128100	not tested	peritrophic matrix structural protein
	TcPMP2-C	GU128101	not tested	peritrophic matrix structural protein
	TcPMP3	GU128102	not tested	peritrophic matrix structural protein
	TcPMP5-A	GU128103	not tested	peritrophic matrix structural protein
	TcPMP5-B	GU128104	not tested	peritrophic matrix structural protein
	TcPMP9	GU128105	not tested	peritrophic matrix structural protein
	TcPMP14	GU128106	not tested	peritrophic matrix structural protein
Phenoloxi-dases	TcLac1	AY884065	No visible phenotype	ferroxidase
	TcLac2A	AY884061	Required for larval, pupal and adult cuticle tanning	tanning
	TcLac2B	AY884062	Required for larval, pupal and adult cuticle tanning	tanning
	TcLLP	GLEAN_15880	Prevent pupal development	unknown vital function
	TcTyr1	*AY884063*	*No visible phenotype*	*Immune-related melanization?*
	TcTyr2	AY884064	No visible phenotype	Immune-related melanization?
	TcTH	EF592178	Required for larval, pupal and adult cuticle tanning	cuticle tanning-related
	TcDDC	EU019710	Delays adult cuticle tanning, darkens body color	cuticle tanning-related
	TcADC	ABU25221	Black body color phenotype	cuticle tanning-related
	TcNAT1	FJ647798	Dark pigment around elytral sensillae	cuticle tanning-related
	Tcebony	FJ647797	Black body color phenotype	cuticle tanning-related
	Tctan	GLEAN_03448	not tested	cuticle tanning-related
Cuticle tanning - related genes	TcYellow-b	GU111762	not tested	pigmentation/sclerotization
	TcYellow-c	GU111763	not tested	pigmentation/sclerotization
	TcYellow-e	GU111765	not tested	pigmentation/sclerotization
	TcYellow-e3	GU111764	not tested	pigmentation/sclerotization
	TcYellow-f	GU111766	Prevents P-A molting	pigmentation/sclerotization
	TcYellow-g1	GU111767	not tested	egg cuticle stabilization
	TcYellow-g2	GU111768	not tested	egg cuticle stabilization
	TcYellow-h	GU111769	pigmentation/sclerotization	pigmentation/sclerotization
	TcYellow-y	GU111770	prevents hindwing melanin production	pigmentation/sclerotization
	TcYellow-1	GU111771	not tested	pigmentation/sclerotization
	TcYellow-2	GU111772	not tested	pigmentation/sclerotization
	TcYellow-3	GU111773	not tested	pigmentation/sclerotization
	TcYellow-4	GU111774	not tested	pigmentation/sclerotization
	TcYellow-5	GU111775	not tested	pigmentation/sclerotization

Category	Gene	Genbank/GLEAN accession #	dsRNA knock-down phenotypes	proposed function
Cuticle protein (CP) genes	RR1 CP	~50 genes	not tested	cuticle structural proteins
	RR2 CP	~50 genes	not tested	cuticle structural proteins
	RR3 CP	~10 genes	not tested	cuticle structural proteins
	CPLC	~15 genes	not tested	cuticle structural proteins
	other CPs*	~15 genes	not tested	cuticle structural proteins
	TcCPAP3-A1	EF125544	not tested	cuticle structural protein
	TcCPAP3-A2	EF125545	not tested	cuticle structural protein
	TcCPAP3-B	EF125544	not tested	cuticle structural protein
	TcCPAP3-C5a	EF125545	not tested	cuticle structural protein
	TcCPAP3-C5b	EF125546	not tested	cuticle structural protein
	TcCPAP3-D1	EF125544	not tested	cuticle structural protein
	TcCPAP3-D2	EF125545	not tested	cuticle structural protein
	TcCPAP3-E	EF125546	not tested	cuticle structural protein
	TcCPAP1-A	EF125546	not tested	cuticle structural protein
	TcCPAP1-B	EF125546	not tested	cuticle structural protein
	TcCPAP1-C	EF125546	not tested	cuticle structural protein
	TcCPAP1-D	EF125546	not tested	cuticle structural protein
	TcCPAP1-E	EF125546	not tested	cuticle structural protein
	TcCPAP1-F	EF125546	not tested	cuticle structural protein
	TcCPAP1-G	EF125546	not tested	cuticle structural protein
	TcCPAP1-H	EF125546	not tested	cuticle structural protein
	TcCPAP1-I	EF125546	not tested	cuticle structural protein
	TcCPAP1-J	EF125546	not tested	cuticle structural protein

*includes tweedle, CPF and CPFL genes

With respect to category **(**1**)** above, insects employ an amazing variety of what appear to be structural protein genes during manufacture of the chitinous matrices. In *T. castaneum* there are approximately 160 cuticle protein genes, encoding approximately 110 RR motif proteins, ~15 "cuticle proteins of low complexity" (CPLCs), 18 "cuticle proteins analogous to peritrophins" CPAPs) and several other minor categories of cuticle proteins, each represented by only one or a few genes (Table 1 and unpublished observations). Why insects should require such a large array of protein structural components for cuticle and peritrophic matrix is still uncertain, but it is becoming clear that the various cuticle proteins are not uniformly expressed, and that different regions of cuticle and peritrophic matrix have different protein composition, probably reflecting the different physical properties and functions needed in different regions of the exoskeleton and digestive sac (Willis, 2010).

A great number and variety of genes are also utilized for chitin-modification and degradation (24 *chitinases*, 4 *N-acetyl glucosaminidases* and 9 *chitin deacetylases*) and for cuticle tanning and pigmentation (26 genes). Of particular interest in the latter category are the 14 *yellow* genes, each of which may be specialized for a unique function. For example, we found that one of the *yellow* genes is required specifically for wing pigmentation (but not that of elytra or body wall) while another *yellow* gene has a specific, vital role in tanning of the cuticle of the adult body wall (Arakane et al., 2010).

3. Summary

In summary, the *T. castaneum* genome project has opened a fast-track to gene discovery in this stored product pest insect for all areas of *Tribolium* biology, two of which are briefly discussed here. These examples reveal the complexity and sophistication of genetic regulation of insect adaptations, but also illustrate the rapid progress towards understanding biological mechanisms made possible by the availability of this genome sequence. Any gene can be quickly categorized as either essential or dispensable by the powerful technique of RNAi. For essential genes, the timing and mode of RNAi-induced death give clues about specific gene functions, supplementing insights gained from protein sequence homology and conserved domain analysis. More subtle and detailed functional inferences can be gleaned by more careful scrutiny of beetles after gene knockdown or by more sophisticated bioassays. Examples include monitoring for changes in responsiveness to pheromone after knockdown of candidate olfaction genes, monitoring for abnormalities in stereotyped premolting behavior after knockdown of

candidate effector genes involved in the endocrine regulation of molting, or monitoring cuticle composition after knockdown of genes with suspected roles in the metabolism or recycling of the exoskeleton. Assessment of gene/protein expression patterns and biochemical studies of purified or recombinant proteins can complement and supplement gene RNAi studies. The resulting improvements in our knowledge of the basic biology of pest insects will fuel the next generation of pest control technologies.

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Investigation of the semiochemicals of confused flour beetle *Tribolium confusum* Jaquelin du Val and grain weevil *Sitophilus granarius* (L.) in stored wheat grain and flour

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Abstract

This investigation sets out to identify specific volatile compounds from both flour infested with the confused flour beetle, *Tribolium confusum* and wheat grain infested with the grain weevil, Sitophilus granarius. These volatiles could help to aid the early detection of infestation by these pests. Volatiles by the infestation of these insect pests were entrained and analysed using Solid-Phase Micro-Extraction (SPME) coupled with gas chromatography-mass spectrometry (GCMS). Several volatile compounds were identified specific to *T. confusum* and *S. granarius*, including the known semiochemicals of *T. confusum*. The *T. confusum* larvae specifically emitted the volatiles 1-octen-3-one, benzeneacetaldehyde and decanal, whilst the adults specifically emitted the volatiles 2-methyl and 2-ethyl-1,3-benzenediols, the known semiochemicals 1-pentadecene, 2-methyl and 2-ethyl-1,4-benzoquinones and a series of yet to be fully identified unsaturated hydrocarbons. Both *T. confusum* adults and larvae emitted 2-methylbutanal and 2-butanone. Furthermore, four volatiles were identified unique to flour infested by *T. confusum*, 3-penten-2-one, 3-octanone, 2-octenal and 2-butyl-1-octanol. The *S. granarius* adults specifically emitted the volatiles 2-methylpropanoic acid and 3-methylbutanoic acid, whilst infested wheat grain produced the following volatile organic compounds, 2-methylfuran, 2-ethylfuran, 2-methyl-1-butanol, 2-ethyl-2-pentenal and 2,5-dimethylpyrazine. We believe these specific volatiles may act as semiochemicals for these insects and could aid in semiochemical monitoring for the early detection of infestation by these insects.

Keywords: *Tribolium confusum*, *Sitophilus granarius*, GC-MS, SPME, Semiochemicals.

1. Introduction

Grain and food products are attacked by pests such as insects, mites and microorganisms during storage. The resulting post-harvest losses are approximately 10-15% worldwide annually (Hodges et al., 1996; Rajendran, 2002; Neethirajan et al., 2007). Infestation by insects encourages growth of fungi including those that produce mycotoxins, and results in contamination of commodities with insect bodies and waste products etc. Some of which are toxic, repulsive or allergenic (Freeman, 1976). Thus, insect infestation detection is very important to ensure the provision of healthy food to consumers, to evaluate the efficiency of pesticide treatment and to work as an early warning for taking suitable control measures.

This investigation sets out to identify volatile organic compounds (VOCs) as markers for the early detection of Confused Flour Beetle, *Tribolium confusum* Jaquelin du Val and grain weevil, *Sitophilus granarius* (L.) infestation in flour and wheat grain. Previous studies have identified VOCs in the headspace above *Tribolium* spp. (Villavarde et al., 2007) and in the headspace above the lesser grain borer, *Rhyzopertha dominica* (L.) (Seitz and Ram, 2004). This investigation was undertaken to discover new volatile compounds and to confirm the presence of compounds previously reported using solid-phase micro-extraction (SPME) to collect and concentrate the headspace volatiles above the samples, with the subsequent analysis by gas chromatography-mass spectrometry (GCMS).

2. Materials and methods

2.1. Insect identification and rearing

Insects were obtained from an organic farm which had a problem with infested grain. The insects were identified at the University of the West of England (UWE, Bristol, UK) and the identification confirmed by the Natural History Museum (London, UK).

Tribolium confusum beetles were reared on wheat flour and wholemeal flour and S. granarius weevils were reared on wheat grain. Moisture content of the grain was estimated using a "Digital Grain Master" moisture meter (Protimeter, Marlow, UK). Glass jars (200 mL), covered with nylon gauze, were used as insect containers and incubated at 25°C ± 2°C under a 14 h light: 10 h dark photoperiod at 70 ± 5% relative humidity (r.h.).

2.2. Collection of VOCs

2.2.1. SPME Fibre Conditioning

SPME fibres (75 µm carboxen/polydimethylsiloxane, Sigma Aldrich, Dorset, UK) were conditioned prior to first use according to the manufacturer instructions (300ºC for 3 h) and reconditioned in between sampling sets (280ºC for 12 min) by heating in the GCMS injector port (Clarus 500, Perkin Elmer, Beaconsfield, UK).

2.2.2. Collection of VOCs from T. confusum

Identification and comparison of the VOCs from *T. confusum* adults and larvae was undertaken using 100 adult beetles and 100 larvae, in two headspace vials (10 ml, Supelco, Poole, UK). VOCs were also collected from flour infested with *T. confusum* beetles (3 g) and non-infested flour (3 g) in headspace vials (10 mL). The SPME fibres were exposed for 16 h to the static headspace of the samples heated at 28°C. Each sample collection of VOCs was repeated three times with new samples. Periodic blank vials were used as controls to ascertain system impurities.

2.2.3. Collection of VOCs from S. granarius

VOCs released by *S. granarius* weevils (100 insects), infested wheat grain (3 g) and non-infested wheat grain (3 g) were collected from 10 ml headspace sample vials (10 ml) using the SPME fibres. The SPME fibres were exposed for 16 h to the static headspace of the samples heated at 28°C. Each sample collection of VOCs was repeated three times with new samples. Periodic blank vials were used as controls to ascertain system impurities.

2.3. GCMS analysis of the SPME fibres

VOCs were analysed using the GCMS system fitted with a split/splitless injector (250˚C, purge off 1.0 min) and separated in the GC using a Zebron-624 column (60 m length x 0.32 mm I.D., 1.40 µm film thickness, Phenomenex, Macclessfield, UK) using the GC oven temperature program (35°C for 5 min, ramped at 7°C min-1 to 250°C for 12 min, run time 47.71 min). The VOC analytes were identified through the MS in full scan mode (17-350 m/z, electron impact ionisation at 70 eV) using the NIST database library (reserves fit hits and Kovat's indices, NIST 2002) and by the comparison of known standard retention times.

3. Results

3.1. Analysis of headspace VOCs from T. confusum

The analysis of the headspace from *T. confusum* showed several of VOCs which were unique to adults compared to the larvae and infested flour samples and were found in all repeat experiments; 2-methyl-1,4-benzoquinone, 2-ethyl-1,4-benzoquinone, 2-methyl-1,3-benzenediol and 4-ethyl-1,3-benzenediol. Two further VOCs, 1-pentadecene and hexadecane, were repeatedly found in both adult and flour infested headspace samples, whilst 2-methylbutanal and 2-butanone were found in both the headspace samples of adults and larvae. A typical chromatogram illustrating the VOCs extracted from the headspace of adults of T. confusum by SPME fibre is shown in Fig. 1.

Analysis of the headspace of wheat flour infested with *T. confusum* showed the presence of four unique compounds; 3-penten-2-one, 3-octanone, 2-octenal and 2-butyl-1-octanol. These were absent in the

headspace samples of non-infested flour. The analysis of the headspace of *T. confusum* larvae samples contained the unique VOCs, 1-octen-3-one, benzeneacetaldehyde and decanal.

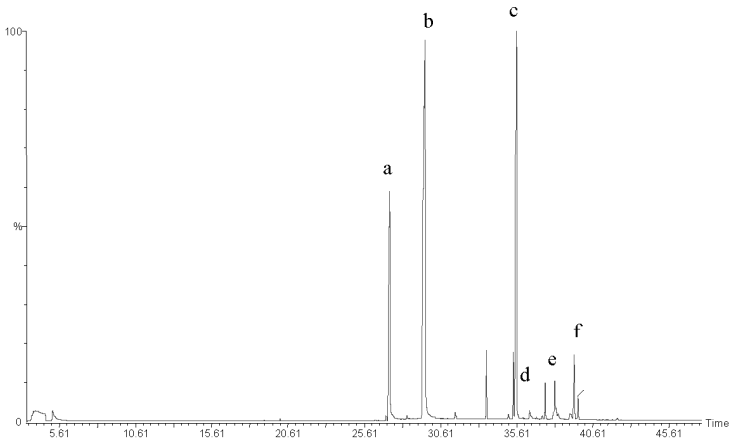

Figure 1 Typical chromatogram illustrating the major VOCs extracted from the headspace above a hundred beetles of *Tribolium confusum*. a = 2-methyl-1,4-benzoquinone, b = 2-ethyl-1,4-benzoquinone, c = 1-pentadecene, d = 2-methyl-1,3-benzeediol, e = hexadecane and f = 4-ethyl-1,3-benzenediol.

3.2. Analysis of headspace VOCs from S. granarius

Two VOCs 2-methylpropanoic acid and 3-methylbutanoic acid were unique to the headspace above *S. granarius* and were repeatedly identified (see Fig. 2).

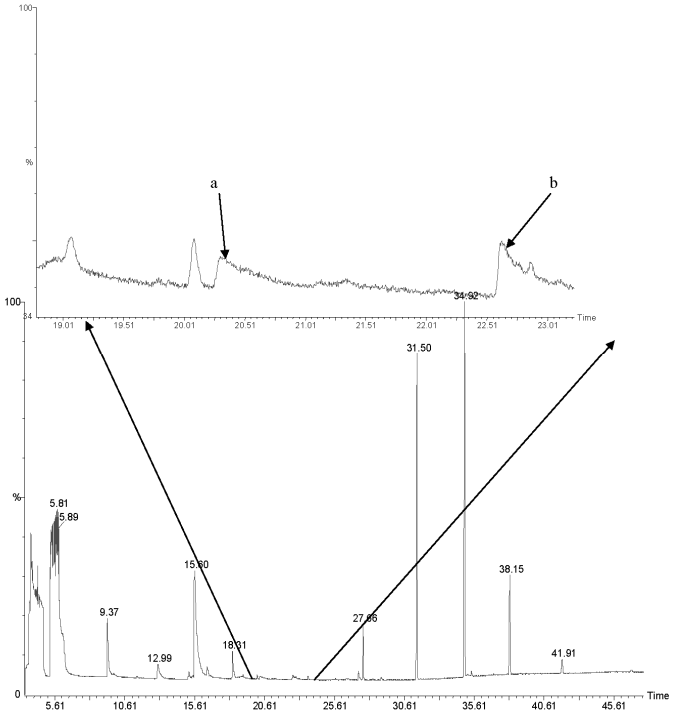

Figure 2 Typical chromatogram illustrating the VOCs extracted from the headspace above a hundred beetles of *Sitophilus granarius*.

Other volatiles were found only in the headspace of wheat grain infested by *S. granarius*; 2-methylfuran, 2-ethylfuran, 2-methyl-1-butanol, 2-ethyl-2-pentenal and 2,5-dimethylpyrazine. These were absent in the headspace samples of non-infested wheat grain.

4. Discussion

4.1. Analysis of headspace VOCs from T. confusum

The analysis of the headspace from *T. confusum* adults showed 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone were present and where found in all the repeat experiments. These quinones were unique to adult headspace samples, but surprisingly where not identified from the flour infested headspace samples. These odorous benzoquinones have been well documented previously (Engelhardt, et al., 1965, Pappas et al., 1996 and Villaverde et al., 2007) and can make infested flour unsuitable for human consumption (Phillips et al., 1984) and even toxic (El-Mofty, et al., 1992). These quinones act as defence secretions for *Tribolium* spp. (Tschinkel, et al., 1975) and are known to be released during overcrowding stressful conditions (Faustini et al., 1986).

1-Pentadecene and hexadecane were found in both adults of *T. confusum* and wheat flour infested with *T. confusum* beetles. 1-Pentadecene is associated with insect odour (Seitz et al., 1996) and is hypothesized as an epideictic (spacing) pheromone (Arnuad et al., 2002). The known conspecific aggregation pheromone of *Tribolium* spp. 2, 4-dimethyldecanal (Arnuad et al., 2002) was not identified in this study. Previously unreported VOCs, 2-methyl-1, 3-benzenediol and 4-ethyl-1,3-benzenediol, were unique to the headspace of *T. confusum* adults. Consequently these VOCs which were produced by adults might be used as biomarkers for detection of *T. confusum* in flour or grain. VOCs unique to flour infested by *T. confusum*, 3-penten-2-one, 3-octanone, 2-octenal and 2-butyl-1-octanol, might also be potential biomarkers for infestation.

According to the available literature there has not been a detailed study of the VOCs produced by *T. confusum* larvae and only methyl fatty acid esters have been reported (Tebayashi et al., 2003). It is therefore significant, that 1-octen-3-one, benzeneacetaldehyde and decanal VOCs, were found in larvae samples and not in adults or laboratory air samples. These too might also be potential biomarkers.

4.2. Analysis of headspace VOCs from S. granarius

The analysis of the headspace from *S. granarius* adults showed 2-methylpropanoic acid and 3-methylbutanoic acid were unique to these samples but not present in the wheat grain infested with S. granarius. The known *S. granarius* attractant 3-methylbutan-1-ol (Germinara et al., 2008) was not identified by this study, however 3-methylbutanoic acid could be the precursor to this and may exhibit a semiochemical response.

VOCs unique to the headspace of the infested wheat grain were 2-methylfuran, 2-ethylfuran, 2-methyl-1-butanol, 2-ethyl-2-pentenal and 2,5-dimethylpyrazine and might also be potential biomarkers.

Some studies confirm that the *S. granarius* produces (2S, 3R)-1-ethylpropyl-1,2-methyl-3-hydroxypentanoate as an aggregation pheromone (Chambers et al., 1996). However the present study did not identify this pheromone, due to possible overcrowding during the sampling.

In summary, this study has identified some unique volatiles previously not identified from these species. Bioassays would be required to confirm if these volatiles act as semiochemicals.

Acknowledgments

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Efficiency comparison of three attractant products against webbing clothes moth *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae) using an adapted four arms olfactometer

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1. Introduction

In patrimonial stock, webbing clothes moth (*Tineola bisselliella* (Hummel), Lepidoptera: Tineidae) larvae may feed year round on woollen textiles and other animal-based products, such as furs and carpets causing economic depreciation of goods disproportionate to the physical damage. Depreciation caused by keratophagous insects including webbing clothes moth approximates one billion dollars annually in the United States alone (Metcalf and Metcalf, 1994). A number of pheromone based attractants can be found on the market and we compared the efficiency of three of them against T. bisselliella.

2. Methods and materials

Webbing clothes moth were reared in plastic boxes with mesh lids at 25 ±1°C, 40-60% r.h. and a 12L:14D inverted photoperiod. Larvae were provided with untreated, untanned, and dry rabbit's pelts with hair (Fig. 1). Every day adult webbing clothes moths were collected and their sex determined through the presence of claspers and aedagus (male) or ovipositor (female).

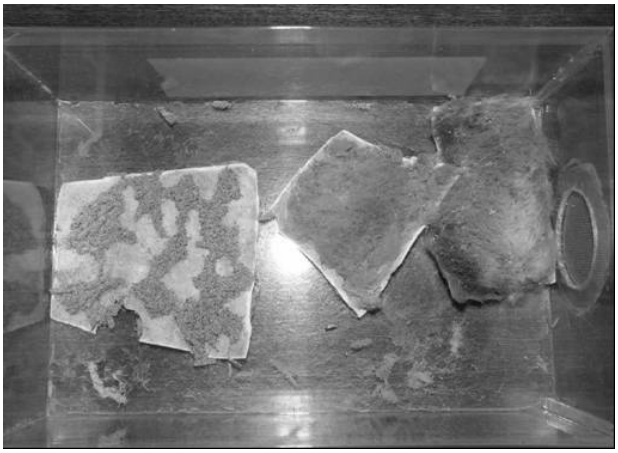
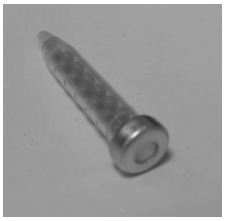


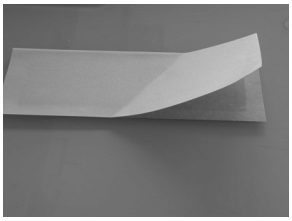
Figure 1 Untreated, untanned and dry rabbit's pelts with hair.

We tested three lures (Fig. 2) : Webbing clothes moth “bullet lure”, Insect limited Inc 16950 Westfield, Park Road, Westfield, IN 46 O74 USA; CAT-QLURE- wcm Russel IPM®, Unit 68,Third Avenue, Deeside Park, Deeside, Flintshire, CH5 2LA United Kingdom; Finicon® FINICON Sticky Pads, Each attractant are supposed to contain webbing clothes moth's sex pheromones, i.e koiganal I ((E)-2-octdecenal) and koiganal II ((E,Z)-2,13-octadecadienal), but they may also contain semiochemical attractants of larval habitat, food, or male aggregation pheromones.



Webbing clothes moth "bullet lure"

CAT-QLURE



Finicon® FINICON Sticky Pads

In order to estimate the efficiency of the three attractants, experiments were assessed in a closed arena olfactometer with four arms without air flow, derived from the Takacs et al. (2001) olfactometer. The arena at the end of each arm contained a sticky trip and one of the three attractants or an empty microcentrifuge tube as a control (Fig. 3). For the five replicates, each attractants were randomly placed and 10 male moths (1 to 4 d old) were released from the petri dish in the center of the main arena. After 24 h, the number of trapped moths for each attractant was noted.

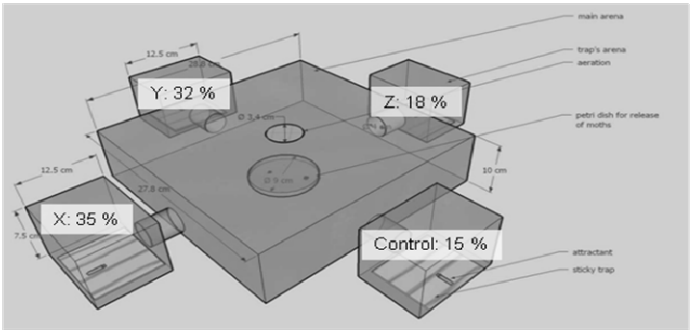


Figure 3 Olfactometer used for tests and percentage of webbing clothes moth trapped for each bait.

3. Results

We noted some trends between the different lures (Fig. 3), but there was no statistical difference between the lures.

4. Discussion

The developed olfactometer can highlight differences in efficiency of different products. Efficacy studies are still ongoing, and we will compare the attraction of males in the presence of products or virgin females. Actually, two products are able to trap one third of the population of insects, which is important but not sufficient to reduce damage enough.

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Pheromone traps for monitoring *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) in the presence of mating disruption

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Abstract

High-dose pheromone lures have proved useful for monitoring some lepidopteran pests in the presence of mating disruption, but not others. We performed experiments in commercial and pilot scale facilities to examine the effect of pheromone dose on detection of Indianmeal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), in the presence of mating disruption. When *P. interpunctella* males were released into 1000 m3 rooms containing traps baited with 0, 1, or 10 mg (Z,E)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac), traps containing 10 mg captured more than those baited with 1 mg in both the presence and absence of mating disruption. Traps baited with 1 mg captured fewer males in the presence of mating disruption than in its absence, but the opposite was observed with traps baited with 10 mg. When males released into 73 m3 rooms were exposed sequentially to blank traps, traps baited with unmated females, and traps baited with 0.1 mg and then 1.0 mg Z9, E12-14:Ac in the presence or absence of mating disruption, 92% of trapped males were captured in female-baited traps in the absence of mating disruption, whereas in the presence of mating disruption 72% of males captured were caught in synthetic pheromone traps. These data suggest that pheromone lures can be used for monitoring *P. interpunctella* in the presence of mating disruption. Implications of these data for mass trapping are also discussed.

Keywords: *Plodia interpunctella*, Mating disruption, Monitoring, Pheromone lures, Mass trapping

1. Introduction

Plodia interpunctella (Hübner) shares (Z,E)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac), as the primary component in its sex pheromone (El-Sayed, 2009) with several other stored product pests of the subfamily Phycitinae. This compound has been used successfully by itself in integrated management of these species, both for monitoring (Nansen and Phillips, 2004; Nansen et al., 2006; Witzgall et al., 2010) and for mating disruption (Ryne et al., 2006, 2007; Sieminska et al., 2009).

Reduced utility of pheromone traps for monitoring can be a barrier to adoption for mating disruption. The degree to which mating disruption prevents use of pheromone traps for monitoring depends on neurobehavioral characteristics of the response of the target species to its sex pheromone. Pheromone lures can be used to monitor the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in the presence of mating disruption, albeit only with higher loads of pheromone in the lure and then at reduced efficiency compared to optimized lures in a non-mating disruption situation (Charmillot, 1990). In contrast, males of the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) are more repelled by higher concentrations of sex pheromone (Roelofs et al., 1973; Cardé and Minks, 1995). Higher pheromone concentrations on lures do not increase the number of males captured, either in the presence of mating disruption or in its absence (Kovanci et al., 2005). Here we report experiments examining the effect of pheromone (Z9, E12-14:Ac) dosage on lures on the number of *P. interpunctella* captured in either the absence or the presence of mating disruption with Z9, E12-14:Ac.

2. Materials and methods

2.1. Insects, lures, and pheromone dispensers

Insects used for these tests came from a laboratory strain of *P. interpunctella* founded with larvae collected at a dried-fruit packer in fall 2007. The laboratory culture was maintained on a wheat brain diet (Tebbets et al., 1978) in walk-in environmental chambers maintained at 26°C, a photophase of 16:8 L:D, and 60% r.h. Unmated males and females were obtained by isolating adults within 30 minutes of emergence. Dissection of many moths demonstrated that females thus obtained were consistently unmated. When unmated females were used as lures, three recently emerged females were sealed into plastic mesh bags. When adults that eclosed on multiple days were used in an experiment, they were held at 10°C until used since previous research showed no loss of fertility at this temperature for two weeks (Johnson et al., 1997). Synthetic pheromone lures containing 0, 0.1, 1 or 10 mg pheromone were prepared by applying 300 µl hexane with the appropriate concentration of Z9,Z12-14:Ac (P6050-93, Bedoukian, Danbury, CT USA) into vacuum-extracted 11 mm gray rubber septa (West Pharmaceuticals, Lionville, PA USA). CheckMate SPM dispensers (Suterra LLC, Bend, OR USA) were used to examine the effect of permeation with Z9, Z12-14:Ac on the number of males captured in traps.

2.2. Experiments

The first experiment was conducted in paired 1000 m3 rooms (floor 12 × 12 m; ceiling 7 m). They belonged to a dried fruit processer and were constructed as fumigation chambers; they are also used for product storage. Each had a small window over the roll-up door, allowing weak daylight and a natural photoperiod. They were not heated or cooled. Temperature loggers (Hobo U10, Onset Instruments, Pocasset, MA USA) placed in the rooms over the test period indicated a minimum 27°C and maximum 36°C temperature. One of the two chambers was treated with 16 CheckMate SPM dispensers, placed at ceiling level at equal intervals on the perimeter of the room. After the pheromone dispensers had been in place for a day, twelve delta traps (LPD, Suterra, Bend, OR USA) were placed in each of the two rooms. Three treatments were assigned randomly to trap positions in the two rooms: no bait, or bait consisting of gray rubber septa loaded with 1 or 10 mg Z9, E12-14:Ac. In each room, two to four jars containing a total of 160 unmated males, aged 0-2 d post eclosion, were placed on the floor near the center, opened, and then the entrance to the room were quickly closed. Three d later, the traps were removed and captured adults were counted.

A second experiment was performed using identical 73 m3 structures (Open bay security offices, Mini Mobile Inc., Phoenix, AZ USA). The interior floor area was 2.4 x 12.2 m, and the ceiling was 2.4 m high. Four wing traps (Suterra LLC, Bend, OR USA) were suspended 1.5 m from the floor, midway between the two long walls, 1.5 m from the two short walls, and 3 m apart. Each long wall had three closed windows covered with blinds, allowing diffuse natural light. When mating disruption was used, two CheckMate SPM dispensers were placed 15 cm from the ceiling on opposite long walls, 3 m from the short wall.

This second experiment consisted of a series of trials examining the number of males captured following the release of forty newly eclosed unmated males from a jar on the floor in the center of the room. Two preliminary trials examined the number of males captured over six nights when both rooms contained traps baited with 1 mg septa, and neither room was treated with mating disruption. In these trials, all males quickly exited the jars; but subsequently we found it necessary to place the release jar on a cafeteria tray covered with mineral oil to avoid ant predation.

Three subsequent trials represented three replications of the second experiment. One of rooms was treated with CheckMate SPM dispensers as previously described, and these were left in place in the same room for the 28 d required for the three trails. In each trial new wing traps were put in the room prior to releasing the males in the late afternoon. Males were released once at the beginning of a series of lure changes, starting with empty traps (no lure). After two nights, plastic mesh bags containing three virgin females were placed in each trap. After an additional two nights the unmated females were replaced with 0.1 mg septa, and after two more nights these were replaced with 1.0 mg septa. After two more nights the traps and septa were removed, and the room was left empty 2 d prior to the beginning of the next trial. Males in traps were counted each morning, and liners were changed when unmated females were removed and replaced with 0.1 mg septa.

The rooms were not heated or cooled, and the temperature inside fluctuated with outdoor temperature and solar radiation. The minimum and maximum temperatures (°C) for trials 1-5 were, respectively: 18, 47; 10, 39, 6, 37, 4, 28; and 4, 28.

2.4. Data analysis

The first experiment was analyzed using a two-way ANOVA, with males per trap (transformed as squareroot[x + 1]) as the dependent variable and mating disruption treatment and septa dose as categorical independent variables. A Tukey multiple range test was used for comparisons. The second experiment was analyzed using a cumulative logit model (Agresti, 2007), with the sequential pheromone treatments (blank, unmated females, 0.1 mg, and 1 mg) as ordinal responses and the mating disruption treatment (presence, absence) as a categorical predictor. This model tests the null hypothesis that there is no difference in ordinal location between the two categories examined. This type of analysis was more appropriate than ANOVA for this experiment because the sequential application of pheromone lure strengths created autocorrelation between these treatments and therefore made ANOVA inappropriate. Student's t-test was also used to compare, between mating disruption treatments, the total number of males captured in traps and the oil trays used to counter ants. For comparison with other studies, percent trap suppression for traps baited with unmated females was calculated as:

% Suppression = {(Countuntreated – Countmating disruption)/Countuntreated} × 100.

Untransformed means and standard errors are used in the figures.

3. Results

In first experiment, a total of 85% of released males were captured in the 1000 m3 room treated with mating disruption dispensers, compared to 71% in the identical untreated room. ANOVA revealed significant differences in males per trap due to pheromone dose on the lure ($F_{2,18} = 38.5$, $P < 0.001$), but not due to mating disruption treatment ($F_{1,18} = 0.03$, $P = 0.86$) or the interaction of lure dose×mating disruption treatment ($F_{2,18} = 2.2$, $P = 0.14$). The number of males captured using blank traps was significantly lower than in traps baited with 1 mg septa ($P < 0.05$) (Fig. 1), and 10 mg-baited traps captured much higher numbers than 1 mg septa-baited traps ($P < 0.001$) (Fig. 1). Fewer males were captured in traps baited with 1 mg septa in the room treated with mating disruption treatment compared to the untreated comparison whereas the converse was true for the traps baited with 10 mg septa (Fig. 1). However, this implied interaction was not significant ($P < 0.05$).

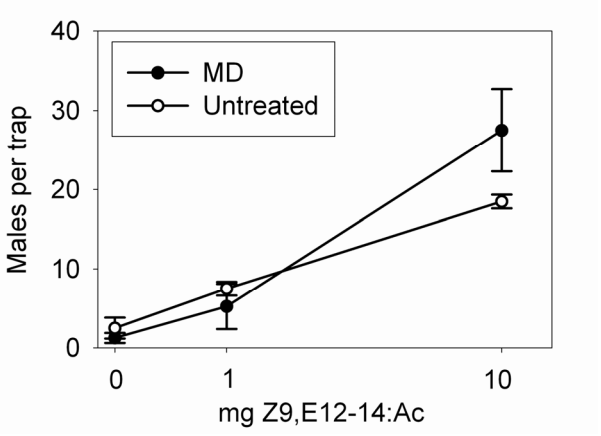

Figure 1 *Plodia interpunctella* males per trap (mean and SE) in empty traps, or traps baited with gray rubber septa loaded with 0, 1 or 10 mg Z9,E12-14:Ac, with or without mating disruption (MD). Traps were exposed simultaneously to 160 males released into each of two 1000 m3 rooms.

For the second experiment, in two preliminary trials examining trap capture with 1 mg septa after 40 males were released into 73 m3 rooms without mating disruption, 38 males were captured each time from the room which was subsequently treated with mating disruption, and 34 and 35 were recaptured

from the other room. These observations suggest that the number of males captured was similar between the two rooms in the absence of mating disruption, and that any possible difference did not result in fewer males captured in the room that subsequently received mating disruption treatment. After applying mating disruption treatment of one of the rooms, fewer males were captured overall in traps the treated room (27 ± 1.7 v. 33 ± 5.4) (mean ± SE) and conversely more were captured in the oil pans placed on the floor to protect the release jar (6 ± 1.7 v. 3 ± 1.9). These differences were not statistically significant ($t4 = 4$; $P > 0.05$). Males captured in traps were significantly more likely to be captured in the blank trap or the trap baited with unmated females in the untreated room compared to the room treated with mating disruption (Wald $\chi 2 = 25.5$, $df = 1$; $P < 0.001$) (Fig. 2).

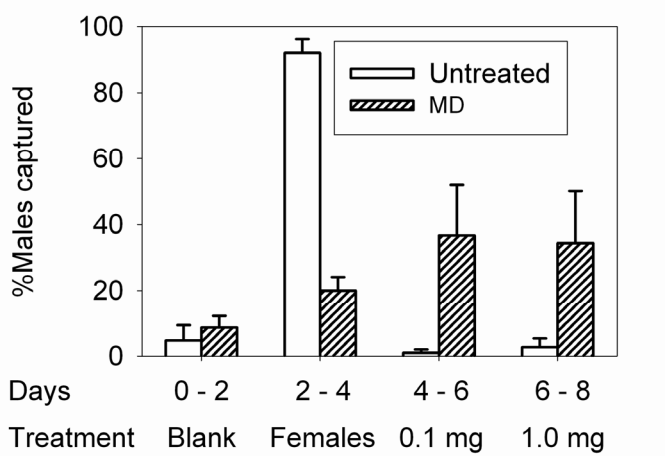

Figure 2 Percent (mean and SE) of *Plodia interpunctella* males captured by various lures in 73 m3 an untreated room, or one treated with mating disruption (MD). Males were exposed successively, over 2 day intervals, to wing traps baited with empty gray rubber septa (blank), unmated *P. interpunctella* females, or gray rubber septa loaded with 0.1 or 1.0 mg Z9,E12-14:Ac.

3. Discussion

These data demonstrate that pheromone permeation resulting in substantial suppression of males captured in traps baited with unmated females, *P. interpunctella* males can be nonetheless be captured in traps baited with septa containing Z9,Z12-14:Ac over a wide range of concentrations. In this study, traps with septa containing as little as 0.1 mg captured males not captured by traps containing unmated females, and trap capture increased as septa loads were increased to 10 mg. Many commercial lures contain 1 mg of Z9,Z12-14:Ac (Nansen et al., 2006). Among lepidopteran orchard pests managed with mating disruption, pheromone lures with loads higher than those usually used for monitoring are useful in the presence of mating disruption against *C. pomonella* but not G. molesta (Roelofs et al., 1973; Charmillot, 1990; Kovanci et al., 2005). These data indicate that *P. interpunctella* is more like *C. pomonella* than *G. molesta* in this regard, and even suggest that standard monitoring lures could provide useful information in structures treated with mating disruption.

The data in this study could alternatively be viewed as suggesting that the mating disruption product used was ineffective. Three observations suggest that this is not the case. First, mating disruption is generally density-dependent; i.e., it is usually more effective at lower population abundance (Cardé and Minks, 1995), and often lower amounts can be used with lower population abundance. We purposely used relatively high population densities to ensure that we could distinguish among treatments. Second, the reduction by mating disruption of the number of males captured in traps baited with unmated females seen in this study, 82%, is similar to the range of reduction in mating seen in a previous study of mating disruption targeted against *P. interpunctella* (Ryne et al., 2001). In that study, which found a range of 80-95% mating suppression over 24 h, no significant differences among treatments were observed when Z9,Z12-14:Ac, alone or with three other components, was applied in small arenas at rates of 0.005, 0.045, or 0.2 mg/d/100 m3 to population densities of 1 or 3 pairs/m3. These densities bracket the density

of males in the 73 m3 rooms in the current study. Subsequent studies in industrial settings found evidence of long-term population reduction of P. interpunctella (Ryne et al., 2007), as well as *Cadra cautella* (Walker) and *Ephestia kuehniella* (Zeller) (both Lepidoptera: Pyralidae) (Ryne et al., 2006; Sieminska et al., 2009), when dispensers emitting 2-3 mg/d were used for every 100 m3 of treated space. Third, while CheckMate SPM dispensers are not currently registered for use in California, data supporting their efficacy has been obtained in other jurisdictions (C.C.R., unpublished data).

In other species of Lepidoptera, an increase in response of males to sex pheromone has been observed for the first several days after eclosion (McNeil, 1991). It is therefore possible that, in the second experiment, age as well as behavioral response to different doses contributed to the observation that traps with baited with synthetic pheromone lures captured males not captured when the traps were baited with virgin females. In the first experiment, however, younger males (0-2 days post eclosion) were exposed to lures loaded with 1 mg of Z9,Z12-14:Ac (the upper dose in the second experiment) and were captured in similar numbers in the presence or in the absence of the mating disruption treatment. Thus the two experiments offer reinforcing evidence that a mating disruption treatment sufficient to substantially suppress male capture in traps baited with virgin females was much less effective at suppressing male capture with lures loaded with 1 mg of Z9, Z12-14:Ac.

This and previous studies provide seemingly contradictory data concerning the effect of concentration of Z9, Z12-14:Ac on attraction of *P. interpunctella* males. On the one hand, septa with pheromone loads of 0.01 to 2 mg all captured males as effectively as calling females when known numbers of unmated males were released in a building with a grid of traps (Nansen et al., 2006). On the other hand, when a 1.46 m3 wind tunnel was used to examine responses to gel drops containing 2.4 or 4.8 mg Z9, Z12-14:Ac, unmated males examined for 15 minutes were significantly less likely to fly upwind, land on a container platform, or make contact with the gel drop with 4.8 compared to 2.4 mg (Nansen and Phillips, 2004). In the current study, using known concentrations of males in a large structure over three nights, we found more males captured in sticky traps baited with septa containing 10 mg Z9,Z12-14:Ac compared to 1 mg. This apparent discrepancy may be due to differences in time and scale between short term wind tunnel assays and assays on a larger scale and over longer time in industrial- or pilot-scale structures. It may also be that *P. interpunctella* males approach concentrated pheromone sources closely enough to be captured on sticky traps, but not closely enough to make contact with lure-and-kill formulations.

A recent simulation study (Byers, 2007) and reviews (El-Sayed et al., 2006, 2009) suggest that, for attractive pheromone formulations, mass trapping or lure-and-kill formulations should reduce populations more efficiently than mating disruption. Particularly for mass trapping, it is useful to maximize the effective attraction radius (EAR) of lures (Byers et al., 1989, El-Sayed et al., 2006). Some previous work has examined lure load for P. interpunctella in the context of optimizing the ability to locate sources of infestation using a trap grid (e.g., Nansen et al., 2006), for which a smaller EAR might be beneficial. However, other studies indicate reduction of population of pyralid stored product moths over time using commercial lures loaded with ≤2 mg Z9,E12-14:Ac with sticky traps (Pierce, 1994) or funnel traps (Trematerra and Gentile, 2010).

» Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Genetic structure of *Tribolium castaneum* populations in mills

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Abstract

We investigated the genetic diversity and differentiation among nine populations of *Tribolium castaneum* using eight polymorphic loci, including microsatellites and other insertion-deletion polymorphisms (="indels"). Samples were collected in food processing/storage facilities located in Kansas, Nebraska, California, Louisiana, Florida and Puerto Rico. Standard population genetic analysis was applied, and an assignment test was used to assign individuals to their genetic population. All loci were polymorphic across populations, with the number of alleles per locus-population combination varying from three to fourteen. Among 72 locus-by-population combinations, 31 deviated significantly from Hardy-Weinberg equilibrium, which was associated with a deficiency in heterozygosity. *Tribolium castaneum* populations show some level of genetic structuring. Genetic differentiation between populations, using F_{ST} estimates, was significant, with F_{ST} varying from 0.018 to 0.149. AMOVA indicated that 8.32% of the variation in allele frequency resulted from comparisons among populations. Genetic distance was not significantly correlated with geographic distance. Correct assignment to the genetic population was possible in only 56% of all individuals. Together, these results revealed that geographically distinct populations of *T. castaneum* had low to moderate levels of genetic differentiation that was not correlated with geographic distance, and the genotypic profile of the individuals did not provide enough information for fingerprinting them with their source population.

Keywords: *Tribolium castaneum*, Population genetics, Genetic structure, F_{ST}, Genetic fingerprinting

1. Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the major pests of wheat and rice mills and has a worldwide distribution. Its distribution and long association with stored food suggests that passive movement through human activities is an important determinant of population structure in both large and small spatial scales. *Tribolium castaneum* is capable of flight and can be captured far from storage locations, but appears to disperse by flight less readily than some other stored-product pests. Monitoring studies in flour mills suggest that *T. castaneum* populations are relatively self contained within individual flour mills and that these populations go through frequent genetic bottlenecks due to regular structural fumigations (Campbell and Arbogast, 2004). This suggests that *T. castaneum* populations within mills may be genetically differentiated populations. Genetic analysis of *T. castaneum* populations from mills would enable us to develop a better understanding of population structure of this important pest, and could potentially be useful in developing tools that would facilitate the identification of sources of infestation in food distribution channels.

The potential for more accurately differentiating populations by using genetic approaches and assigning individuals to their source population exists due to the recent advances in *T. castaneum* molecular studies. The recent genome sequence (*Tribolium* Genome Sequencing Consortium, 2008) makes possible the development of more appropriate molecular markers (Demuth et al., 2007) by providing an extensive collection of markers for screening, from which selection can be made based on characteristics such as polymorphism, codominance and abundance throughout the genome.

Here, we summarize part of a study investigating the genetic structure of nine *T. castaneum* populations collected from wheat and rice mills throughout the USA using microsatellites and other insertion-deletion polymorphisms (i.e., indels). Specifically, we wanted to determine if there was genetic structure

to these populations, if levels of differentiation were associated with geographic distance, and if it was possible to accurately assign individuals to their source population.

2. Materials and methods

2.1. Sample collection

Tribolium castaneum was collected from rice and wheat mills across the United States and the territory of Puerto Rico (Table 1). Beetles were collected using Storgard Dome traps baited with *Tribolium* spp. pheromone lure and food oil (Trécé, Adair, OK, USA). A sample from each location (≥30 beetles) was obtained from a collection of individuals captured in multiple traps distributed throughout each facility during the same monitoring period (~2 wks) and should represent an unbiased proportion of the population present at the period of collection. After being collected, beetles were kept in 1.5 mL centrifuge tubes with 75% ethanol, and frozen at -80°C until the DNA could be extracted.

Table 1 Collection details and summary statistics for the nine populations of *Tribolium castaneum.*

Population sample ID	Location	Sampling period	Sample size	H_{exp}*
SaCA1	Sacramento, CA	July, 2007	32	0.64 (0.15)
SaCA2	Sacramento, CA	July, 2007	31	0.67 (0.10)
FrNE	Fremont, NE	July, 2007	32	0.67 (0.12)
OmNE	Omaha, NE	July-Aug, 2007	31	0.66 (0.15)
MhKS	Manhattan, KS	Aug, 2007	30	0.60 (0.20)
HdKS	Hudson, KS	Feb-Mar, 2005	36	0.61 (0.19)
LcLA	Lake Charles, LA	Aug, 2007	32	0.68 (0.11)
TpFL	Tampa, FL	July, 2007	32	0.68 (0.14)
GnPR	Guaynabo, PR	Aug, 2007	32	0.73 (0.12)

*H_{exp}, expected heterozygosity. Values represent mean and standard deviation across all loci.

2.2. DNA extraction and fragment analysis

A modified protocol for DNA extraction and PCR amplification was developed to deal with issues associated with using beetles collected from pheromone traps. Genomic DNA from individual beetles was extracted from entire specimen homogenates using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). Genotypes were determined based on eight unique polymorphic loci including six microsatellites (MS1 through MS6) and two other insertion-deletion polymorphisms (ID1 and ID2). These molecular markers, which were either a result of our own screening of the *T. castaneum* genome or based on the literature (Demuth et al., 2007), were unique, distributed across multiple linkage groups, and confirmed to be polymorphic. For high-throughput genotyping, fluorescent labelled PCR fragments were produced using a M13 oligonucleotide adaptor sequence attached to the 5' end of the forward primers that allowed the incorporation of the fluorescent dye to the fragments. Allele sizes at each locus were scored using an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA) and GeneMarker version 1.85 software (SoftGenetics, State College, PA, USA).

2.3. Statistical analysis

For each population-by-locus combination, the expected and observed heterozygosities were calculated using GDA v. 1.1 (Lewis and Zaykin, 2001). Linkage disequilibrium and deviation from Hardy-Weinberg equilibrium were tested using GENEPOP v. 4.0 (Raymond and Rousset, 1995). For multiple comparisons, sequential Bonferroni was applied to determine the significance level (Rice, 1989). The genetic structure of the populations of *T. castaneum* was determined on the basis of analysis of molecular variance (AMOVA) using Arlequin v. 3.11 (Schneider et al., 2000) with two levels of hierarchy, among and within populations. Since the presence of null alleles and the failure to account for their presence can underestimate the within-population genetic diversity and overestimate differentiation among populations (Avise and Dakin, 2004), we used the software Freena (Chapuis and Estoup, 2007) for the estimation of F_{ST} values. Isolation by distance was investigated with Mantel test using semi-matrices of genetic distance ($F_{ST}/1-F_{ST}$) and of geographic distance (ln (km)) as implemented in GENEPOP. Finally, we used an assignment approach to verify the likelihood of correctly assigning individuals to their population of origin. The assignment test was carried out using the software GENECLASS 2 (Piry et al., 2004).

3. Results

All loci included in this study segregate independently, since no significant linkage disequilibrium was observed in any of the pair-wise comparisons. Parameters used to describe the genetic diversity of *T. castaneum* populations showed a wide range of values for most loci and populations. There was a great level of individual genetic variability within populations for most loci. Observed heterozygosity ranged from 0.063 to 0.839 and tended to be, in many cases, lower than the expected. These values were within the range found for other populations of *T. castaneum* (Demuth et al., 2007; Drury et al., 2009), and other species of beetles (Brouat et al., 2003; Schrey et al., 2008). Expected heterozygosities varied among populations, with values ranging from 0.60 to 0.73 (Table 1). Many locus-by-population combinations (31 of 72) showed significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction ($P < 0.01$), caused by a deficit in heterozygotes.

All eight loci were polymorphic in all populations, with the number of alleles per locus within populations varying from three to fourteen. Allele frequencies were highly variable among populations regardless of allele size or locus with frequencies ranging from 0.00 to 0.867. Most alleles were found to be common to more than one population, but when considering all loci at least one private (i.e., only occurring in one population) allele was present in each population. Private allele frequencies within populations ranged from 0.0156 to 0.391. The population from Puerto Rico, GnPR, had the greatest number of private alleles (total of eight). Null alleles (i.e., undetectable alleles due to factors such as mutations at the primer sites) were estimated to be present in all loci tested (mean frequency in each population ranged from 0.08 to 0.16).

Analysis of molecular variance (AMOVA) showed a significant level of genetic differentiation ($P < 0.01$) among the populations (among populations: d.f. = 8, sum of squares = 88.36, variance component = 0.14; within populations: d.f. = 571, sum of squares = 921.26, variance component = 1.61): among populations accounted for 8.32% of the total variance. After correction for the presence of null alleles, corrected F_{ST} values ranged from 0.018 to 0.149 (Table 2, upper diagonal), with the Global pair-wise F_{ST} for all loci and population pairs being 0.082. To evaluate how much of the variation among populations could be explained by the distance between mills, the correlation between genetic and geographic distance was evaluated. There was no significant correlation between geographic distance (Table 2, lower diagonal) and genetic distance ($F_{ST}/1\text{-}F_{ST}$) using Mantel test ($P = 0.61$). This resulted in a lack of isolation by distance which means that the increase in geographic distance did not result in an increase of genetic distance in most cases. For example, the F_{ST} value for the pair-wise comparisons between FrNE and MhKS (distance = 247 km), LcLa and TpFL (distance = 1072 km), and SaCA1 and FrNE (distance = 2148 km) were 0.069, 0.064, and 0.059, respectively.

Table 2 Pair-wise F_{ST} values (upper diagonal) and geographic distances in km (lower diagonal) for populations of *T. castaneum* with a global F_{ST} of 0.082.

	SaCA1	**SaCA2**	**FrNE**	**OmNE**	**MhKS**	**HdKS**	**LcLA**	**TpFL**	**GnPR**
SaCA1		0.051	0.059	0.097	0.106	0.094	0.078	0.107	0.073
SaCA2	< 0.3		0.027	0.075	0.081	0.093	0.059	0.109	0.069
FrNE	2147.7	2147.7		0.071	0.069	0.060	0.018	0.078	0.025
OmNE	2194.7	2194.7	49.9		0.089	0.145	0.107	0.149	0.094
MhKS	2152.6	2152.6	247.0	236.4		0.149	0.091	0.118	0.096
HdKS	1990.1	1990.1	412.9	422.2	217.3		0.075	0.116	0.073
LcLA	2746.7	2746.7	1277.2	1249.6	1042.5	1007.2		0.064	0.038
TpFL	3787.1	3787.1	1967.4	1922.3	1806.5	1880.4	1071.7		0.040
GnPR	5762.5	5762.5	3852.4	3803.7	3731.9	3834.2	3030.0	1975.5	

To determine how well individual beetles could be assigned to their population of origin, we used a GENECLASS-based assignment test. Unfortunately, only 56% of the individuals were correctly assigned. When the Bayesian probability was averaged among all individuals within a population, the values ranged from 0.429 ± 0.06 to 0.596 ± 0.06. These values indicate that the likelihood of correctly assigning individuals to their population of origin varies among the populations in this study.

4. Discussion

Results of this study provide evidence of genetic differentiation among populations of *T. castaneum* in different mills. Even though significant among-population variance was detected, it was relatively low (8.32%) considering our expectations of low gene flow at least among some of the populations we used in this study. Conversely, the significant and high within-population variance detected by the analysis of molecular variance (AMOVA) is a characteristic of microsatellites because of their highly polymorphic nature (Carbonnelle et al., 2007), which may increase the genetic diversity within a population and may reduce the power of differentiation among populations. Analysis of other systems has also detected low but significant among-population variance when using these types of markers (Paupy et al., 2004; Roos and Markow, 2006).

Drury et al. (2009) analyzing microsatellites from other populations of *T. castaneum*, including populations from other countries, found F_{ST} values that ranged from 0.0289 to 0.353 with a Global pair-wise F_{ST} of 0.18. When they selected one population from Africa, Central America, South America, and North America and compared them, the average pair-wise F_{ST} was 0.09. Although they used a larger number of different microsatellite loci, the Drury et al. (2009) study F_{ST} values were only slightly greater than in our study, when including only populations in the USA (F_{ST} = 0.127). In both studies, most pair-wise comparisons had low to moderate levels of differentiation. According to Balloux and Moulin (2001), a value lying between 0-0.05 indicates little genetic differentiation; 0.05-0.15, moderate differentiation; 0.15-0.25, great differentiation; and above 0.25 indicates very great genetic differentiation. A difference between these two studies is that all of our beetles were collected directly from natural populations, while in Drury et al. (2009) there were populations that came from laboratory colonies, some of which had been collected more than 20 years earlier.

The low genetic differentiation among populations was also indicated by the lack of relationship between geographic distance (ln (km)) and genetic distance (F_{ST}/1-F_{ST}). Drury et al. (2009) also did not find a significant correlation between genetic distance and geographic distance, even though they had a large range of geographic distances. These findings could indicate that our markers are not appropriate for detecting population differentiation at these scales or that sufficient individuals are moving among populations even at a national and global scale that sufficient genetic differentiation cannot occur. Across these broad geographic distances this movement is likely to be human aided dispersal. Anthropogenic transport of commodities such as flour has been shown to play an important role in mixing populations of stored-product species (Ryne and Bensch, 2008). Our population locations were selected so that we would have different predicted levels of potential human movement among locations. For example, we predicted greater differentiation among populations from rice mills and wheat mills or between West Coast and Midwest mills, but this was not observed. Other factors could also play important roles in determining levels of differentiation, such as, the maternally acting selfish genes (MEDEA) (Beeman et al., 1992). Another possibility is that the likelihood of genetic mixture of these populations is increased by a combination of human aided dispersal of infested materials for long distances and active dispersal by the beetles themselves from surrounding areas into the mills.

The lack of strong differentiation among populations contributed to our inability of assigning all the individuals to their population of origin with accuracy (only 56% were correctly assigned). Although our inability to assign individuals may have been caused by the lack of unique genotypes shared by individuals of the same populations, it could also have resulted from the presence of null alleles. Null alleles can affect assignment tests by reducing the power of the tests and consequently the proportion of correctly assigned individuals, and this effect will be stronger if the total number of loci is low (Carlsson, 2008). It is still possible that alternative markers or loci can be found that could eventually be used for assigning beetles to source populations. A better understanding of population structure and gene flow can improve our pest management programs by determining the level of movement of individuals among populations and the spatial scale of movement. Moreover, it can help with the evaluation of IPM program effectiveness and resistance management by allowing the discrimination between individuals that originate from rebounding of local populations, or other founder populations.

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Molecular markers for *Psocoptera* species identification

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Abstract

The genus *Liposcelis* (Psocoptera: Liposcelididae) is commonly associated with stored products all over the world. Species identification is not an easy task because most psocids are very small and morphologically similar. In this study we examined partial cox1 gene sequences (COI) as barcodes and AFLP (Amplified Fragment Length Polymorphism) markers for psocid species characterization and distinction. About 30 specimens of males and females of each of the following species were analyzed: *Liposcelis bostrychophila*, *L. brunnea*, *L. corrodens*, *L. entomophila*, *L. fusciceps*, *L. paeta*, *L. pearmani*, and *L. rufa*. DNA extraction followed the standard protocol from the Qiagen DNeasy tissue extraction kit. DNA quantification by nanodrop was between 60 and 200 ng of DNA. A good PCR amplification was obtained by both techniques. The primers used for COI were HCO and LCO; for AFLP we used the dominant markers FAM, NED, and JOE. After the selective amplification, the DNA sequences and fragments were analyzed. The sequences of COI are still being studied and will be deposited in the GenBank. The AFLP markers grouped the species in the same subgroups formed by morphological characters. We concluded that both molecular techniques represent useful tools for the identification and distinction of psocid species.

Keywords: AFLP, Liposcelididae, Psocids, Stored-product pests.

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Tricorynus rudepunctatus (Pic) (Coleoptera: Anobiidae): diagnosis and damage

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Abstract

The objective of this research was to identify and study a species of Anobiidae that causes great damage and is a cause of concern as an urban pest in Brazil. This species has been found infesting wood, furniture, doors, books, insect collections, tea, dried fruits, handcrafts, and many other commodities. Inspections were done in houses and storehouses in the city of Curitiba, PR, Brazil in order to collect objects and materials that present signs of anobiid attack. The only species identified was *Tricorynus rudepunctatus* (Coleoptera: Anobiidae). There is only one reference to this species in the central region of Brazil. Another anobiid, the book pest *Tricorynus herbarius* has been recorded attacking books and historical documents and *Tricorynus* sp. attacking forest trees, but it was not recorded in our survey. Usually, the damage caused by *T. rudepunctatus* is mistaken with damage by termites; and when the insect is collected it is frequently misidentified as *T. herbarius* or as the cigarette beetle, *Lasioderma serricorne* or even as *Stegobium paniceum*, the drugstore beetle. Some morphological characters useful to identify *T. rudepunctatus* are: oval body about 2.7 mm long; dark brown with smooth hairs all over the body; head concealed under the pronotum; 10-segmented antenna with the three apical segments forming a 3-segmented loose club; elytra with two grooves at the posterior edge; fore femur with a transversal line on its anterior face; pro and mesotibia with two distinct striae; metasternum longitudinally carinate in the middle. Adults and larvae bore inside the materials, forming galleries and producing a coarse powder.

Keywords: Anobiids, Insect identification, Morphological characters, Urban pest.

1. Introduction

The members of the genus *Tricorynus* Waterhouse, 1849 (Coleoptera: Anobiidae: Dorcatominae) were previously included in the genus *Catorama* Guérin-Meneville, 1850. According to White (1965), the name *Catorama* was used for more than 100 years, however it was synonymized with the older name *Tricorynus*, which was described by G. R. Waterhouse in 1849, based on the type species *Tricorynus zeae* from Barbados Islands.

There are 131 species of *Tricorynus* described for the world in the genus, most of them occurring in the southwest United States; in Texas, New Mexico, Arizona, and California. The fauna from Central and South America has not been well studied and may contain many more new species of *Tricorynus*, as pointed out by Maurice Pic who described numerous species of Coleoptera from the Americas in the early 1900s. Since his descriptions were very brief and vague, most of Pic's names for the species of *Trycorinus* were lately reviewed and reassigned by White (1965, 1981).

The complexity of the taxonomy of the genus *Tricorynus* has been clearly demonstrated in the paper by White (1965). The author mentions that only about one third of the 6000 specimens loaned from North America could be determined at species level, and about 40% of them were misidentified. Eleven species of *Tricorynus* were mentioned by White (1981) as occurring in Brazil, as well as *Tricorynus herbarius* (Gorham, 1883) reported by Silva et al. (2004), but there have been no further studies on their distribution or bionomics.

There is a lack of references concerning the behavior and biotic associations among the species of *Tricorynus*. Most publications refer to *T. herbarius*, the Mexican book beetle, as an urban pest damaging books, leather, stored products, furniture and other wood goods. Another species, *Tricorynus tabaci* (Guérin-Méneville, 1850), is mentioned as an economically important pest feeding on tobacco seeds in the tropics (Peck, 2009).

Species of *Tricorynus* are usually confused with each other and with other anobiid genera and species such as the cigarette beetle *Lasioderma serricorne* (F., 1792) and the drugstore beetle *Stegobium paniceum* (L., 1761). People usually mistaken the damage by *Tricorynus* as that caused by termites.

The species *Tricorynus rudepunctatus* (Pic, 1904) is included among the species described by Pic from the county of Jataí, state of Goiás, in the Midwest region of Brazil, and reviewed by White (1981). In this paper, we identified the anobiid species causing extensive damage in southern Brazil as *T. rudepunctatus*. This species is a small brown beetle, oval in shape and with smooth hairs covering the body, morphologically similar with many other anobiids, which results in frequent misidentification. Similar to other members of the subfamily Dorcatominae, this species conceals head and appendices (legs, mouthparts, and antennae, except for the last antennal segment) under the body, allowing it to bore and hide in the wood and other materials.

The objectives of this study were to collect, identify and present morphological data about *T. rudepunctatus* found infesting and causing extensive damage in wood doors, furniture, books, insect collections, stored products, and other house goods in southern Brazil.

2. Materials and methods

Materials infested with anobiids were collected sporadically in houses and commercial buildings in the city of Curitiba, state of Paraná, southern Brazil, over two years. The insects were removed from wood (doors, furniture), straw chairs, crafts made with different materials, stored food (tea, soybeans, barley, packed foods), and insect collections (from the dead insect carcass and in the cork or foam plastic bottom). All collected material were taken to the laboratory and the insects were either pinned or fixed in 70% ethanol after being boiled in 20 volumes of oxygenate water. Adult specimens were dissected to analyze genitalia and different body parts. Vouchers were deposited in the Pe. Jesus Santiago Moure Entomological Collection (DZUP) of the Universidade Federal do Paraná.

Adults were dried and mounted on stubs and analyzed under electronic scanning microscope in the Electronic Microscopy Laboratory. Descriptions based on morphological characters observed by optical and scanning microscopy are presented as well as an adapted key based on White's (1981) key.

3. Results

All samples analyzed that were damaged by anobiids contained exclusively *T. rudepunctatus*. A detailed description based on external adult morphology analyzed by optical and scanning microscopy follows. Some larva characters are also given. Morphological characters and damaged materials are illustrated and a brief key contrasts adults of *T. rudepunctatus* with other *Tricorynus* species mentioned for Brazil.

Parasitoids in the Bethylidae family were recorded in our samples associated with this anobiid species; however, they are not considered good control agents because they sting painfully forming long lasting rash in people and domestic animals.

3.1. Description of T. rudepunctatus

3.1.1. Larvae (Fig. 1.)

They are C-shaped scarabaeiform, creamy white, integument is soft; head is distinctly sclerotized and hypognata. Body is subcylindrical, not particularly sclerotized, but with small dorsal hooks on most segments; three pairs of well developed legs with distinct claws. The head capsule is suboval and larger at the middle portion; there are many long setae and the area behind the epistome is well pigmented; the antennae are reduced without distinct segments; the stemmata are apparently absent. Mouthparts are well developed; mandibles with one or two teeth; lacinia lobed with two spines, about the same size as the galea, which is also lobed with strong spines at the apex; maxilar palps are claw like with three articles. Ten abdominal segments are present; thoracic and abdominal spiracles vary in shape and size. Pre-tarsus is sclerotized with setae; the claws are curved and the arolium may or may not be present.

Figure 1 Larva of *Tricorynus rudepunctatus*.

3.1.2. Adults (Fig. 2 a, b, c, d)

Small, about 2.7 mm, subcylindrical, oval almost globular beetles; reddish brown dorsally with head and abdomen slightly darker than metasternum; antennae, palps, and tarsi orange. Body covered with fine, silky hairs; dorsal cuticle with small punctures uniformly distributed mixed with sparse large punctures; large punctures are present on the head, laterally on the pronotum, on the elytra (sparse at the base), metasternum and abdomen.

Head is opisthognathus, deflexed under the pronotum, round with two rows of very close punctures usually coalescent. There is a distinct suture under the insertion of the antennae, this margin is carinate and extend diagonally towards the anterior margin of the eyes. Compound eyes large, round, well developed, but weakly bulging.

The antennae are 10-segmented with a loose 3-segmented club, sparsely pubescent, inserted right below the bases of the mandibles on the subocular suture. The first antennal segment is reddish brown punctuated and pubescent, triangular and twice longer than wide, larger at the apex; it is visible when the insect is resting. All the other antennal segments are yellowish; the second being much smaller than the first one, broadening towards the apex, length less than 2x the width. Segments 3 to 7 are cylindrical; some of them bear one or two long straight hairs. Segments 8 and 9 are triangular, broadening towards the apex, about the same size as the first one; segment 10 is large, narrow at the base and at the apex, and broad in the middle (Fig. 2 d).

The clypeus is distinct with an arcuate dorsal suture. The labrum is slightly reduced between the bases of the mandibles. The outer margins of the mandibles are arcuate, glabra and shiny, whereas the middle of the mandibles is depressed and pubescent. There are two teeth, the external is acute and the internal is obtuse. The maxillary palp is 3-segmented; the apical segment has parallel sides, 1.5 to 5x longer than wide. The labial palp is 3-segmented, with the apical segment triangular, 1 to 2x longer than wide.

The pronotum is hood like concealing the deflexed head, anterior margin distinct forming an almost straight angle with its laterals; the posterior margin is sinuous and may reach laterally the sides of the elytra. The pronotum is punctuated with larger punctures on the sides. The scutellum is triangular with narrow bases and arched sides. The prosternum is transverse, 5x wider than long, concave, and has a small ventral groove to hold the antennae. There is a fringe of long straight hairs and the coxal cavities are opened. The mesosternum is wide and horizontal, curved ventrally forming a hook like projection over the middle coxae separating them. The metasternum is carinate at the anterior third, and concave in the middle where a metasternal hook rests. There are large punctures on the central part.

The elytra cover the abdomen completely, with two distinct lateral striae or grooves on the elytral apex. The dorsal surface of the elytron is sculptured with punctures; the small ones are uniformly distributed, whereas the large ones are sparse on the bases and more distinct and closer together on the elytral apex. There are 9 or 10 rows of longitudinal yellowish striae along the surface of each elytron.

The legs are 5-segmented, uniformly covered with silky hairs. The first tarsomere is the longest, the other five are shorter and equally large. The fore coxae are triangular and contiguous; the middle and hind coxae are separated and transverse; the femur has a distinct transversal stria on the dorsum-anterior surface; the tibia is flat and has two grooves or striae.

The abdomen is five-segmented; the first segment is partially covered by the hind coxae and depressed laterally where the legs rest; the other four segments are distinct with straight or bisinuated sutures; there are uniform punctures on the surface.

The male genitalia is trilobed; the lateral lobes are curved externally, with toothed apices; the stylus is inserted laterally up to the middle portion; they are larger at the apex or about cylindrical and pubescent. The sides of the median lobe may be parallel, sinuate or narrow apically with long and sparse hairs laterally at the apex. The most important diagnostic characters of the male genitalia are the internal processes and the median lobe. The processes are either hook like or shaped as a tubercle; sometimes they bear two or three spines.

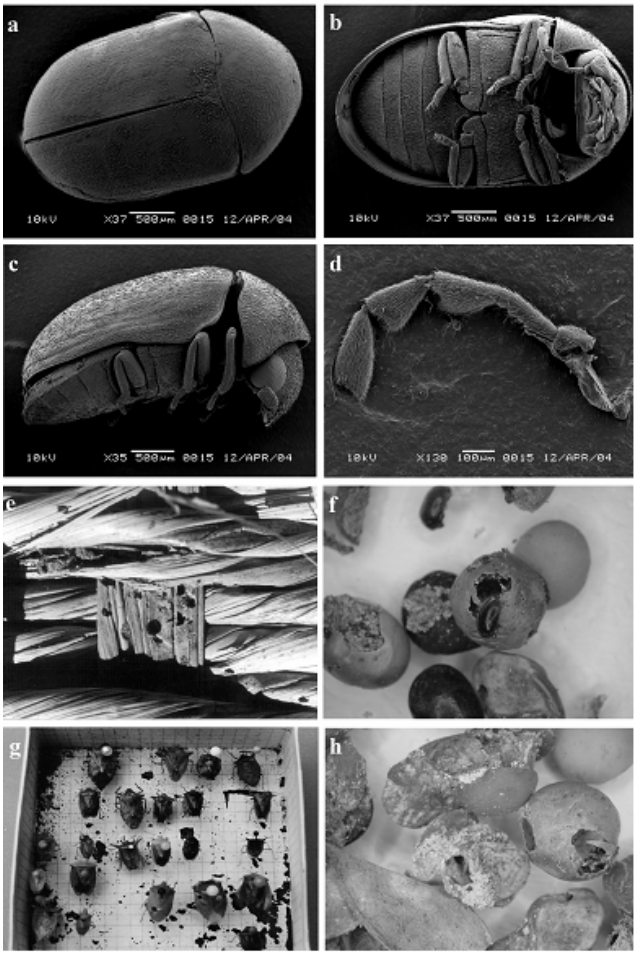

Figure 2 Adult of *Tricorynus rudepunctatus*: (a) dorsal view; (b) ventral view; (c) lateral view; (d) antenna. Damage caused by larvae and adults of *Tricorynus rudepunctatus* (e) straw; (f) soybean; (g) insects and cork bottom; (h) puparium formed with secretion holding damaged soybean seeds.

3.2. Damage (Figures 2 e-h).

Larvae and adults were found boring into the wood of doors and furniture, straw chairs and handcrafts, all sorts of grains, cereal and oil seeds, tea, dried fruits, and insect collection. They produce a coarse powder; the larva makes a pupal case with rest of materials glued together with a hard secretion.

4. Discussion

Our results showed that the identity of the anobiid species observed damaging wood, straw, and all sorts of stored materials in Curitiba, Paraná, is *T. rudepunctatus*. Thus, it has been erroneously assumed that the household damaging species in Brazil is exclusively *T. herbarius*, probably because reference collections and adequate descriptions are not available. There is only one reference to this species in Brazil – specimens from the state of Goiás, included in the Pic's material studied by White (1981).

Silva et al. (2004) reports the occurrence of the bibliophagous anobiid *T. herbarius* and presents biological data for this species reared on different diets. It was not possible to check the material studied by Silva to confirm the identification. The presence of *T. herbarius* was recorded in the libraries of Rio de Janeiro, São Paulo and Minas Gerais that hold many historical books (Guimarães, 1989). There are early reports on the attack of *T. herbarius* in books, mentioning those by Faria (1919), Sawaya (1955), Lelis (1980), and Carrera (1981).

The diagnostic characters of *T. rudepunctatus* are the metasternum rounded front to back, distinctly carinate at middle, eyes separated by about 1.7x vertical diameter of an eye, pronotum bulging above anterior margin, 9 or 10 rows of longitudinal yellowish striae along the surface of each elytron; it is usually smaller than *T. herbarius*. The main characters of *T. herbarius* that can be used to distinguish it from *T. rudepunctatus* are: metasternum not carinate, eyes separated by 1.8–2.4x vertical diameter of an eye, elytra with large punctures.

Based on the key by White (1981) for tropical species of *Tricorynus*, a brief key is presented below to distinguish among the 11 species he mentioned for Brazil plus *T. herbarius* mentioned by Silva et al. (2004).

Further studies are needed to determine taxonomic status, biological parameters bionomical data, and geographical distribution of *Tricorynus* species in Brazil. It is also necessary to establish effective and safe measures to control *Tricorynus* species attacking bibliographical materials, art pieces, furniture, and food in houses and public spaces.

All the 12 species assigned in the key are overall morphologically similar to most anobiids; they have 1 or 2 distinctly impressed lateral grooves at apical half of each elytron. Beyond this point, the species can be separate by the characters indicated in the key that follows.

1. Elytron with 1 of the lateral grooves distinctly impressed, or with lower groove stronger than the upper groove	2
- Elytron with 2 impressed grooves, about equally deep	4
2. Head just above eye with a deep, arcuate, transverse groove, pronotum with acute anterior angle, pubescence with a golden reflection, elytron at apex with a fine, impressed groove, above it with a stria of aligned punctures, length 3.7 mm.	*T. fulvopilosus*
- Not as above	3
3. Elytral apex at side with a 2nd upper groove, metasternum carinate at middle, large elytral punctures separated on an average by a little over diameter of a puncture, eyes separated by about 1.7x vertical diameter of an eye, pronotum at side bulging, length 3 mm	*T. distinctus*
- Elytral apex at side with but 1 groove, eyes smaller, weakly bulging, separated by nearly 2x vertical diameter of an eye, length 1.7-1.9 mm.	*T. unisulcatus*
4. Anterior tibia with 2 distinct grooves, metasternum distinctly, longitudinally carinate at middle, eyes separated by about 1.7x vertical diameter of an eye, pronotum bulging above anterior margin, body about 1.9 x as long as wide, metasternum rounded front to back, length 2.7 mm	*T. rudepunctatus*
- Tibia same as above; metasternum not carinate	5
5. Lateral elytral striae distinct at apex but not indicated at level of metasternum	
- Lateral elytral striae distinct at apex and weakly to clearly indicated at level of metasternum by shallow grooves or aligned punctures	6 9

6. Elytral apices distinctly produced, outline of elytral apex when see from above as a broad "W", eyes separated by 1.7x vertical diameter of an eye, pronotum at side inflated, length 2.3 mm — *T. caudatus*
- Elytral apices evenly round, otherwise not as above — 7

7. Pronotum at side with large punctures only, separated on an average by more than diameter of a puncture, small punctures absent, head with large punctures only, eyes separated by 1.5x vertical diameter of an eye, length 2.6 mm — *T. subplicatus*
- Pronotum at side not as above, head not as above, eyes separated by 1.6–2.0x vertical diameter of an eye — 8

The species *T. reitteri* and *T. minutissimus* share the following characters before they are separated by couplet 8: abdominal sutures not impressed, segments nearly flat front to back, punctuation at side of pronotum clearly to obscurely dual, length 2.0–2.4 mm, mesosternal hooklike process not produced, eyes smaller, weakly bulging, separated about 2x vertical diameter of an eye, punctuation at side of pronotum clearly dual, large punctures much larger than small punctures and denser.

8. Body primarily dark brown but with elytral apex, head, and abdomen more or less red brown, length about 2.4 mm — *T. reitteri*
- Body primarily red brown but with metasternum a little darker than remainder, length about 2.0 mm — *T. minutissimus*

9. Punctures of head clearly dual, of small, dot like punctures and larger, rimmed punctures, punctures at side of pronotum above anterior margin so dense that they are largely confluent — *T. brasiliensis*
- Punctures of head of one size, irregular in size, or obscurely dual — 10

The species *T. convexus*, *T. cribratus*, and *T. herbarius* share the following characters before they are separated by couplet 10: punctures at side of pronotum dual, distinctly impressed and clearly of 2 sizes.

10. Length about 4.0 mm, dark brown nearly throughout, apex of 5th abdominal segment narrowly produced — *T. convexus*
- Length about 2.4–3.5 mm, red brown nearly throughout, apex of 5th abdominal segment not produced — 11

11. Elytra with large punctures on disk showing no tendency to alignment in bands, eyes separated by 1.6x vertical diameter of an eye, length 2.8 mm — *T. cribratus*
- Elytra with large punctures on disk showing weak to distinct tendency to alignment in bands, large punctures of metasternum smaller, sparser laterally, not quite attaining side, metasternum behind anterior margin on each side of middle with a narrow, elongated fovea, eyes separated by 1.8–2.4x vertical diameter of an eye, length 2.7–3.5 mm — *T. herbarius*

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Hylotrupes bajulus (L.) (Col., Cerambycidae): nutrition and attacked material

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Abstract

Hylotrupes bajulus, attacks softwood utilising the cellulose contained in wood walls as food. The fibre is digested in variable percentages, depending on the type of analysis, 20 to 48% and, according to some authors, without the assistance of intestinal symbiotic microorganisms. Furthermore, there is published work referring to *Hylotrupes*, concluding that "starch" … "plays no role in the nutrition of the larvae". Nevertheless, considering that attacks of this species decrease with wood seasoning increasing and having been demonstrated, and that "lignin degradation products of spruce wood do not influence larvae development", it is possible to suppose that cell walls alone are not sufficient to feed this wood boring species. Furthermore, *Hylotrupes* larvae have chisel shaped mandibles, similar to those of powder post beetle larvae that feed on starch and need to pulverise the wood to access the cellular content. Preliminary research suggests an utilization of wood fibre as well as of starch by larvae of *H. bajulus*.

Therefore, the purpose of this research is to test the degree of digestion of wood fibre from different sources (sapwood or heartwood) and the possible role of symbiotic microorganisms. Larvae of *H. bajulus* were grown on synthetic diets made of purified wood fibre and/or starch as main components supplied with mineral and vitamin. Substrates and frass were analysed for fibre fractions, starch and acid insoluble ash, the latter used as an indigestible marker. Larvae purified DNA was analysed by means of metagenomics approaches carried out by direct retrieval and analysis of 16S rRNA gene sequences free of cultural bias in order to discover the bacterial diversity from larva alimentary channel alone. Larvae of *H. bajulus* seem be able to digest either fibre or starch, and a role for symbiotic bacteria is supposed.

Keywords: Cellulose, Starch, Frass, Mouth apparatus, Mandible

1. Introduction

The house longhorn beetle *Hylotrupes bajulus* L. (Coleoptera: Cerambycidae) is an important pest of structural timber. It mainly occurs in roof spaces, where summer temperatures become high enough to permit flight and egg laying. *Hylotrupes bajulus* larvae usually feed in the sapwood of the coniferous genera Pinus, Picea and Abies, but, in the late stages of infestation, can also penetrate in heartwood (Eaton and Hale, 1993). It is an insect of considerable size, reaching, as an adult, 20-30 mm in length. The larva, cream coloured, has the typical form of the Cerambicidae larvae and, fully grown, measures about 22-25 mm.

The female oviposits in cracks within the wood, choosing almost exclusively conifer wood, as it is attracted by α-pinene and β-pinene, volatile substances present in these plants. However, attacks on hardwood trees (e.g. poplar and willow) have also been recorded though it is suspected that these are probably due to misidentification of the larvae, very similar to those of *Trichoferus holosericeus* Rossi: the lignin of hardwoods, in fact, contains a toxic substance that does not allow the development of *Hylotrupes larvae* (Cymorek, 1981). Development lasts from 1 to 7-8 years, with peaks of up to 17 years, depending on the temperatures.

Damage can be detected even in very old structures, but, in this case, involves parts of the structure replaced in more recent years (Cymorek, 1973), as attacks of this Cerambicidae occur predominantly during the first 80 years of the use of the timber, subsequently decreasing markedly, and disappearing after 100 years.

Few insects can digest wood with their own enzymes and they are not very effective in their action. Precisely for this reason it seems that most wood-boring insects have evolved complex symbiosis with micro-organisms specialized in the degradation of wood components (Battisti, 2001).

Hylotrupes seems not to exploit the action of endo-symbiotic microorganisms for the digestion of cellulose: in fact, Falck (1930) states that it secretes an enzyme, cellulase, and can digest about 20% of the cellulose and hemicelluloses of attacked softwood, the remaining indigestible 80% being expelled with the faeces. Its long development cycle, like that of many wood-borers, clearly attests the difficulty in obtaining energy from cellulose and in using the few proteins present in the woody tissues in which it usually lives (Battisti, 2001). In fact, if proteins are added to the diet of the larvae, there is an acceleration of growth (Schuch, 1937) showing that lack of proteins in the diet can be a limiting factor. In the thirties and forties, research carried out on *H. bajulus* nutrition showed that, notwithstanding that this species digests cellulose (Falck, 1930), there are no micro-organisms in the gut and therefore the cellulase is endogenous (Muller 1934; Mansour and Mansour-Bek, 1934). Parkin (1940) concluded that Cerambycidae are able to use cell contents and carbohydrates of the cell wall including cellulose. Cazemier et al. (1997) demonstrated β-glucosidase and carboxy methylcellulasis (CMC-ase) activity in the whole *Hylotrupes* gut (but very high in the foregut), while only a low number of bacteria were present only in the midgut, suggesting the endogenous nature of the cellulolytic enzymes. Höll et al. (2002) found about the same amount of alpha-glucans (considered mainly starch) in faeces and in the alimentary substrate while a significantly greater amount of beta-glucans (result of partial break-down of cellulose during digestion of a wood diet) was present in the faeces. They concluded that starch might "play no role in the nutrition of the larvae" and that the presence of a high amount of beta-glucans in faeces indicates the digestion of cellulose and hemicelluloses and, at the same time, "a surplus uptake of wood into the digestive tract in order to acquire compounds such as vitamins and reduced nitrogen".

Concerning the assessment of *Hylotrupes* larvae microbiota biodiversity, it is well known that several micro-organisms cannot be grown readily in pure culture, and that culturing does not capture the full spectrum of microbial diversity of a given environment (Handelsman, 2004). For this reason, several culture-independent methods were designed to describe the phylogenetic diversity in several environments. Among the methods developed to gain access to the genetics and physiology of uncultured micro-organisms, metagenomics, the isolation of bacterial genomic DNA from an environment followed by its direct analysis, has emerged as a powerful identification technique (Handelsman, 2004).

In this study we applied a metagenomic approach to the analysis of the woodworm's gut microbiota. The aim of the present study was to investigate the alimentary habits of this insect, focusing on starch as a possible key nutrient for larvae and the presence of bacteria in the gut.

2. Materials and methods

2.1. Diet experiment protocol

In order to determine the use of starch, cellulose and hemicelluloses by the *Hylotrupes* larvae, four diets were prepared with different percentages of starch (0, 3, 14) and different fibre sources (heartwood and sapwood): sapwood-starch 0%, sapwood-starch 3%, heartwood-starch 3%, sapwood-starch 14%.

The artificial diet was prepared scraping off the wood in a fine powder, washing it to remove starch, drying it in an oven and mixing it with starch in the specified proportion, minerals and vitamins (1.5%), albumin (6%) and water and arranging it in a square container. After desiccation in a microwave oven, artificial diet cakes were sawn into blocks measuring cm 5 x 5 x 1. Each one was prepared with a hole of the same diameter as that of the larvae and weighed.

The larvae used in this experiment were about 6 month old and were obtained from laboratory-breeding blocks maintained in a temperature-controlled room at 27 ± 2°C and 75 ± 5% r.h. The larvae were extracted from breeding blocks, left in Petri dishes without food for 24 h, weighed, and transferred to the different substrates (1 larva/ block). Each block was isolated in a ventilated container and placed in a temperature-controlled room at 27 ± 2°C and 75 ± 5% r.h. Eight larvae were used for each diet.

After 30 d, the larvae were taken off the blocks, checked for vitality and weighed. The frass produced by each larva during this period of time was weighed, too. Both the frass and the substrate were analysed to assess starch and fibre content. Fibre fractions (neutral detergent fibre= NDF; acid detergent fibre= ADF; acid detergent lignin = ADL) were analyzed according to Goering and Van Soest (1970), and starch by an enzymatic method (AOAC Method 996.11). Hemicelluloses content was calculated as NDF minus ADF, and cellulose as ADF – ADL. All the data were expressed on a dry matter basis.

2.2. Statistical analyses

The statistical processing of the weight of frass product and the weight difference between beginning and end of the larvae feeding using the four different diets was performed using the ANOVAs one-way (univariate) method with the aid of the SPSS 15.0 program: analysis with the diets as the dependent variable was performed for the larvae percentage of weight increase and frass weight (independent variables).

2.3. DNA analyses

Fully grown larvae of H. bajulus taken from laboratory-breeding, were dissected, the separated guts were then isolated in Eppendorf tubes and frozen at -20°C. Prokaryotic and Eukaryotic DNAs were extracted from single guts and the purified DNA was used as target DNA for amplification of 16S rRNA gene.

The selective amplification of bacterial ribosomal RNA gene was achieved by using two universal primers for V2-V3 region of 16SrRNA gene: HDA1-GC (CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G-ACT CCT ACG GGA GGC AGC AGT)/HDA2 (GTA TTA CCG CGG CTG CTG GCA C) (Walter et al., 2000) used in the reaction mix (Megamix, Labogen s.r.l) at 0,5 µM final concentration.

A 35-cycle PCR will run in a GeneAmp 9700 (Applied Biosystems, Foster City, CA) using the following profile: 94°C for 1 min, 56°C for 1 min, and 68°C for 60 sec.

The resulting amplification product was a mixture of different bacterial ribosomal gene sequences.

DGGE (Denaturing Gradient Gel Electrophoresis) analysis was performed with a DGene System (Bio-Rad Hercules, California) using 0.8 mm 8% polyacrylamide gel ratio of acrylamide to bisacrylamide, (37.5:1), containing a 35 to 55% gradient of urea and formamide for amplicons obtained with primers Hda1-Hda2. In order to identify at the species level bacteria producing the DNA pattern in DGGE analysis, bands were excised from the stained gels with a sterile scalpel and eluted in 20 µL of sterile water overnight at 4°C. One mL of the eluted DNA of each DGGE band was used as target DNA in PCR reaction using the conditions described above. The resulting PCR products were purified using Wizard® SV Gel and PCR clean-up system (Promega Madison, WI, USA) and sequenced.

The amplified 16S rRNA gene were ligated into the pGEM-T easy vector (Promega) and then transformed by heat shock into chemically competent Escherichia coli cells strain JM109 (Promega). The colonies were screened for α-complementation of β-galactosidase by using X-gal and IPTG. Positive colonies, containing the plasmid with the ribosomal RNA gene, were selected (Fig. 1). Plasmid DNAs were extracted and the inserts were sequenced using the BigDye v3.1 sequencing kit (Applied biosystems). The sequences were loaded and ran on the ABI Prism 3100 Genetic Analyzer (Applied Biosystems) (Fig. 2). Finally sequence data were used in search for the closest known relatives to the partial 16S rRNA sequences obtained, using the BLAST and RDP programs.

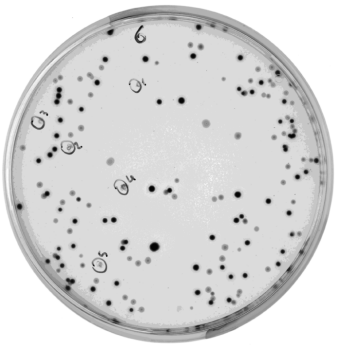

Figure. 1 A Petri dish showing blue and white colonies. The white colonies (positive) contain the plasmid with the desired fragment while the blue colonies (negative) contain the self-ligated plasmid.

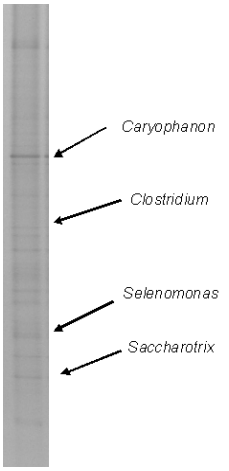

Figure 2 DGGE profile obtained from GI *Hylotrupes* larvae: band 1 corresponds to Caryophanon, band 2 to Clostridium, band 3 Selenomonas, band 4 to Saccharotrix.

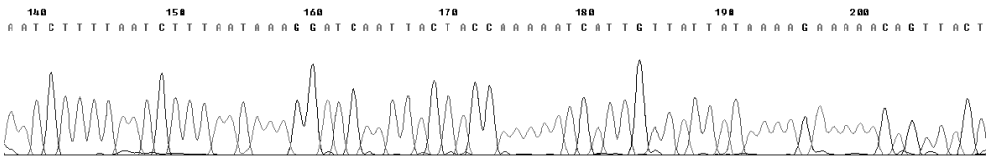

Figure. 3 Example of sequence obtained using the Genetic Analyzer ABIPrism 3100.

3. Results

3.1. Diet experiment

The statistical results of the diet experiment are summarized in Table 1. Larvae fed on diets poor in starch (0 and 3%) showed a weight variation (reduction of about 20%) statistically different (significant at P=0.01) from that (increase of about 0.6%) recorded in those fed on the diet rich in starch (14%).

Table 1 Summary table of analysis of variance on the average values of key variables examined in the various theses. F = F of Fischer; ns = not significant; * = significant per p< 0.05; ** = significant for p< 0.01. Mean values followed by different letters are different from the SNK (Student Neunman Keuls) for P<0.05. The values expressed as percentages were transformed for the compilation of statistical differences, in their angular values.

Diets	initial weight larvae (g)	weight increase (%)		frass weight (g)	
Sapwood 0%	0.2856	-17.73	b	2.839	a;b
Sapwood 3%	0.2865	-22.44	b	4.387	b
Heartwood 3%	0.3331	-17.78	b	3.516	a;b
Sapwood 14%	0.3265	0.632	a	2.052	a
F (significance)	0.288 ns	8.948 **		3.054 *	

The results of chemical analyses are reported in Table 2; all values are expressed as a percentage. NDF values show a similar quantity of fibres in the substrate and frass for all types of diet. The NDF value is obviously slightly higher in the frass because the insect used the soluble components present in the plant cells and therefore the frass-fibre is more concentrated. ADL values were used as indigestible markers in the digestibility estimate, as the amounts of acid-insoluble ash were negligible. The values of starch digestibility in the diets with 0% and 3% of starch were omitted because the amounts were too low to perform a correct analysis. In the diets with 3% of starch the digestibility of all fibre constituents is about the same. The fibre digestibility decreases with increasing amounts of starch in the diet.

Table 2 Digestibility of different fibre fractions and starch in the different diets. The values of starch digestibility in the diets at 0% and 3% of starch were omitted because the amounts were too low to perform a correct analysis; neutral detergent fibre= NDF; acid detergent fibre= ADF.

Fibre fractions	Sapwood 0%	Sapwood 3%	Heartwood 3%	Sapwood 14%
NDF	17.0	9,4	8,6	1,8
ADF	15.3	7,6	5,8	-0,1
Hemicellulose	28.8	16,6	18,9	8,7
Cellulose	23.0	11,5	8,6	-0,2
Starch	-	-	-	11,1

3.2. DNA analyses

DGGE profiles allowed us to identify bands related to the genera Caryophanon, Clostridium, Selenomonas, Saccharotrix. While, considering a partial sequence of about 450 bp of the ribosomal fragment and excluding the uncultured sample record presented in the database, we found sequences showing good homologies (usually with high coverage and max identity greater than 98%) with database records related to several genera: Streptococcus, Staphylococcus, Lactobacillus, Propionibacterium, Pseudomonas, Pelomonas, and Actinobacterium bacteria. Some sequences showed good homologies, but with 96% of max identity, with Gemella bacteria. Finally, with 90% of max identity, we also found some relations with Clostridium and Anaerosporobacter bacteria.

4. Discussion

Hylotrupes larvae, like those of *T. holosericeus*, the other Cerambycid that attacks timber, have chisel shaped mandibles (Schmidt & Parameswaran, 1977), similar to those of powder post beetle larvae that feed on starch and need to pulverise the wood to access the cellular content. The frass they produce is composed of extremely fine wooden fragments derived from the insect tunnelling mixed with faeces (elements cylindrical in shape). The fact that the frass is not made up of by faeces alone, as it is in the case of Anobiidae, proves that the larva does not ingest all the material it tunnels. This could suggest the idea that the larva operates a choice during feeding activity, separating what is necessary from what is not. In fact it seems that the wood powder/faeces proportion is much higher in larvae fed with starch-deprived diets. This is also the reason why we have analysed the whole frass and not only the faeces.

Contrary to what was suggested by Höll (2002), our results indicate that starch is utilized by Hylotrupes larvae as a nutrient source. In the 14% starch diet the larvae gained weight while with the other diets they lost weight. In addition, the total frass produced was less than in the other diets, even if not always significantly. This could demonstrate a lower tunnelling activity of the larvae in the case of higher starch content because of the adequate nutrients in the alimentary substrate.

A depressive effect of higher starch content on fibre digestibility is evident (from 17% of NDF digestibility in sapwood diet with 0% of starch to 1.8% in sapwood diet with 14% of starch) and is probably due to the presence of starch as a more readily available food source than cellulose and hemi-celluloses. This agrees with findings in higher animals with symbiotic micro-organisms (ruminants). However, two mechanisms can be postulated: a depressive effect of starch on symbiotic microorganisms with fibrolytic activity or reduced secretion of fibrolytic animal enzymes in the presence of significant quantities of starch in the diet. In addition, it cannot be excluded that starch availability lowers ingestion of fibrous fragments and then fibre digestion. Nevertheless, a certain digestibility of hemi-celluloses is maintained also in starch-rich diets and this could represent a relevant source of energy for the larvae, besides the starch.

DNA analysis highlights the presence of bacterial DNA in the Hylotrupes larval gut, confirming the results of Cazemier et al. (1997). Metagenomics involves the direct analysis of bacterial DNA extracted from a certain sample bypassing the need for culturing. Considering this, the bacterial 16S rRNA genes were selectively amplified from the extracted DNA by means of some universal primers, producing a mixture of fragments with different sequences that represents a picture, even though partial, of the real bacterial complexity in the starting samples. This mixture of fragments was analysed using two different methodologies: 1) DGGE followed by sequencing of some excised bands, and 2) cloning the PCR

products into a suitable vector, transforming them into a E. coli host strain and finally sequencing the resulting transformant clones.

The combined use of these two approaches shows that the bacterial diversity from Hylotrupes's gut is surprisingly high: indeed we determined the presence of bacteria related to several genera.

It is not surprising that we obtained different results with the different approaches considering that these analyses are still at the beginning, and up to now only a few DGGE bands and single clones (globally 32) have been sequenced.

Concerning the ribosomal DNA analysis, only a fragment of the 16S ribosomal RNA gene, was analysed, obtaining preliminary data. We think that more information about the genera and eventually the species present in Hylotrupes gut will be obtained by a complete sequencing of the different clones analysed.

Relating to the genera Gemella, Clostridium and Anaerosporobacter, it must be noted that the max identity is not very high, ranging from 90 to 96%. This could be due to the fact that the 16S sequence we found belongs to species that are not present in the available sequence databases, yet. Therefore, for these, we will not probably be able to find the true genera, but only the most similar to the obtained sequences, even completing the sequencing.

Finally with a preliminary analysis carried out using primers specific for cellulase encoding genes we also found some indirect evidences of the presence of the cellulosolytic species Bacteroides cellulosolyticus (unpublished data).

The presence of cellulosolytic bacteria and of bacterial cellulases ensures that the larva can digest cellulose at least by means of bacterial activity, in contrast to what has been suggested by the studies conducted so far.

Further studies will be needed to confirm the preliminary data obtained (separated faeces and wood powder analysis, cloning and sequencing etc).

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Resistance management and the ecology of *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) in subtropical Australia

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Abstract

Subtropical Australia is a demonstrated hotspot for phosphine resistance in stored product pests. *Rhyzopertha dominica* and *Tribolium castaneum* are common pests of stored grain in this region and management of these pests is increasingly impeded through the spread of resistance to phosphine, the most desirable control method. A number of field-oriented studies were conducted from the 1970's to 1990's to understand the ecology of these pests in subtropical Australia, including seasonal abundance, flight and population growth in stored grain. To manage the evolution and spread of resistance we require an understanding of movement of these beetles among foci of infestation. This paper presents preliminary analyses of two aspects of new research on these species: (1) a trapping program using pheromones to investigate beetle numbers in spatial and temporal contexts, and (2) characterisation of beetles leaving infested farm silos.

Adults of both species were trapped throughout the year with the lowest numbers corresponding to the coldest part of the year. The coldest trapping period had mean maximum and minimum temperatures of 21.1 and 3.5°C respectively. Trapping also revealed distinct differences between the two species, both in terms of numbers caught and where they were caught. In general, more R. dominica were caught than *T. castaneum*, similar numbers of *R. dominica* were caught near farm silos and in paddocks at least 1 km from the nearest silo, and more *T. castaneum* were caught near silos than in paddocks. Individual adults intercepted flying from farm silos are being characterised in the laboratory, and results to date show that these adults are long-lived, the females have mated before emigrating and are highly fecund. By undertaking research of the type summarised here we aim to develop an understanding of how these two species interact with their environment and how these interactions influence resistance development.

Keywords: Ecology, *Rhyzopertha dominica*, *Tribolium castaneum*, Australia

1. Introduction

The subtropical grain growing region of eastern Australia, comprising southern Queensland and northern New South Wales, is a demonstrated hotspot for phosphine resistance in stored product pests. Two of the most serious pests of stored grain, *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst), are common pests of stored grain in this region and management of these pests is increasingly impeded through the spread of resistance to phosphine, the most desirable control method. In 1997, a new type of resistance was detected in *R. dominica* in southern Queensland (Collins et al., 2002). After selection of the field strain the resistance factor was ca. 600 times for adults exposed to phosphine for 48 h (Collins et al., 2002), compared with a resistance factor of ca. 30 times for a strain considered to be typical at the time (Daglish, 2004). Currently, about 5% of *R. dominica* populations sampled from southern Queensland and northern NSW contain individuals with this strong resistance (P.J. Collins, personal communication), but the potential of the problem is illustrated by the Brazilian experience where strong resistance is much more common (Lorini et al., 2007). Currently 92% of *T. castaneum* samples from the region contain phosphine resistant individuals although the strength of resistance is lower than in *R. dominica* (M.K. Nayak, personal communication).

An understanding of the ecology of pest insects is critical to effective pest management (Walter, 2003). A number of field-oriented studies were conducted from the 1970's to 1990's to understand the ecology of these pests in subtropical Australia, and provided information such as seasonal abundance, flight and

population growth in stored grain (Sinclair and White, 1980; Sinclair 1982; Sinclair and Haddrell, 1985; White, 1988; Daglish, 2005). To manage the evolution and spread of resistance in *R. dominica* and *T. castaneum*, in particular, we require an understanding of their ecology, especially the movement of these beetles among foci of infestation. This paper presents preliminary analyses of two aspects of new research addressing this need: (1) a trapping program using pheromones to investigate beetle numbers in spatial and temporal contexts, and (2) characterisation of beetles leaving infested farm silos.

2. Materials and methods

Trapping was undertaken in a grain growing area in southern Queensland, Australia; where farmers typically grow a winter crop of wheat and a summer crop of sorghum. Bureau of Meteorology 98 year averages show that the coolest and hottest months are typically July (diurnal range 3.6-19.3°C) and January (diurnal range 19.5-33.2°C) respectively. Although rain can fall throughout the year it falls predominantly during summer.

Lindgren four-funnel traps (Contech, Delta, BC, Canada) baited with species-specific pheromone lures (Trece, Adair, OK, USA) were used to monitor flight activity of *R. dominica* and *T. castaneum* in the farm environment. Traps were located either near farm silos (silo traps) or at least 1 km from the nearest stored grain along fences dividing paddocks (paddock traps). Traps were located 1.5 m from the ground and trapped beetles were preserved in propylene glycol. Trapping occurred for one week periods at intervals of 4-6 wk from November 2008 to October 2009. Trap catches were transferred to alcohol in the laboratory, the numbers of *R. dominica* and *T. castaneum* adults were counted.

The mating status, longevity and fecundity of beetles intercepted emigrating from farm silos containing infested wheat were determined in the laboratory at 25°C, 55% r.h. A transparent plastic cone was attached to the base of each farm silo and beetles leaving from the bottom hatch area fell into a glass jar at the base of the cone. Individual beetles were removed immediately and put into individual containers of wheat (10 g). The containers of beetles were taken by car to the laboratory within 48 hours of collection during which time they were stored at ambient conditions. On arrival at the laboratory the containers were placed into controlled environment rooms. *Tribolium castaneum* individuals were placed into 250 g of wheat. The beetles were checked at weekly intervals for mortality and any live beetles were transferred to fresh wheat. Dead *T. castaneum* were sexed based on the presence of the sub-basal setiferous puncture on the anterior femur (Halstead, 1963), and R. dominica were sexed based on the genitalia (Potter, 1935). All containers of wheat in which beetles had been present were incubated for 11 wk and then sieved for the adult progeny.

3. Results

Bureau of Meteorology temperature data are shown in Figure 1. Mean monthly temperatures during the study tended to match long-term means except for maximum temperatures from July to October 2009 which were up to 5.1°C higher than average.

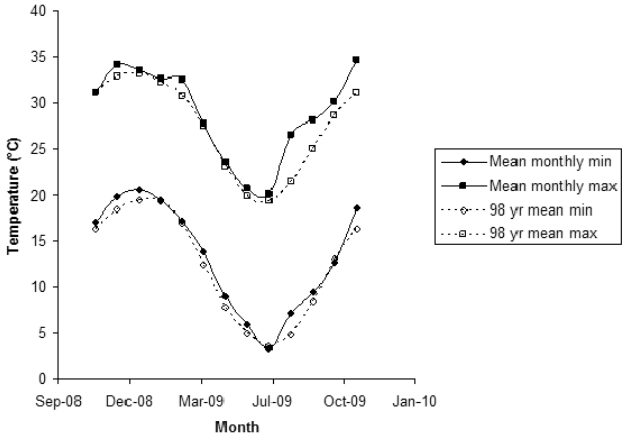

Figure 1 Ambient temperature data for Miles, Australia (source: Australian Bureau of Meteorology).

Figure 2 shows the results of trapping for *R. dominica* in seven sites where traps were located near farm silos and eight sites where traps were located in paddocks. Beetles were trapped throughout the study although few were trapped when traps were set in the winter months of June and July 2009. The mean minimum and maximum temperatures were 7.5 and 20.5°C during the June trapping period and 3.5 and 21.5°C during the July trapping period. The corresponding results for *T. castaneum* are shown in Figure 3. As with *R. dominica*, beetles were trapped throughout the study although few were trapped when traps were set in the months of June and July 2009. The results for *T. castaneum* contrast greatly with those of *R. dominica* in two ways. Mean trap catch tended to be lower and there was an obvious difference between the two categories of trap site, with more beetles being trapped in silos sites than paddock sites.

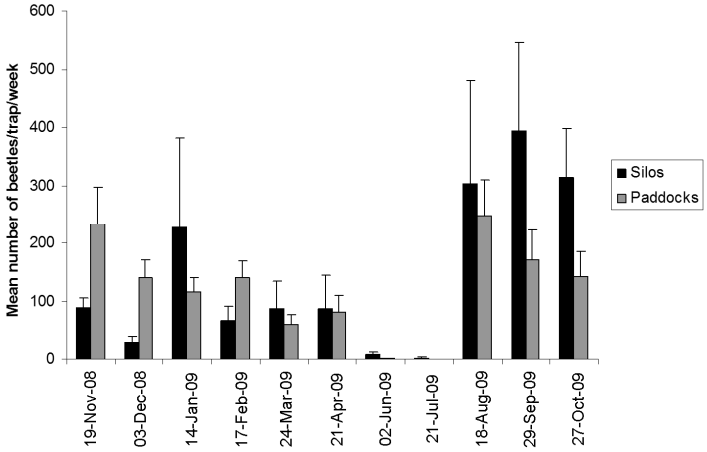

Figure 2 Number (mean & SE) of *Rhyzopertha dominica* adults trapped during 1 wk periods in traps placed near farm silos (n = 7) or in paddocks (n = 8).

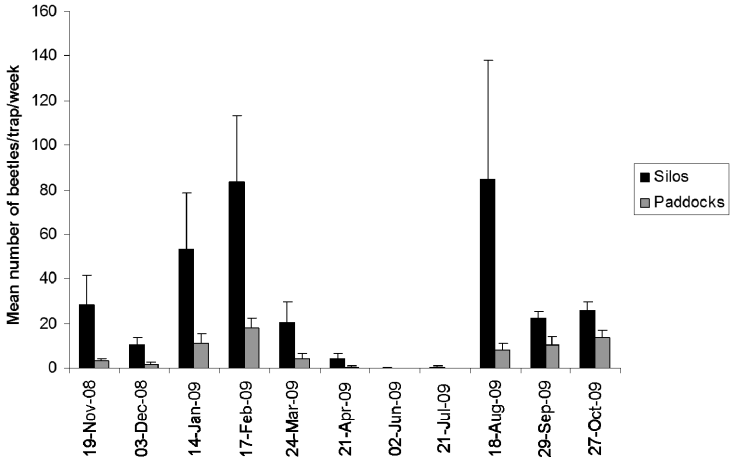

Figure 3 Number (mean & SE) of *Tribolium castaneum* adults trapped during 1 wk periods in traps placed near farm silos (n = 7) or in paddocks (n = 8).

For both species mean trap catch was positively correlated with the variance, indicating that frequency distributions were skewed to the right, and that logarithmic transformation would be needed to normalise the data. There was a strong linear correlation ($r11 = 0.935$, $P < 0.01$) between the geometric mean number of *T. castaneum* caught in paddocks and near silos, and approximately three times as many beetles were caught near silos (Fig. 4). Linear correlation was clearly inappropriate for *R. dominica* (Fig. 5) so a non-parametric test was used (Kendal's rank correlation coefficient).

The test showed that there was a weak but significant correlation ($\tau = 0.382$, $P < 0.05$) between the geometric mean number of *R. dominica* caught in paddocks and near silos.

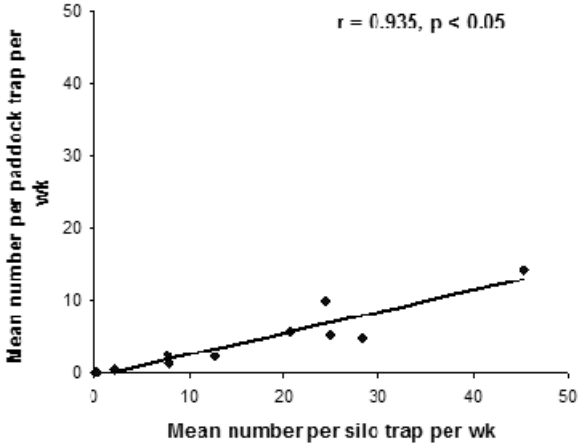

Figure 4 Comparison of geometric mean number of *Tribolium castaneum* adults trapped during 1 wk periods in traps placed near farm silos (n = 7) or in paddocks (n = 8).

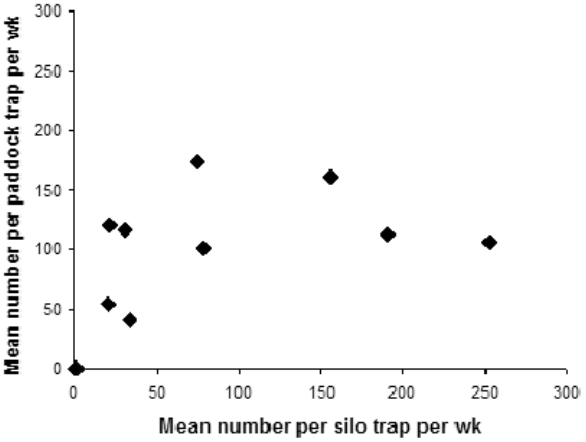

Figure 5 Comparison of geometric mean number of *Rhyzopertha dominica* adults trapped during 1 wk periods in traps placed near farm silos (n = 7) or in paddocks (n = 8).

The study characterising individual beetles emigrating from farm silos has not been completed but preliminary results are informative. The results shown in Table 1 are based on observations on 60 *R. dominica* and 46 *T. castaneum* on wheat at 25°C and 55%rh, yielding data on 31 *R. dominica* females and 28 *T. castaneum* females. Most females had mated before leaving silos, and the average female could reproduce for at least 7 wk without re-mating.

In the case of *R. dominica*, the mean number of offspring per live female declined linearly during the first 7 wk (y = 49.6x – 4.2, r2 = 0.954, F = 104.4, P < 0.001), but this trend was not evident in *T. castaneum*.

Table 1 Reproduction of *Rhyzopertha dominica* and *Tribolium castaneum* females collected emigrating from a farm silo and maintained separately on wheat (25°C, 55% r.h).

Species	Week in Labaratory	Percentage of initial female population producing offspring	Percentage live females producing offspring	Offspring per live female (mean ± SE)
R. dominica[1]	1	96.8	100.0	46.1 ± 3.0
	2	90.6	93.5	39.3 ± 2.4
	3	90.6	96.6	37.2 ± 2.9
	4	84.4	96.4	35.7 ± 2.7
	5	75.0	96.0	28.6 ± 3.1
	6	65.6	87.5	21.5 ± 2.9
	7	56.3	87.7	21.8 ± 3.0
T. castaneum[1]	1	75.0	75.0	4.4 ± 0.8
	2	78.6	81.5	8.2 ± 1.0
	3	78.6	81.5	11.0 ± 1.3
	4	82.1	88.5	13.2 ± 1.4
	5	78.6	84.6	10.1 ± 1.2
	6	75.0	84.0	10.2 ± 1.1
	7	67.9	76.0	8.4 ± 1.3

4. Discussion

The results of pheromone trapping suggest species specific patterns of abundance and distribution for *R. dominica* and *T. castaneum* in subtropical Australia. *Rhyzopertha dominica* has a wide spread distribution of adults that does not appear to be closely linked with stored grain while *T. castaneum* has a much more aggregated pattern of distribution centred around storages. Our results contrast with those of Sinclair and Haddrell (1985) who found no relationship between catch with grain and sticky traps of beetles and nearby infestations except over short distances (less than 100 m).

Two possible explanations of the results obtained so far for *R. dominica* are that beetles are moving significant distances, or that they are reproducing in non-grain hosts away from grain storage. These alternatives are not mutually exclusive and both would have significant implications for managing phosphine resistance. Studies in the USA using pheromones have demonstrated that *R. dominica* can be trapped in open fields and forested sites (Edde et al., 2005; 2006), and there is some evidence that *R. dominica* uses acorns as a non-grain food source resource in the USA (Jia et al., 2008). There is no similar evidence, however, for *R. dominica* using non-grain food sources in Australia.

Changes in trap catch numbers may reflect both changes in abundance or changes in the conditions for flight. Cox et al. (2007) estimated flight thresholds of 20 and 25°C for *R. dominica* and *T. castaneum* respectively in the laboratory, and in Wright and Morton (1995) estimated a threshold for *R. dominica* of 16°C based on diurnal trapping data. Adults of both species were trapped throughout the year although numbers were lower during the two coldest trapping periods, when ambient maximum temperatures were less than 25°C. The large increase in trap catches of both species in August 2009 coincided with unusually high ambient temperatures. Although we have not analysed trap catch data in relation to weather variables, these observations suggest that trap catches in the current study reflect, at least in part, the influence of ambient temperatures on flight.

It would be useful to characterise the beetles being trapped in the rural landscape, both for ecological modelling and modelling resistance development. Preliminary results obtained so far for beetles intercepted leaving farm silos are revealing. The results show that most *R. dominica* and *T. castaneum* females had mated before leaving silos, and that the average female could reproduce for at least 7 wk at moderate temperature without re-mating. This shows the potential of individual females to colonise grain, and suggests a faster spread of resistance genes than would occur if most emigrating females were virgins.

In this paper we have reported preliminary findings from research on the ecology of *R. dominica* and *T. castaneum* in subtropical Australia. We have shown that flight occurs for most of the year, and that beetles are not restricted to the immediate environment around farm storage. Our results suggest that female beetles leaving infested farm are likely to have mated before leaving and are capable of reproducing for many weeks without mating again. Future ecological research will include studies on dispersal, colonisation of grain, the frequency of phosphine resistance, and further characterisation of beetles trapped in the rural landscape. We aim to develop an understanding of how these two species interact with their environment and how these interactions influence resistance development.

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On automatic bioacoustic detection of stored product pests: the case of *Sitophilus oryzae*

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Abstract

The present work reports research efforts towards the development and evaluation of a unified framework for automatic bioacoustic recognition of specific insect pests. Our approach is based on capturing and automatically recognizing the acoustic emission resulting from typical behaviour, i.e., locomotion, feeding, etc., of the target pests. After acquisition the signals are amplified, filtered, parameterized and classified by advanced machine learning methods on a portable computer. Specifically, we investigate an advanced signal parameterization scheme that relies on variable size signal segmentation. The feature vector computed for each segment of the signal is composed of the dominant harmonic, which carries information about the periodicity of the signal, and the cepstral coefficients, which carry information about the relative distribution of energy among the different spectral sub-bands. This parameterization offers a reliable representation of both the acoustic emissions of the pests of interest and the interferences from the environment. We illustrate the practical significance of our methodology using *Sitophilus oryzae* (Rice Weevil – RW), (Coleoptera: Curculionidae). This approach led to 100% successful detection.

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A systemic approach of qualitative changes in the stored wheat ecosystem: prediction of deterioration risks in unsafe storage conditions in relation to relative humidity, infestation by *Sitophilus oryzae* (L.), and variety influence

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Abstract

A multidimensional laboratory trial was carried out to identify how key overall quality traits of different common wheat varieties change during storage, to understand their interactions in the process of deterioration, and finally to reveal underlying trends of critical storage conditions that may endanger grain quality. A large set of qualitative criteria were followed on grain batches of three wheat varieties with various qualities for food processing, which were stored for 160 d at 22-23°C, under two different relative humidities (r.h.), and with or without infestation by the rice weevil *Sitophilus oryzae*. All variables involved in quality components assessment were recorded at 42-d periods. From the Pearson's product moment correlation matrix, it was observed that the quality traits that correlated significantly to biotic variables (insect and fungal species dynamics) were: moisture content, hL mass, seed viability, and fat acidity of extracted flour. The interactions between biotic deteriorative factors and qualitative trait changes revealed through principal component analysis (PCA) were significant between four factors explaining the major part of qualitative criteria variance: storage duration; moisture content; hidden infestation density; fungal contamination level. The rate of increase of insect population was significantly different among varieties. PCA revealed that the significant difference in qualitative deterioration pattern among the three varieties was not related to their hardness, but to a different r.h. affinity. The germination rate was the qualitative criteria the more early declining during storage. The technological properties of extracted flour from each variety were significantly affected only when insect density exceeded 1000 insects per kg, a situation only observed in hot-spots. This work highlighted the trends of variation in quality traits of wheat varieties when stored under critical conditions. It can be used in IPM approaches to predict the susceptibility of a wheat variety to insect and fungi damage during storage.

Keywords: Common wheat, Variety, Qualitative change, Insect pest, Fungal spoilage, Multivariate analysis

1. Introduction

The interactions between quality biodeterioration factors (insects and fungi) and the quality traits that determine the commercial value of a grain batch are complex. Variable qualitative damage may occur according to storage conditions and the intrinsic sensitivity of the species and variety of cereal to these pests, as we have shown in previous work (Bekon and Fleurat-Lessard, 1992; Fourar, 1994; Fourar and Fleurat-Lessard, 1997). The conditions of safe storage may dramatically change when grain batches kept in good and safe storage conditions in wheat-producing countries are exported to other countries with much warmer climates and sometimes no availability of the adequate means to manage storage pests. Thus, to develop a better understanding of the susceptibility of different wheat varieties or cultivars to qualitative deterioration during storage, the objectives of this research were: i/comparison of the storability of different wheat varieties currently cultivated in France; and ii/identification of the major deterioration factors of the overall quality traits in order to understand the role of their interactions in the deterioration rate, especially for the more sensitive wheat quality traits.

2. Material and methods

A multidimensional laboratory trial was carried out to identify what key factors of the overall quality traits change, to understand their interactions in the process of deterioration, and finally to reveal underlying trends of critical storage conditions that may endanger grain quality retention. A large set of qualitative criteria were followed on grain batches from three wheat varieties with various qualities for cereal food processing. Wheat was stored for 160 d at 22-23°C, under two different relative humidities (r.h.), and with or without infestation by the rice weevil *Sitophilus oryzae* (L.). Variables involved in quality determination were recorded approximately every 40 d to build the multivariate data matrix (Tab. 1).

Table 1 List of quality traits of wheat grain and reference of each analytical method used for their quantification in the present study.

Grain Quality trait analyse	acronym	Analytical method	Reference
1. Sanitary and soundness condition			
Adult insects counting	Insect_AD	Sieving – NF-V 03-742	AFNOR, 1982
Insect hidden infestation counting	Insect_HI	Radiograph-ISO 6632-4	AFNOR, 1982
2. Microbiological spoilage			
2.1 Qualitative analysis: Rate of fungi-contaminated kernel	Cont_Rate	Ulster's method	Cahagnier and Richard-Molard, 1997
2.2 Quantitative analysis: Isolation and identification of fungal colony-forming-unit (CFU) per g	Fungi_Q	NF V08-011	AFNOR, 1996a
3. Germination			
Germinative capacity	Germ_Cap	ISTA rules for seed testing	ISTA, 1999
4. Physical-chemical condition			
Moisture content (wet basis)	MC	Oven-drying practical method NF V 03-707	AFNOR, 1982
Kernel hardness	Hardness	Hardness point-meter	Hardness meter notice
Hectolitre mass (or volumic mass)	hL_M	NF V 03-719	AFNOR, 1996b
5. Biochemical composition			
Nitrogen and protein content	Prot	Dumas method PR NF EN ISO 16634	
Lipid acidity (or fat acidity)	Lipid_Ac	NF V 03-712	AFNOR, 1982
6. Technological criteria (or fitness)			
6.1 Laboratory mill processed products			
Hagberg index (falling number)	Hagb_Ind	NF V 03-703	AFNOR, 1982
6.2 Alveographic parameters			
Baking strength (W)	W	ISO method 27971	ISO, 2008b
Other parameters (P, G, P/L)	P, G, P/L	ISO method 27971	ISO, 2008b
7. Statistical analyses			
Multivariate explanatory analyses		Multiple correlation - PCA	Xlstat®

In this experimental design, we applied the "fixed-effect-modelling" approach in which several qualitatively and logically distinct variables were measured on the same grain samples. This is a covariance analysis situation where several treatments (different grain varieties and storage conditions) were applied to the objects of the experiment (grain samples) to see if the response variable values changed. This is also a situation in which several variables are measured on each individual. The statistical analysis of the quantitative data used the explanatory procedure of principal component analysis (PCA). The multidimensional statistical analysis and chart plotting were achieved with Xlstat® (Addinsoft, Paris, France) software.

In our experiment, multivariate data were expressed in a matrix form with p columns (measured variables) and n rows (individual grain sample partitioned into different classes (or "dependent variables"): variety, r.h. level, insect presence or absence). The data processing software allowed the

calculation of simple and multiple correlation coefficients, and the analysis of covariance to model by multiple correlation the evolution of each dependant variable as a function of the explanatory variables. The Pearson' s product moment correlation matrix of the correlations between all the dependant and explanatory variables was built to appreciate their complex interactions, which were then graphically represented using two major components of PCA.

3. Results and discussion

3.1. Change in quality traits with storage time

From the Pearson's product moment correlation matrix, it was observed that the variables that correlated significantly to biotic variables (insect and fungal species dynamics) were: moisture content, 1000-grain mass, seed viability, fat acidity and Hagberg index of extracted whole flour. The interactions between biotic deteriorative factors and qualitative trait changes revealed through principal component analysis (PCA) were significant with four factors explaining the major part of the variance of qualitative criteria: storage time, moisture content, hidden insect infestation density, and fungal contamination level (Tab. 2). The rate of increase of insect population was significantly different among varieties (Tab. 3 and 4). PCA revealed that the significant difference in qualitative deterioration pattern observed among the three varieties was not related to their hardness, but rather to a different r.h. affinity. The qualitative traits which were more early affected during the storage period were germination rate and energy.

Table 2 Pairwise correlations between all variables relating quality attributes and "deterioration factors" of three wheat varieties stored during 160 d at 22-23°C, under two different r.h. conditions, and with and without an infestation with *Sitophilus oryzae* (Pearson' s product moment correlation matrix).

Variables	Hard-ness	PROT	RH_ Equi	Infested	Time	hL Mass	MC	Insect AD	Insect HI	Fungi Qu	Cont Rate	Fat Acid	Hagb Ind
Hardness	1												
PROT	-1.000	1											
RH_Equi	0.000	0.000	1										
Infested	0.000	0.000	0.000	1									
Time	0.000	0.000	0.000	0.000	1								
hL Mass	-0.158	0.158	-0.267	-0.246	-0.541	1							
MC	-0.086	0.087	0.286	0.256	0.671	-0.850	1						
Insect AD	-0.055	0.056	0.087	0.431	0.260	-0.249	0.364	1					
Insect HI	0.005	-0.005	0.073	0.330	0.389	-0.866	0.764	0.073	1				
Fungi Qu	0.203	-0.203	0.144	0.172	0.195	-0.790	0.613	-0.028	0.773	1			
Cont Rate	0.367	-0.368	0.195	0.046	-0.615	0.108	-0.311	-0.193	-0.194	0.214	1		
Fat Acid	0.033	-0.032	0.164	0.240	0.438	-0.911	0.818	0.145	0.917	0.909	-0.091	1	
Hagb Ind	0.676	-0.675	-0.007	0.004	-0.116	-0.073	0.038	-0.084	0.097	0.156	0.149	0.086	1

Values in bold are different from 0 at signification level alpha = 0.05

Table 3 Equations of exponential models predicting the rate of increase of the density of *Sitophilus oryzae* adults during 124 d development of two couples on the three wheat varieties at two r.h. levels: comparison of parameters derived from the calculated rate of increase in the different experimental conditions.

Variety	Equilibrium r.h. (%)	Exponential rate of increase equation	Net multiplication rate after 124 d	Natural rate of increase per d (rm)	Correlation coefficient R^2
Caphorn	75	y = 0.518285 e0.054221x	144	0.0401	0.87
	85	y = 0.2252 e0.0709x	494	0.0500	0.94
Apache	75	y = 0.1538 e0.0778x	794	0.0538	0.97
	85	y = 0.2769 e0.0747x	973	0.0555	0.98
Crousty	75	y = 0.2607 e0.0684x	419	0.0487	0.95
	85	y = 0.1491 e0.078x	789	0.0538	0.98

Table 4 Equations of exponential models predicting the rate of increase of the density of *Sitophilus oryzae* hidden infestation (all stages) during 124 d development of two couples on three wheat varieties at two r.h. levels: comparison of parameters derived from the calculated rate of increase under the different experimental conditions.

Variety	Equilibrium r.h. (%)	Exponential rate of increase equation	Net multiplication rate after 124 d	Natural rate of increase per d (rm)	Correlation coefficient R^2
Caphorn	75	y = 15.314 e0.0275x	463	0.0495	0.68
	85	y = 9.1155 e0.0361x	801	0.0539	0.91
Apache	75	y = 7.0759 e0.043x	1464	0.0588	0.96
	85	y = 10.398 e0.0427x	2072	0.0616	0.93
Crousty	75	y = 21.906 e0.0284x	741	0.0533	0.81
	85	y = 57.586 e0.023x	998	0.0557	0.92

From the alveograph® test results, measured only once at the end of the storage period, it was shown that storage of any of the three varieties at 75% r.h. did not significantly modify the flour baking strength (W), in spite of a small reduction of the swelling parameter (G) (Tab. 5). Flour extracted from infested samples compared to uninfested samples stored under the same r.h. conditions, had a clear reduction in W and G. In infested series, the extreme insect damage reached at the end of the storage period induced an imbalance of the r.h.eological parameters of the extracted flours. Thus, the technological properties of flour extracted from each variety were significantly affected only when insect population density exceeded 1000 adult insects per kg, a situation in practice only observed in hot-spots.

Table 5 Comparison of the alveographic parameters measured on flour extracted from the three wheat varieties before and after 160 d storage at constant temperature (22-23°C), at two r.h. levels and with and without an infestation by *Sitophilus. oryzae*.

Variety	Before storage			After 160 d of storage Conditions											
				75% r.h. no insects			75% r.h. with insects			85% r.h. no insects			85% r.h. with insects		
	W	G	P/L	W	G	P/L	W	G	P/L	W	G	P/L	W	G	P/L
Caphorn	240	18.1	1.39	270	17.7	1.68	195	12	5.07	280	18.1	1.55	185	12	4.97
Apache	255	24.5	0.51	235	23.8	0.52	105	15.1	1.41	165	19.4	0.72	NA	NA	NA
Crousty	190	27.4	0.25	120	20.6	0.44	120	19.7	0.55	135	23.3	0.32	155	14.8	1.84

3.2. Interactions between the studied variables

Although the hardness criterion is considered by many researchers as being related to varietal susceptibility to insect attack (Russel, 1962; Dobie, 1974; Juliano, 1981; Horber, 1983; Fourar, 1994), but even though the three varieties tested had large differences in hardness this was not the major factor involved in a lower level of susceptibility to insect attack and S. oryzae population multiplication. This contradiction could be clarified by multiple correlation modelling of each monitored qualitative criteria as a function of related explanatory variables. Thus, the variation in grain moisture content of the three wheat varieties can be modeled by four explanatory variables: r.h. equilibrium level, storage duration, insect infestation, and variety (Tab. 6). Storage time and variety influence were the most important variables explaining the major amount of variance explained by multiple linear regression. The variable `Hardness' did not show any dependence with the variation in insect density (neither adult nor hidden infestation). Thus, it could be shown that the hardness characteristic did not significantly influence the susceptibility of the three wheat varieties to the weevils, but that this susceptibility was rather related to capacity for r.h. absorption of grain.

Table 6 Multiple correlation modelling of qualitative traits (dependent variables) changes during storage time of three wheat varieties kept during 160 d in experimental storage at two different r.h. and with and without an infestation by *S. oryzae* (four quantitative explanatory variables and one qualitative variable: variety). The parameters of the linear (polynomial) models are indicated as "value" with the level of significance of the deviation from the mean (the constant term in the equation was omitted).

Explanatory variables	**# F-test value**	**Hardness**			**RH Equilibrium**			**Insect infestation**	
Dependent variables		**Value**	**t**	**Pr. > [t]**	**Value**	**t**	**Pr. > [t]**	**Value**	**t**
Moisture content	21.263***	-0.012	-1.21	0.233	7.779	3.485	0.001***	0.695	3.115
Germination capacity	6.411***	0.02	0.123	0.9	-60.4	-1.614	0.114	-10.71	-2.86
Adult Insect density	3.181*	-2.888	-0.451	0.654	9905	0.663	0.511	490.86	3.283
Insect hidden infestation	3.843**	-0.886	-0.028	0.98	4258.5	0.573	0.569	1918	2.582
Fungi germs quantity	1.334 NS	41.69	1.392	0.171	7015	1.003	0.322	840.5	1.202
Seed contamination rate	12.262***	0.162	3.813	< 0.001***	19.583	1.978	0.055	0.458	0.463
Lipid acidity	3.577**	0	0.139	0.89	0.071	1.282	0.21	0.01	1.872
HL mass	6.9***	-0.041	-1.37	0.178	-16.33	-2.337	0.024*	-1.506	-2.155
Hagberg Index	24.86***	5.178	8.225	<0.001***	-12.292	-0.084	0.934	0.729	0.05

continue

Explanatory variables	**# F-test value**	**Storage time**			**Variety (global)**			
Dependent variables		**Pr. > [t]**	**Value**	**t**	**Pr. > [t]**	**Value**	**t**	**Pr. > [t]**
Moisture content	21.263***	0.003**	0.02	8.172	<0.001***	0.965	4.073	<0.001***
Germination capacity	6.411***	0.007**	-0.171	-4.125	<0.001***	-8.192	-2.161	0.045*
Adult Insect density	3.181*	0.002**	3.276	1.98	0.05*	121.5	0.766	0.448
Insect hidden infestation	3.843**	0.0013**	25.025	3.044	0.004**	1353.8	1.717	0.093
Fungi germs quantity	1.334 NS	0.236	10.551	1.363	0.18	441.78	0.595	0.555
Seed contamination rate	12.262***	0.646	-0.069	-6.256	<0.001***	-2.13	-2.027	0.049*
Lipid acidity	3.577**	0.068	0	3.214	0.003**	0.09	1.539	0.131
HL mass	6.9***	0.037*	-0.037	-4.728	<0.001***	-0.266	-0.359	0.722
Hagberg Index	24.86***	0.961	-0.352	-2.165	0.036*	107.21	6.87	<0.001***

*** = P ≤ 0.001; ** = P ≤ 0.01; * = P ≤ 0.05. – # F value of multiple regression model (5, 42 df)

One of the original results of this study was to show that the greater sensitivity of the Apache variety to the multiplication and the damage caused by *S. oryzae* was primarily associated with the intrinsic physical-chemical property of this variety to equilibrate aw and r.h. at a higher level of moisture content than the two other varieties, for the same r.h. in the grain samples storage enclosure. Figure 1, which represents the circle of the correlations between all the measured quantitative variables showed a first component (F1) that contained 40.7% of the variability. The proximity of the factors of qualitative deterioration with one of the ends of the axis F1, highlights a strong negative impact of four variables representing the "deterioratives forces" (Tipples, 1995) (variable: Time, MC, Insect_HI, Fungi_Q) on three major criteria of grain sanitary condition (Fat_Acid, Germ_Cap and hL_M). The three variables related to sanitary condition (MC, Insect_HI and Fungi_Q) were positively correlated to the storage period (Time), indicating that lengthening the storage period simultaneously increased the grain's hydration level, that in turn increased insect hidden stages density (in the case of infested series), and enrichment of storage fungi germs. Yet, a significant evolution of fungal contamination level was only observed during the last month of storage and was induced by the huge insect infestation level.

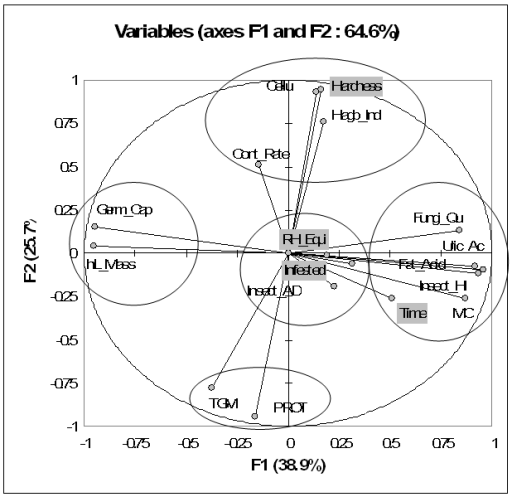

Figure 1 PCA: circular diagram of correlations between all variables (dependent and *explanatory* variables; these later within a shaded frame) revealing the interactions in qualitative trait changes occurring during a 160-d storage period (*Time*) of three wheat varieties of different hardness (*Hardness*), at two different r.h. levels (*RH_Equi*), and with or without an infestation by *Sitophilus oryzae* (*Infested*).

The second component (vertical axis) of the PCA, which included nearly 21% of the global variance, was directed by the physical-chemical variables related to intrinsic quality: hardness, protein content, and Hagberg index (Hi). The hidden infestation density was positively correlated with some dependent variables (MC, Lipid_Acid, Fungi_Q) and negatively with others (Germ_Cap and hL_M). The correlation with Time as the main explanatory variable related to insect hidden infestation variance was also highlighted through the PCA diagram.

3.3. Concluding remarks

In our study, the differences in susceptibility of wheat varieties to adverse storage conditions (high ambient r.h., high temperature and insect infestation) could be related to the dynamics of the interactions between 'deteriorative forces' and qualitative traits. The prediction of quality traits deterioration risks for different wheat varieties should be useful in building preventive strategies of grain storage management, especially in Mediterranean or tropical countries having imported wheat for long-term storage or strategic reserves.

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Conscent PE mating disruption system is an effective alternative to methyl bromide for the control of stored product moths

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Abstract

Stored-product moths, *Plodia interpunctella*, *Ephestia kuehniella*, *Ephestia cautella*, *Ephestia elutella* are a serious threat to stored-food commodities. These moths are mostly controled using chemical insecticides. Recently, the residual effects of synthetic insecticide have become a serious concern to consumers. Therefore, an alternative measure is being sought. The object of the present study was to induce mating disruption and subsequently a reduction of the population of the aforementioned storage moths. Conscent PE, a steady release solid dispenser emits pheromone component Z-9, E-12-Tetradecadien-1-yl acetate. This dispenser was tested consecutively for five years in a real life situation in the United Kingdom. This mating-disruption system is specifically designed for use in enclosed spaces leading to an increase of pheromone concentration in the air. Solid dispensers containing 100 mg of sex pheromone Z-9, E-12-Tetradecadien-1-yl acetate were applied every 8 meters in a food factory. Complete absence of the pest was achieved after 12 month of continuous application. Pheromone traps showed zero captures and three monthly biological assessment indicated the absence of any breeding. There was also a dramatic decline in reported customer complaints and the twice yearly fumigations with methyl bromide were deemed unnecessary. The dispensers were found to be effective for up to 13 weeks.

Keywords: Consent PE, Mating disruption, Pheromone, *Plodia interpunctella*, *Ephestia kuehniella*, *Ephestia cautella*, *Ephestia elutella*.

Behavioural effects of pheromone-based control system, ExosexTM SPTab, on male Indianmeal moth, *Plodia interpunctella*

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Abstract

We report on laboratory studies to examine behavioural effects of a new pheromone-based control system for stored product moths, Exosex™ SPTab auto-confusion, on virgin male *Plodia interpunctella*. The SPTab comprises a compressed electrostatic powder tablet, containing the female sex pheromone (Z,E)-9,12-Tetradecadienyl acetate; designed to be an attractive source to males and disrupt the mate finding ability of several stored moth pest species.

Our aims were to examine the effects of SPTab contact on male ability to locate females and to be attractive sources to other males. Observations of behavioural effects were made in a moth flight tunnel. Virgin male *P. interpunctella* were treated on SPTabs weathered from 1-8 weeks. After treatment, males were either immediately released downwind of calling females in the upwind section of the flight tunnel, or caged individually for 1 to 48 h before release. The ability of treated males to act as false lures for other males was measured by treating males on different age SPTabs as before, caging them and then placing them upwind of untreated males. In all studies males were scored on making source contact.

Males treated on the SPTab and immediately released downwind of calling females showed a 96.7% reduction in their ability to contact the females. Males held for 48 h before release still showed a significant reduction in source contact compared to untreated controls. Treated males tested after 3 h, were as attractive as calling females with >75% of flights resulting in source contact. Significant reduction in female source contact was still evident when males were treated on SPTab weathered for 4 weeks. Males treated on SPTabs weathered for up to 8 weeks were significantly attractive to untreated males, and equal to calling females.

The results indicate that contact with SPTab significantly reduces 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. The Exosex SPTab has novel methods of causing mating disruption.

Keywords: Mating disruption, *Plodia interpunctella*, Exosex SPTab, Sex pheromone, Flight tunnel.

1. Introduction

Plodia interpunctella (Hübner) (Indianmeal moth), herein referred to as IMM, is a common pest of food manufacturing and stored product areas all over the world. Since treatment of stored products with methyl bromide is to be phased out worldwide by 2015, alternative treatments for stored-product pest protection are being urgently sought (Vassiliou, 2008). Sex pheromones have potential for indoor moth pest management. Adult male IMM, and several other Pyralid species, will positively orientate towards a source of (Z,E)-9,12-tetradecadienyl acetate (ZETA), a compound identified as part of the sex pheromone released by the female (Brady et al., 1971; Kuwahara et al., 1971), by following the plume of sex pheromone upwind to the receptive female. Mass trapping whereby multiple sticky or funnel traps with a pheromone lure are positioned to capture as many moths as possible have been used to reduce moth populations (Cox, 2004). A limitation of this system is that only males of the species are targeted. An alternative moth control technique that has been tested in food processing and storage is the use of sex pheromones to cause mating disruption, which requires the environment to be saturated with pheromone from multiple fixed point sources (Ryne et al., 2006). These point sources can disrupt the response of male moth antennae by causing sensory fatigue or by competing with pheromone plumes produced by calling females (Cardé and Minks, 1995).

Research has shown that electrostatically charged powders can adhere to insect cuticles and be used as carrier particles for active ingredients (Armsworth et al., 2006) such as entomopathogens, insecticides and pheromones. Sex pheromones can be mixed into EntostatTM (Exosect Ltd, Winchester, UK), a proprietary electrostatic wax powder derived from the Brazilian wax palm *Copernica cerifera* Martius (Palmae). Auto-confusionTM pheromone control systems have been developed from this technology. The concept behind auto-confusion is that contact by male moths to the pheromone loaded powder allows the powder to adhere to the moth, moths then leave the point source carrying the powder and confusing pheromone with them. Exosect claim that once they have 'collected' powder, male moths become unable to locate female pheromone plumes and become mobile pheromone point sources, possibly attracting other males, thus reducing the number of fixed point sources needed to distribute the pheromone. EAG tests with male *Lobesia botrana* (Denis and Schiffermüller) treated with pheromone loaded Entostat showed that antennal responses to sex pheromone were reduced in males compared to untreated males, thus they had a reduced response to calling females (Nansen et al., 2007). Baxter et al. (2008) showed that a significant amount of Entostat powder adhered to IMM when artificially treated, and thus could be used as a carrier for pheromone dissemination.

Exosex SPTab is a pheromone product for the control of IMM and other *Pyralid* species, developed by Exosect Ltd. It is an Entostat powder blend, loaded with 10 mg of ZETA, and compressed into tablets. Exosex SPTab and traditional mating disruption systems likely differ in their modes of activity because the lower concentration of pheromone in SPTab allows the males to make contact with the point source (Huggett, unpublished data). Traditional mating disruption dispensers mask female pheromone plumes by flooding the air with pheromone, and creating false trails to confuse male moths (Cardé and Minks, 1995). If pheromone release rates are too high, contact is unlikely to occur. This work aims to determine how long the SPTab reduces the ability of male IMM to follow a female pheromone plume once they have contacted SPTab, and whether they become attractive sources for other males. It also aims to determine whether weathering of SPTabs reduces their effectiveness; as SPTab is deployed for 8 week intervals. Studies were carried out in a moth behavioural flight tunnel.

2. Materials and methods

For all studies SPTabs were placed into a 31 x 31 x 100 cm stainless steel wind-tunnel, at a constant 25ºC for a maximum of 8 weeks before treating males. IMM were 2 days old for experiments, cultured as per Baxter et al. (2008). All moths were virgins, and separated into gender groups at pupal stage. Gender groups were kept in heated vivariums in separate rooms to prevent pheromone habituation. All flight work was carried out in a custom built wind tunnel similar to a design described in Nansen et al. (2007) with dimensions of 0.75 x 0.75 x 2.5 m, a wind speed of 0.3 m/s and a temperature of 24ºC.

Virgin males for all tests were artificially treated on SPTabs by inverting a glass vial containing individual males onto the SPTab, leaving them for 5 s and re-capturing them in a wire mesh tea strainer release cage (Salt and Pepper Company Ltd, Monmouth, UK). In all studies males were released in the downwind section of the wind tunnel, they were timed and their ability to contact the upwind source was recorded. Each moth was given a maximum 5 min to achieve source contact before it was removed from the wind tunnel. For all studies SPTabs were placed into a 31 x 31 x 100 cm stainless steel wind-tunnel, wind speed 0.5 m/s, temperature a constant 25ºC, for a maximum of 8 weeks before treating males.

2.1. Female source location at different time lags after treatment on 1-week-weathered-SPTab

The purpose of this study was to determine the effect of time since SPTab contact on ability of males to find calling females. Males were treated on an SPTab weathered for one week and either immediately tested (0 h time lapse) or caged individually for 1, 2, 3, 6, 24 or 48 h, and then released in the downwind section of the flight tunnel with two caged calling females in the upwind section. Untreated males were tested as a control source. For each time lapse, and for controls, 30 males were tested.

2.2. Treated male source location at different time lags following treatment on 1-week-weathered SPTab

The purpose of this study was to determine the effect of time since SPTab contact on ability of treated males to attract untreated males. Male IMM were treated and placed in individual mesh cages upwind of untreated males at either 0, 1, 2, 3, 6, 24 or 48 h following treatment on a 1-week-weathered SPTab. Two calling females were tested as a positive control source, and two untreated males were tested as a control

male source. For each time lapse since treatment, 20 untreated male IMM were tested for their response to each source by releasing them individually in the downwind section of the flight tunnel.

2.3. Female source location after treatment on SPTabs weathered 1-4 weeks

The purpose of this study was to determine the effect of weathered SPTab contact on ability of males to find calling females. Males were treated on tablets weathered for either 4, 3, 2 or 1 week, left for 1 h in release cages, released in the down-wind section of the flight tunnel and then their ability to source contact calling females was evaluated. Untreated males were tested as a control source. For each age of tablet and for the controls, 20 males were tested.

2.4. Treated male source location following treatment on SPTabs weathered 1-8 weeks

The purpose of this study was to determine the effect of weathered SPTab contact on ability of treated males to attract untreated males. Male IMM were treated and placed in individual mesh cages upwind of untreated males 1 h following treatment on a 1, 2, 3, 4, 5, 6, 7 and 8-week-weathered SPTab. Two calling females were tested as a positive control source, and two untreated males were tested as a control male source. For each time lapse since treatment, 20 untreated male IMM were tested for their response to each source by releasing them individually in the downwind section of the flight tunnel.

2.5. Statistical analysis

For all experiments the proportion of positive source contact outcomes was compared to the total number of flight observations for each group using the Chi-squared parametric k proportions test; Monte Carlo method. The Marascuilo procedure was also used if the Monte Carlo simulation rejected H0, as the Marascuilo procedure compares all pairs of proportions, which enabled the pairwise differences to be identified. Statistical analysis was performed using XLSTAT (Addinsoft™, New York, NY, USA).

3. Results

3.1. Female source location at different time lags after treatment on 1-week-weathered-SPTab

In the untreated control group, 85% of males made contact with calling females. Immediately following treatment (time lapse 0 h), and 3 h following treatment, source contact with females was reduced to 33% and 0%, respectively (Fig. 1). Source contact was still lower than controls at 3.3% and 46.7% at 24 h and 48 h following treatment, respectively. Treatment of males on SPTab had a significant effect on source contact (Chi-square = 91.216, DF = 7, $P < 0.0001$) (Fig. 1). Males treated on SPTab weathered for one week and left for 0, 1, 2, 3, 6 and 24 h before flight made significantly fewer source contacts with calling female IMM compared to the untreated control male moths. There was no significant difference between the untreated males and males tested 48 h after treatment. There was no significant difference in proportion making source contact between moths released 0 and 1 h after dosing. Releasing males 2, 3 and 6 h following treatment resulted in significantly fewer source contacts than control moths and moths released 0, 1, and 48 h after treatment. There were no significant differences in proportion making source contact between moths released 24 h following dosing and those released 0, 1, 2, 3 and 6 h after dosing.

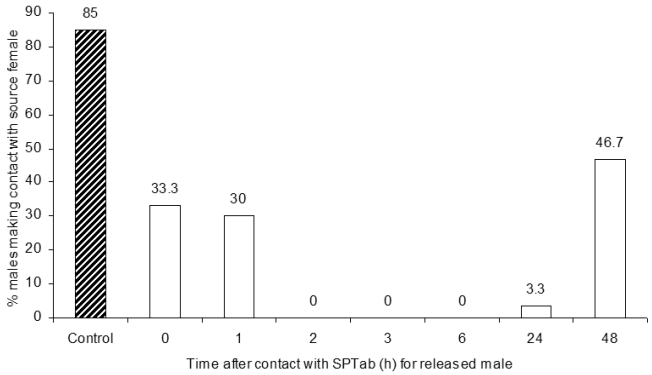

Figure 1 Percentage of virgin *Plodia interpunctella* males making female source contact at different time lags after treatment on 1-week-weathered-SPTab.

3.2. Treated male source location at different time lags following treatment on 1-week-weathered SPTab

In the female control source group 80% of males made contact. No males made contact with untreated control males (Fig. 2). Between 0 and 6 h following treatment the proportion of males making source contact with a treated male was up to 85%. No source contact occurred 24 and 48 h following treatment of the male source. Treatment of source males on SPTab had a significant effect on source contact (Chi-square = 101.157, DF = 8, P < 0.0001). Percent contacts with source males left 0, 1, 2 and 3 h after treatment were not significantly different to the percent contacting calling females. Contacts were significantly fewer than the female control group, but the same as control males, if the treated male source was placed upwind 6, 24 and 48 h following treatment on SPTab.

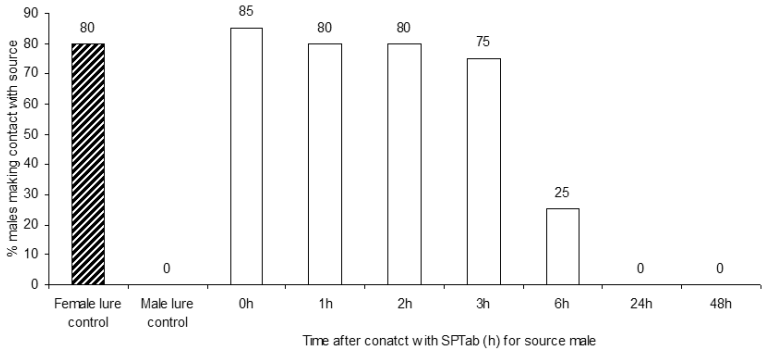

Figure 2 Percentage of untreated virgin male *Plodia interpunctella* making source contact with a male treated on a 1-week-weathered SPTab.

3.3. Female source location after treatment on SPTabs weathered 1-4 weeks.

A maximum of 20% of males treated on SPTabs weathered for either 1, 2, 3 or 4 wks made contact with the female pheromone source compared to 80% in the untreated male control group (Fig. 3). Treatment of males on SPTabs had a significant effect on source contact (Chi-square = 35.020, DF = 4, P < 0.0001). Source contact by released male moths was significantly decreased following treatment on 1, 2, 3 and 4 week weathered SPTabs.

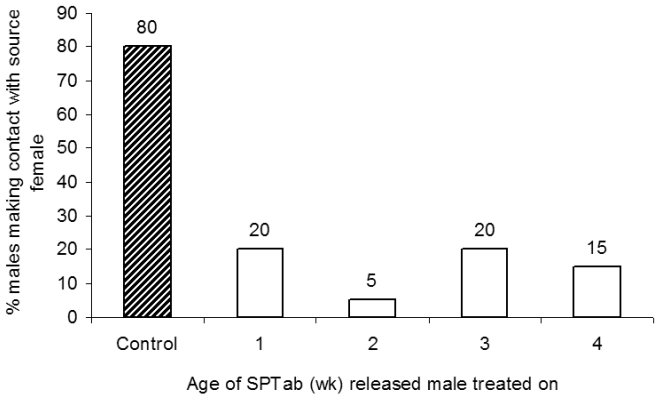

Figure 3 Percentage of *Plodia interpunctella* males making female source contact after treatment on SPTabs weathered 1-4 weeks.

3.4. Treated male source location following treatment on SPTabs weathered 1-8 weeks.

In the female control group, 80% of released males made contact. No males made source contact with the male control group (Fig. 4). Up to 90% of male moths made contact with treated male sources when the sources were treated on SPTabs weathered for 1, 2, 3, 4, 5, 6, 7 and 8 weeks. Treatment of male sources on SPTabs had a significant effect on source contact (Chi-square = 71.604, DF = 9, P < 0.0001). There was no significant difference in source contact between all the treated groups and the positive female control source group, but significantly more contact for all those groups than in the untreated control male source group.

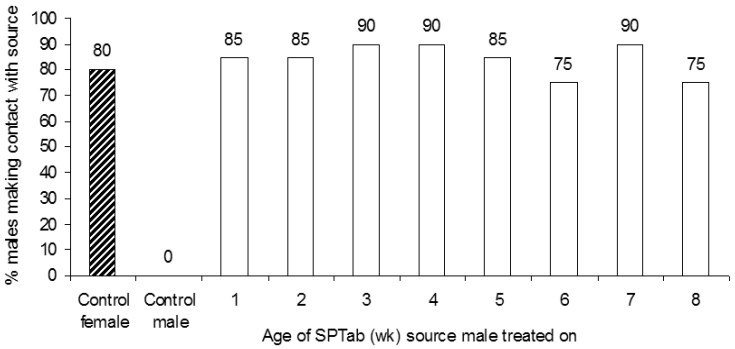

Figure 4 Percentage of untreated virgin male *Plodia interpunctella* making source contact with a male treated on 1 to 8-week-weathered SPTabs.

4. Discussion

This study has shown that very low concentrations of pheromone, if placed into an appropriate carrier, can successfully disrupt the mate-finding behaviour of male IMM once contact with the carrier has been made, a process termed auto-confusion. Contact of male IMM with SPTab reduces the ability of the males to locate females for over 24 hours. After 24 h, the pheromone from the powder may have completely volatilised, or the powder may have been removed by grooming, allowing the males to locate pheromone plumes again, from either calling females or SPTabs. Baxter et al. (2008) showed that 89.8% of Entostat was lost from a male IMM 48 h following artificial treatment in a dosing tube. The delay in males finding females was effective when males had made contact with SPTabs weathered for up to 4 weeks. Males that contacted SPTab were also highly attractive sources of pheromone to other males for over 3 hours following SPTab contact, causing males to follow a 'false' pheromone plume. Attraction to these 'false' sources was equal to two calling females even if the SPTabs had been weathered for up to 8 weeks. Untreated males were not at all attractive to other males; attraction to treated males must have been because of pheromone transfer with the powder, which adhered to the male cuticle after contact with SPTab. The attraction of males to an SPTab treated male also shows that the single major component of the female pheromone, ZETA, can elicit the full suite of flight behaviour, despite Zhu et al. (1999) indicating the minor components were required.

If males follow false male lures, or are unable to locate females, then mating is either delayed or prevented altogether. Even delayed mating reduces the chances of female moths laying fertile eggs (Huang and Subramanyam, 2002) thus a significant effect on the population is possible.

The inability of males to locate females following SPTab contact may have been due to sensory habituation. Stelinski et al. (2005) showed that habituation to pheromone following exposure to pheromone in the air can take up to an hour, and in this study a stronger reduction in source contact was seen in males left for 2 h than if tested immediately. The habituation effect observed when males are exposed to high pheromone concentrations in air (Stelinski et al., 2005) could be extended with the SPTab system due to males actually being in direct contact with the pheromone after visiting the dispenser.

Traditional mating disruption systems release large quantities of pheromone, which can cause sensory habituation in males, masking of female pheromone plumes and false trail following (Cardé and Minks, 1995). The lower amounts of pheromone released from SPTab compared to traditional mating disruption dispensers and monitoring lures (Storm, unpublished data) means that trap shutdown is not observed, thus allowing the continued use of monitoring traps (Pease and Storm, 2010).

This study has shown that the mode of action of SPTab is auto-confusion. SPTab may have similar effects on other Pyralid moths attracted to ZETA. This system could be a safer, greener alternative to pesticide sprays in indoor storage and food processing environments.

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Study on volatile compounds in rice by HS-SPME and GC-MS

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Abstract

Headspace solid phase micro-extraction (HS-SPME), together with gas chromatography and mass spectrometry, were used to investigate volatile compounds in rice. Experimental conditions including extraction time, temperature, the amount of sample and equilibrium time, were optimized. Optimal conditions were that a 20 g sample was heated for 30 min at 80°C prior to headspace absorption, and then extracted for 30 min with HS-SPME. The volatile compounds in indica and japonica rice were alcohols, aldehydes, ketones, esters, hydrocarbons, organic acids, as well as heterocyclic compounds. Aldehydes were the most abundant making up around 30% of volatiles by weight.

Keywords: Headspace solid phase micro-extraction, Rice, Gas chromatographic and mass spectrum, Volatile substances

1. Introduction

Rice is the main grain staple for about half of the world's population and China is one of world's three main rice producers. Paddy or rough rice is the main security reserve crop in China (LONG Qilin, 2004), so that it may be held in storage for considerable periods. As paddy respires actively its quality may decline during storage. Therefore, research on volatile components of rice and exploring new method of rapidly evaluating the quality of rice in storage has important practical significance for the guidance of scientific storage of paddy.

The solid-phase micro-extraction (SPME) method was developed by Arthur and Paw (1990) in the last century. SPME is a technology of sample pre-treatment, which combines sampling, extracting, concentrating and injection. Combined with gas or liquid chromatography, it can be effectively used to analyze trace organic compounds in samples. It has good repeatability and a detection limit up to μg/L (MA Jipin et al., 2002). SPME with the characteristics of simple preparation, rapid analysis, low cost, and without organic solvents, is widely used in the food, environmental, chemical, and pharmaceutical industry.

The changes during storage of volatile components in rice have been little studied and reported (Maga, 1984; Ling ,1988; Zhou et al., 2005). A total of 12 group components in rice were separated by gas chromatography by Zhou et al. (2005), but no volatile components were identified. The volatile carbonyl compound (VCC) composition and content were analysed using column chromatography by Ling (1988). In this paper, we identify and classify the volatile components of rice, and compare indica and japonica varieties, using solid-phase micro-extraction and GC-MS.

2. Materials and methods

2.1. Experimental material

Paddy samples: Two indica rice samples were collected from Hubei and Guangdong provinces and two japonica samples collected from Heilongjiang Province and Liaoning provinces in China.

2.2. Main instruments

Solid-phase micro-extraction device and extraction head (CAD-PDMS 75μm, PDMS 100μm), the United States Supelco, Inc.; Gas-MS analyzer, Thermo Electron Corporation; Laboratory test Huller, type JLGJ4.5, Zhejiang Taizhou Food Instrument Factory; Water bath, Shanghai Shenxin Experimental Instruments Co. Ltd; Extraction bottles with Cypriot.

2.3. Sample preparation

Brown rice sample preparation: The paddy was cleaned of impurities then husked. The resultant brown rice was placed in a ziplock bag and mixed before use.

Headspace solid-phase extraction: 20g brown rice was placed in 100mL glass bottle, and water added in a ratio of 1:2 was added. The glass bottle was put in heated water bath until there was an equilibrium between the gas in the top space and the liquid in the lower part. The head of the solid-phase micro-extraction fiber was inserted into the glass bottle through a hole in the top. After the extraction period was complete, the fiber was removed and inserted quickly into the injection hole of a gas chromatogram, and desorbed at 250°C for 5min in splitless mode.

2.4. Gas chromatographic conditions

Column is DB5-MS capillary column (30m × 0.25mm × 0.25μm); Carrier gas helium with flow rate 1.0mL/min; Initial column temperature 50°C, and heated to 165°C with 10°C/ min, keeping 5min, and then to 250°C with 15°C/min, keeping 3min; Non-split injection.

2.5. MS conditions

Interface temperature is 280°C; Ion source is the EI.Ion at 230°C; Electron energy is 70ev; Mass scan range(M/Z) is 35-350amu.

2.6. Data Processing

The volatile components of unknown samples were identified by computer through searching and matching NIST and Weily MS libraries. The volatile components were quantified using peak area normalization.

3. Results and discussion

3.1. Optimization of extraction method

There are many factors that can have a direct impact during the headspace-solid phase micro-extraction process these include the equilibrium time, extraction time and temperature.

3.2. Selection of extraction temperature

Solid-phase micro-extraction and the enrichment of the sample is a dynamic equilibrium process. The temperature is the most important parameters affecting the distribution coefficient, which is a thermodynamic constant. Raising the temperature can concentrate the gas phase of gas-liquid equilibrium, increasing the volatile compounds that reach the headspace and the fiber surface, but the adsorption process of SPME is generally an exothermic reaction, so increasing temperature will reduce the absorption capacity of the fiber coating. It is therefore best to keep the extraction medium at a higher temperature, while the extraction fiber surface should maintain at a lower temperature. The different nature of the compounds is also an important consideration for the extraction process. When the conditions for the equilibrium temperature, equilibrium time, extraction time, and sample weight are set at 80°C, 30 min, 30 min, and 20 g respectively, extraction temperatures of 50, 60, 70, 80, and 90°C were selected to explore the impact of extraction temperature. The results showed that the optimal extraction temperature is 80°C (Fig. 1).

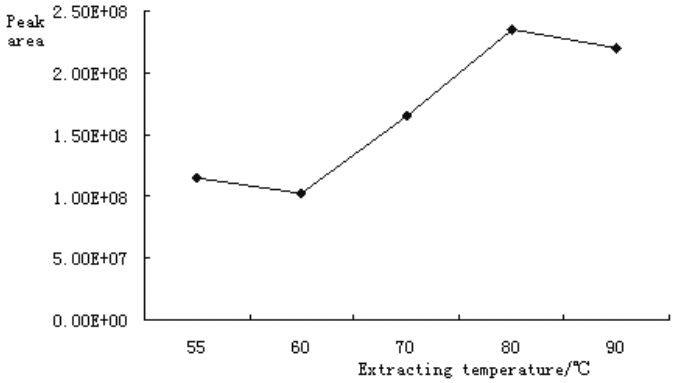

Figure 1 Effect of different extraction temperatures on total peak area.

3.3. Selection of equilibrium time

The gas phase partial pressure of each component is not only affected by temperature, but also has a close relationship to the equilibrium time. When the conditions for equilibrium temperature, extraction time, extraction temperature and sample weight were set at 80°C, 30 min, 80°C, and 20 g respectively, the equilibrium times of 10, 25, 40, 60, and 75 min were selected for testing. The results showed that 60 min was the optimal equilibrium time (Fig. 2).

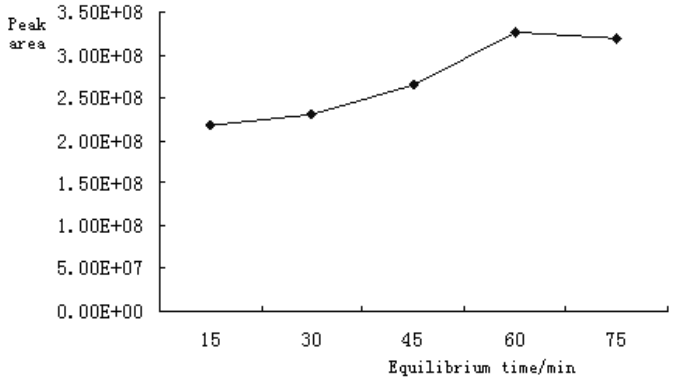

Figure 2 Effect of different equilibrium times on the peak area.

3.4. Selection of extracting time

When conditions of equilibrium temperature, equilibrium time, extraction time, extracting temperature, and sample weight were 80°C, 30 min, 80°C, and 20 g respectively, extraction times of 10, 25, 40, 55, and 70 min were selected to test the effect of extraction time on the total peak area. The total peak area grows as extraction time increases (Fig. 3) and is maximal at 55 min. It then tends to balance and the impurity peaks increase rapidly after 55 min. In order to avoid impurity peaks, 50 min was adopted as the optimal extraction time.

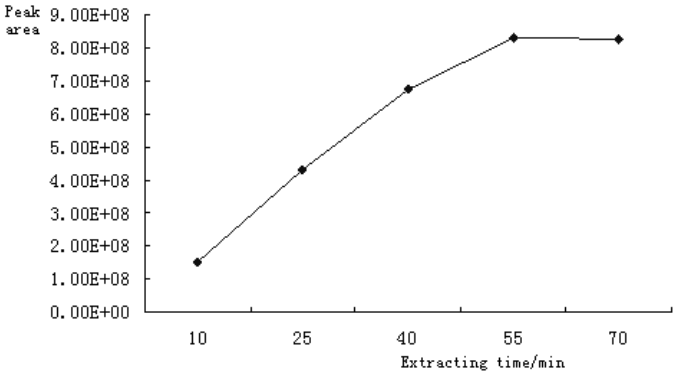

Figure 3 Effect of different extraction times on total peak area.

3.5. Choice of sample amount

When conditions for equilibrium temperature, equilibrium time, extraction time, extraction temperature were set at 80°C, 30 min, 30 min, and 80°C respectively, the sample weights of 10, 15, 20, 25, and 30 g were selected to test how sample weight affected total peak area. A sample weight of 20 g gave the greatest peak area (Fig. 4) and so was adopted as the optimal sample size.

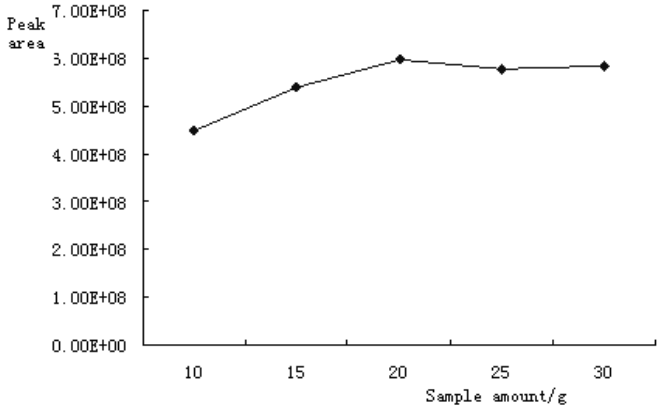

Figure 4 Effect of different sample amounts on total peak area.

3.6. Precision test

Using the optimum conditions for solid-phase micro-extraction, the same sample was analysed five times. The relative standard deviation of the total peak height was 2.54%, and the relative standard deviation of the total peak area was 4.31% (Tab. 1). This shows that the method has good precision.

Table 1 Reproducible experimental analysis results of volatile components in rice.

Test times	The total peak height	The total peak area
1	236714734	711688705
2	232831897	711763421
3	229953884	689801699
4	242762815	745467910
5	243723859	663439100
mean	237197438	704432167
SD	6027595	30351355
RSD (%)	2.54	4.31

3.7. The GC-MS total ion peaks map of volatile components in rice

Solid-phase micro-extraction can fully extract the volatile components in rice which can then be well separated (Fig. 5 and 6). This shows that solid-phase micro-extraction is a feasible pre-processing technology for the volatile components in rice.

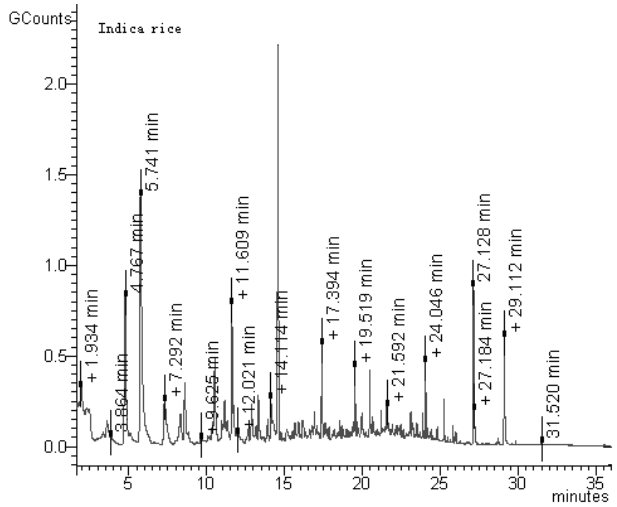

Figure 5 The GC-MS total ion peaks map of volatile components in indica rice.

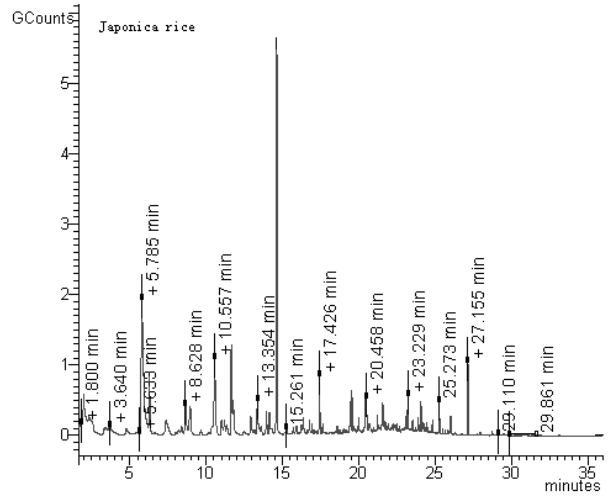

Figure 6 The GC-MS total ion peaks map of volatile components in japonica rice.

3.8. The overall composition of volatile components in rice

Rice contains alcohols, aldehydes, ketones, esters, hydrocarbons, acids, and heterocyclic volatile compounds (Fig. 7). Hydrocarbons compounds were the most numerous, followed by aldehydes, alcohols and ketones, organic acids, esters and heterocyclic components. Aldehydes and hydrocarbons make up the highest proportion of volatiles compounds by weight in rice followed by alcohols, ketones and heterocyclic (Fig. 8). In indica and japonica rice the total aldehyde content was very similar, 32.22% and 33.47% respectively, with hydrocarbon content only a little less so at 24.34% and 29.15%. In the case of total alcohol indica rice at 9.1% was almost double japonica at 5.6%.

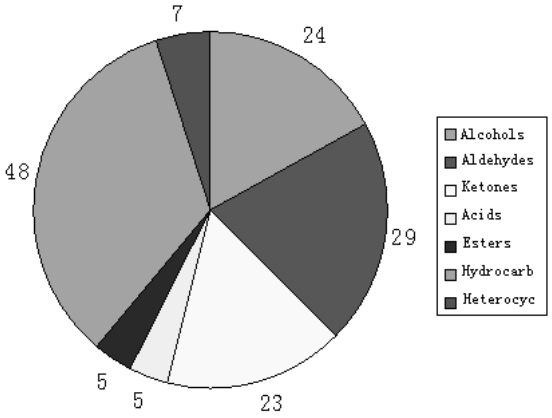

Figure 7 The numbers of various volatile compounds in rice.

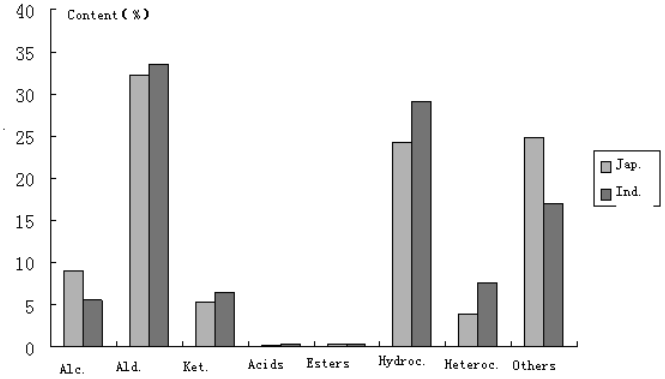

Figure 8 The proportion of various volatile components in rice.

3.9. Volatile alcohols

There were a total of 22 different volatile alcoholic compounds detected in the rice, of which there were 19 in indica and 13 in japonica (Tab. 2). The most abundant volatile alcohols in indica rice were n-hexyl alcohol, n-octanol and 2-hexyl-1-octanol, while in japonica they were n-octanol, 2- hexyl-1-octanol, and 3,7,11-trimethyl-1-12 alcohol.

Table 2 The different volatile alcohol compounds in rice and the relative content (%).

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
1	n-hexanol	3.35	0.17	—	—
2	n-heptanol	0.26	0.15	0.12	0.34
3	n-octanol	1.24	0.40	0.30	1.53
4	2-hexyl-1-octanol	1.14	0.67	0.09	1.01
5	1-octene-3-ol	0.58	0.32	0.17	—
6	2-ethyl-1-decanol	—	1.43	2.35	—
7	2-butyl-1-octanol	0.78	—	—	—
8	n-nonanol	0.46	—	—	—
9	2-ethyl-1-decanol	0.59	—	—	—
10	2-methyl- Undecanol	0.74	—	—	—
11	n-Dodecanol	0.20	0.15	—	0.33
12	3,7,11-trimethyl-1- Dodecanol	0.01	1.65	2.09	0.07
13	tetradecanol	—	—	—	0.04

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
14	6,10,13-trimethyl-tetradecanol	0.29	—	—	—
15	10-pentadecene-1-ol	—	—	0.268	0.48
16	n-hexadecane	0.76	0.69		0.12
17	3,7,11,15 -tetramethyl-2-hexadecimalen-1-ol	—	1.23	0.79	—
18	n-heptadecanol	0.13	—	—	—
19	10-heptadec en-1-ol	—	0.11	—	—
20	n-eicosanol	0.21	—	—	—
21	pentacosene	—	—	1.12	—
22	phytol	0.50	—	—	—

3.10. Volatile aldehydes

A total of 28 different volatile aldehydes were detected of which there were 18 in indica and 23 in japonica (Tab. 3). Both indica and japonica contained pentanal, hexanal, heptanal, 2-heptene aldehyde, octanal, nonanal, decyl aldehyde and benzene formaldehyde. The most abundant aldehyde was hexanal, which on average accounted for 13.31% of the aldehydes (averaging 14.69% for indica and 1.93% for japonica), followed by nonanal which accounted for an average of 7.93%. Pentanal, hexanal, heptanal, octanal, nonanal, decyl aldehyde, and benzene formaldehyde were present at relatively high levels.

Table 3 The different volatile aldehyde compounds in rice and the relative content (%).

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
1	3-methyl-2- butyraldehyde	—	—	—	0.11
2	pentanal	0.33	0.29	0.32	1.45
3	2,4-pentadiene aldehyde	0.02	—	—	—
4	hexanal	13.5	15.9	9.27	14.59
5	2-heptene aldehyde	0.095	—	0.07	0.16
6	2,4-hexadiene aldehyde	—	—	—	0.204
7	2-ethyl -2-hexenal	—	0.44	—	—
8	heptanal	3.12	0.79	0.93	2.33
9	2-heptenal	0.65	0.48	0.42	0.92
10	octanal	4.0	1.13	1.18	5.09
11	2-octenal	0.63	0.51	0.49	—
12	2-butyl-2-Octenal	—	1.48	1.03	—
13	2,3,7-trimethyl-Octanal	—	0.53	—	—
14	nonanal	10.0	2.53	2.96	16.25
15	nonnenal	0.52	—	—	0.51
16	2,4-nonadienal	—	—	—	0.17
17	decyl aldehyde	2.46	0.53	0.47	2.18
18	2-decenal	—	—	—	1.07
19	undecane	0.46		0.11	0.47
20	citral	—	—	—	0.05
21	dodecanal	—	—	—	0.19
22	2,4-diene dodecanal	—	—	0.11	0.13
23	tridecanal	0.26	—	—	—
24	tetradecanal	0.26	—	—	0.23
25	hexadecanal	0.16		0.12	0.52
26	benzene formaldehyde	1.49	1.0	1.41	1.24
27	4-methyl benzene formaldehyde	—	—	0.04	0.07
28	benzene acetaldehyde	—	—	0.09	—

3.11. Volatile ketones

A total of 23 different volatile ketones were detected of which there were 19 in indica and 13 in japonica rice (Tab. 4). Even within the two rice varieties, there was considerable variation in the types of ketone detected. The ketones content was much lower than the aldehyde content.

Table 4 The different volatile ketone compounds in rice and the relative content (%).

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
1	acetone	0.97	—	—	—
2	4-hydroxy-4-methyl-2-pentanone	0.01	—	—	—
3	2-heptanone	—	0.95	—	—
4	6-methyl-2-heptanone	0.16	0.48	—	—
5	heptene-dione	—	0.286	—	—
6	6-methyl-5-ene-2-heptanone	1.28	—	—	—
7	3,5-heptadiene-2-one	0.21	0.15	—	—
8	2-octanone	—	0.35	0.91	0.71
9	2,3- octandione	—	—	0.32	0.88
10	3-octene-2-one	—	0.46	0.45	—
11	3-octdiene-2-one	—	—	—	0.06
12	6-methyl-5-heptanone	—	—	0.691	1.83
13	5-ethyl-6-methyl-2- heptanone	—	—	0.08	0.39
14	6-methyl-3,5-heptadiene-2-one	—	—	0.39	0.76
15	2-nonanone	—	0.169	—	—
16	3-nonene-2-one	—	—	0.25	—
17	2-undecanone	—	0.32	0.24	—
18	6,10 -dimethyl-2-undecanone	0.67	—	0.51	0.72
19	6,10-dimethyl-5,9-undecandione	0.291	—	—	—
20	2-dodecanone	—	0.56	0.55	0.12
21	2-tridecanone	—	—	0.17	0.19
22	2-pentadecanone	0.26	—	—	0.11
23	6,10,14-trimethyl-2-pentadecanone	2.60	0.41	0.79	1.99

3.12. Volatile hydrocarbons

A total of 48 volatile hydrocarbons volatile were detected in the rice (Tab. 5). Both indica and japonica contained 2,6,10-trimethyl-12 alkane, pentadecane, 2,6,10-trimethyl-15alkane, hexadecane, heptadecane, 19 and 20 alkyl alkanes all of which were relatively abundant, averaging 1.39%, 1.75%, 3.14%, 2.60%, 2.52%, 1.62% and 3.52%, respectively.

Table 5 The different volatile hydrocarbon compounds in rice and the relative content (%).

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
1	propane	—	—	—	0.02
2	3,5-dimethyl-octane	0.01	—	—	—
3	3-ethyl-2-methyl-heptane	1.12	—	—	—
4	nonane	—	—	—	0.25
5	decane	—	—	—	0.48
6	2,6-dimethyl-decane	0.70	—	—	—
7	2,4,6-trimethyl-decane	—	—	0.17	—
8	undecane	1.70	—	0.04	0.06
9	2,5-dimethyl-undecane	0.17	0.05	0.05	—
10	dodecane	—	0.53	0.18	0.11
11	2-methyl-dodecane	0.15	—	—	—
12	2,6,10-trimethyl- dodecane	0.42	3.45	1.45	0.25
13	tridecane	0.67	—	—	1.59
14	5-methyl-tridecane	0.22	0.22	0.18	—
15	tetradecane	—	1.544	—	—
16	2,6,10-trimethyl-tetradecane	—	—	0.56	—
17	pentadecane	1.72	0.06	2.96	2.28
18	5-methyl-pentadecane	—	—	0.591	—
19	2,6,10-trimethyl-pentadecane	1.48	3.03	7.82	0.25
20	hexadecane	1.07	3.43	4.24	1.69
21	3-methyl-hexadecane	—	—	0.90	—
22	2,6,10,14-tetramethyl-hexadecane	—	—	1.72	—
23	heptadecane	3.17	0.92	5.72	0.26
24	2,6-dimethyl-heptadecane	0.19	1.22	0.28	—
25	2,6,10,14-tetramethyl-heptadecane	—	1.47	1.55	—

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
26	octadecane	—	3.99	—	0.58
27	nondecane	0.45	3.74	1.83	0.46
28	eicosane	0.27	3.31	10.0	0.47
29	docosane	0.19	—	—	—
30	tricosane	—	0.97	—	—
31	tetracosane	—	3.22	2.13	—
32	3,5,23-trimethyl-tetracosane	—	—	2.09	—
33	hexacosane	—	0.46	—	0.25
34	heptacosane	—	—	0.59	—
35	octacosane	0.29	—	—	—
36	triacontane	0.41	0.36	0.61	
37	dotriacontane	0.15	0.20	—	—
38	17-hexadecyl-tetratriacontane	—	—	0.75	—
39	hexatriacontane	0.22	0.59		
40	1,1-dimethyl-2-octyl-cyclobutane	0.12	—	—	—
41	alkyl-cyclopentane	—	—	—	0.09
42	nonyl-cyclohexane	—	0.34	—	0.17
43	undecyl-cyclohexane	—	—	1.21	—
44	pentadecyl-cyclohexane	0.13	1.49	—	—
45	heptadecyl-cyclohexane	—	—	0.46	—
46	toluene	—	—	—	0.68
47	1,2,3-trimethyl-benzene	—	—	0.02	—
48	1-ethyl-2-methyl-benzene	0.16	—	—	0.26

3.13. Other volatile compounds

Five organic acids, five esters, and seven heterocyclic compounds were detected (Tab. 6). There were the big differences in the organic acids and esters of the two rice varieties. The heterocyclic components in both indica and japonica contain 2-pentyl-furan and methyl-naphthalene, with average contents of 3.37% and 0.80% respectively.

Table 6 The other volatile components in rice and the relative content (%).

N	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
	Organic acids				
1	acetic acid	—	0.02	0.19	0.14
2	propyl acid	0.13	—	—	—
3	2,3-dihydroxy-succinic acid	—	—	0.04	—
4	3,7-dimethyl-hexanoic acid	—	—	—	0.084
5	palmitate	0.09		0.09	0.12
	Esters				
1	formic acid hexate	—	—	0.35	—
2	hexanoic acid dodecate	—	—	0.24	—
3	phenyl acetic acid-4-tridecate	—	0.05	—	—
4	acetic acid tetradecate	—	—	—	0.19
5	oxalic acid-cyclohexyl methyl nonate	—	0.56	—	—
	Heterocyclic Compounds				
1	2-methyl-furan	—	—	—	0.74
2	2-pentyl - furan	3.31	2.63	2.15	5.41
3	2-methyl-5-isopropyl-furan	0.24	—	0.10	0.28
4	2,3-dihydrobenzofuran	—	—	—	1.53
5	methyl-naphthalene	0.30	1.22	1.40	0.28
6	methoxy-phenyl-oxime	—	—	0.94	2.38
7	indole	—	—	—	0.06

4. Conclusions

Headspace solid phase micro-extraction, together with gas chromatography and mass spectrography, were used to study for volatile compounds in rice. The optimal conditions for extraction were a 20 g sample heated for 30 min at 80°C prior to headspace absorption, and then extracted for 30 min with HS-SPME.

The volatile compounds in rice are alcohols, aldehydes, ketones, esters, hydrocarbons, organic acids, as well as heterocyclic compounds. Aldehydes were the main volatile compounds. Hexanal is the most abundant, averaging 13.31%; followed by nonanal, averaging 7.93%.

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External egg morphology of common stored-product pests from the families Anobiidae (Ptininae) and Dermestidae (Coleoptera)

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Abstract

External egg morphology of some common stored-product pests from the families Anobiidae (*Ptininae*: *Ptinus*, *Niptus*) and Dermestidae (*Trogoderma*, *Reesa*) based on optical and scanning electron microscope (SEM) micrographs are presented. Diagnostic characteristics are described. Differences in the eggs of these families, genera and some species were found with respect to shape, size and surface structures.

Keywords: Egg morphology, SEM micrographs, Stored-product pest identification

1. Introduction

Some species from the family Anobiidae, subfamily Ptininae (*Ptinus*, *Niptus*) are common stored-product pests and can develop in a wide variety of substrates of both plant and animal origin (grain, seeds, drugs, dried insects and skin). These pests are frequently found in grain stores (Stejskal et al., 2003) and other locations such as mills, warehouses, museums and households. They are classified as secondary pests, but under optimal conditions, they can build up large populations, e.g., *Niptus hololeucus* (Faldermann) (Weidner, 1982). These pests are known to infest and develop in pet food and also in rodent baits (Stejskal et al., 1994). Their natural habitats include bird nests, animals and insects, which can be the source of stored commodity infestations if they are in the vicinity of the stores. Dermestid beetles from the genera *Trogoderma* and *Reesa* are stored-product pests that mainly damage stored grain and seed, but are also associated with other dried materials of plant or animal origin (Stejskal and Kučerová, 1996; Rees, 2004). The most important pest is *Trogoderma granarium* Everts, formerly classified as a quarantine species (Stuart et al., 1993; Stejskal and Kučerová, 1996).

In spite of the fact that the identification of adults and larvae is difficult, descriptions for diagnostic purposes are available for many species from the aforementioned families (Weidner, 1982; Gorham, 1991; Peacock, 1993; Rees, 2004). There are no data that include descriptions of the eggs of these organisms. Rather, the descriptions consist of brief notes without SEM micrographs of select species from these genera, as reported by Le Cato and Flaherty (1974). Therefore, the aim of this paper is to present preliminary results of the measurements and external morphology of the eggs based on detailed microscopic examination to enable better identification of these most common stored product species in the Czech Republic from above mentioned genera.

2. Materials and methods

Studied species were reared at one of the following two conditions: 20ºC and 75% relative humidity (r.h.) on a mixture of whole and ground wheat, wheat germ, yeast, oak flakes and granular dog food (Ptininae), or 25ºC and 75% r.h. on a mixture of ground wheat, yeast and oak flakes (Dermestidae). Isolated females were allowed to oviposit in small containers (4 cm deep, 3 cm inner diameter) containing a small amount of food. Eggs were collected with a brush, gently cleaned in a drop of distilled water and used for optical microscopic examination (stereomicroscope Nikon SMZ 800 – Nikon spol. s.r.o., Prague, Czech Republic, Axioscope Zeiss – Carl Zeiss spol. s.r.o., Prague, Czech Republic). Size measurements were taken using a light microscope with an objective micrometre. A minimum of 30 eggs were measured for each species. Measurements taken included length (L), width (W) and L/W ratio of eggs. The eggs used for scanning electron microscopy were placed on stubs covered with double-sided sticky tape and sputter-coated with platinum in a Sputter Coater (model SDC 050 – Balzers s.r.o., Prague, Czech Republic). The egg surface (chorion) was then studied with a JSM 6400 (JEOL (Europe)

S.A., Prague, Czech Republic) scanning electron microscope (SEM) at magnifications of 200 to 20,000 x. Approximately 10-30 eggs were examined with the SEM for each species.

3. Results

The dimensions (µm) of the eggs studied are shown in Table 1.

Table 1 The size dimensions (mean ± S.D.) of eggs (µm).

Ptininae:	**Length (L)**	**Width (W)**	**L/W ratio**
Ptinus fur	546 ± 23	361 ± 22	1.52 ± 0.15
Ptinus tectus	437 ± 22	303 ± 18	1.45 ± 0.11
Niptus hololeucus	638 ± 43	474 ± 27	1.35 ± 0.10
Dermestidae:			
Trogoderma granarium	592 ± 40	251 ± 38	2.37 ± 0.23
Trogoderma glabrum	584 ± 47	236 ± 13	2.49 ± 0.28
Reesa vespulae	698 ± 49	247 ± 7	2.82 ± 0.18

3.1. Family Anobiidae (subfamily Ptininae = formerly family Ptinidae)

Ptinus fur L.: The shape of the eggs is irregular oval, considerably variable (Fig. 1a), with one end sometimes moderately or distinctly pointed (Fig. 1b). The eggs are opaque white in colour. Microstructures are formed from irregular dome-shaped protuberances that densely cover the entire surface of the eggs (Fig. 1c). Micropyles and aeropyles were not observed on the egg surface.

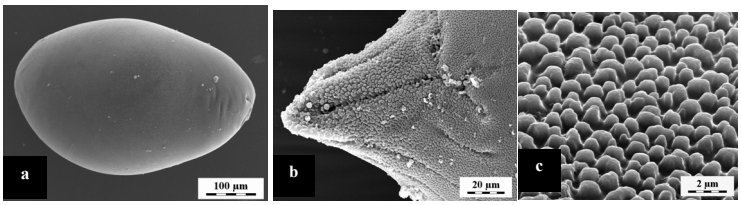

Figure 1 *Ptinus*: a) entire egg, ; b) end of the egg; c) surface structure.

Ptinus tectus Boieldieu: Egg shape is irregular oval (Fig. 2a) with more-or-less rounded ends (Fig. 2b). The eggs are opaque white in colour. Microstructures are composed of irregular dome-shaped protuberances that cover the entire surface of the eggs, but they are markedly larger and less dense at the posterior end (Fig. 2c). On the top of the same end, there is also a slightly visible area with 5 - 6 hollows, which resemble micropyle openings (Fig. 2d).

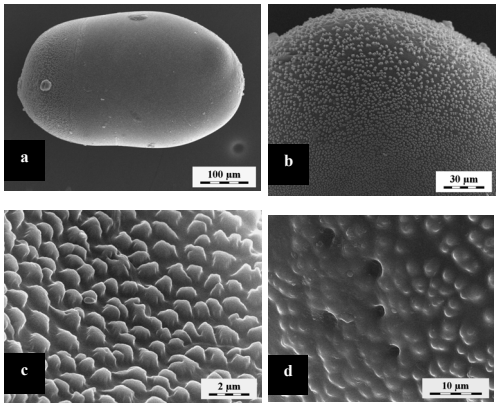

Figure 2 *Ptinus tectus*: a) entire egg; b) posterior end of the egg; c) surface structure; d) detail of the top of posterior end.

Niptus hololeucus (Faldermann): Egg shape is oval to broadly ovoid (Fig. 3a) with both ends rounded (Fig. 3b). The eggs are opaque white in colour. The entire surface of the egg is covered with rugged, pointless protuberances of uniform density (Fig. 3c). Micropyles and aeropyles were not observed on the egg surface.

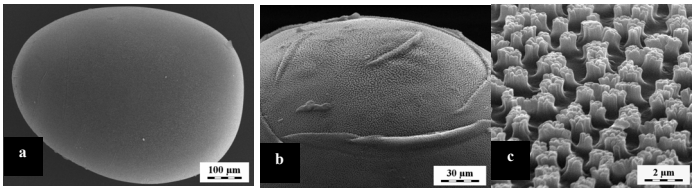

Figure 3 *Niptus hololeucus*: a) entire egg; b) end of the egg; c) surface structure.

3.2. Family Dermestidae

Trogoderma granarium Everts: The shape of the eggs is cylindrical (Fig. 4a). One end is usually moderately broader and terminated with fraying fibres (Fig. 4b). The eggs are creamy white in colour. The chorion surface is slightly wrinkled and has a microstructure created by longitudinal protruding ridges (Fig. 4c). Micropyles and aeropyles are absent on the egg surface.

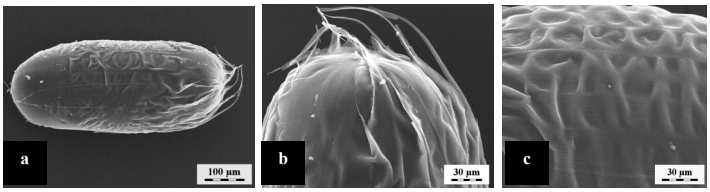

Figure 4 *Trogoderma granarium*: a) entire egg; b) end of the egg; c) surface structure.

Trogoderma glabrum Herbst: The general appearance of these eggs, including chorion structures (Fig. 5c), is similar to T. granarium, however, the shape is on average more elongate with slightly tapering ends (Fig. 5a,b).

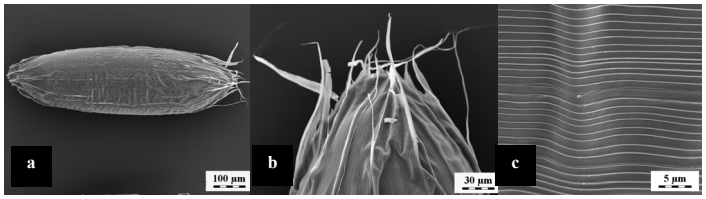

Figure 5 *Trogoderma glabrum*: a) entire egg; b) end of the egg; c) surface structure.

Reesa vespulae Milliron: The egg shape is cylindrical (Fig. 6a) with slightly tapering ends. One end is terminated with fraying fibres. (Fig. 6b). The eggs are opaque creamy white in colour. The chorion surface is wrinkled and microstructures create longitudinal protruding ridges, similar to Trogoderma spp. (Fig. 6c). Micropyles and aeropyles are absent on the egg surface.

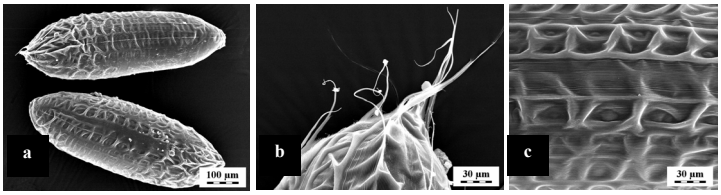

Figure 6 Reesa vespulae: a) entire egg; b) end of the egg; c) surface structure.

4. Discussion

The diagnostic characteristics reported here enable the accurate differentiation between eggs of both studied groups (ptinids and dermestids) through comparisons of their shape, size (L/W ratio) and surface structures (Table 2). Significant differences among studied species from the subfamily Ptininae were detected with respect to egg size and the character and density of surface microstructures. The egg shape is less useful for differentiating species, because of its considerable variability. Identifying both *Trogoderma* species is not consistently possible; the size measurements overlap and surface structures look similar. *Ressa vespulae* eggs are significantly larger than *Trogoderma* species eggs, but surface microstructures are considerably similar. The openings facilitating sperm penetration (micropyles) and respiration (aeropyles), which are frequently present in the insect chorion (Chapman, 1998; Kučerová, 2002) and provide useful taxonomic characters, were not found in the eggs studied (except possibly in the case of P. tectus).

Table 2 Morphological egg differences between families.

Family	Egg shape	Surface microstructure
Anobiidae (Ptininae) (*Ptinus, Niptus*)	Irregular oval to ovoid with rounded or one end pointed, quite variable	Surface covered with dome-shaped or rugged protuberances
Dermestidae (*Trogoderma*, *Reesa*)	Cylindrical with more or less rounded or slightly tapering ends, one end usually terminated with fraying fibres	Surface wrinkled with longitudinal protruding ridges

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Intra and interspecific variation assessment in Psocoptera using near spectoscopy

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Abstract

Several species of Psocoptera are associated with and damage grains and other stored products, books, historical documents, and insect collections. Their small size and lack of expressive morphological variation make it a difficult group for species identification. The spectra of adult males and females of 10 psocid species from the genus Liposcelis were obtained by near infrared spectroscopy (NIRS) and analyzed. Each specimen was placed on a diffuse reflectance accessory of a NIR spectrometer to obtain the respective spectrum, using ten replicates for each species or sex. All spectra were analyzed by combined methods of multivariate analysis using the technique of crossed validation for the multivariate models. The analysis discriminated the species without significant overlapping among the species spectral patterns. The NIRS also revealed variation in the metabolomic profile of males and females; however, it is still possible to distinguish the species using only males or females or even from mixed sex samples. NIRS technique proved to be a powerful tool to discriminate species both at intra and interspecific levels based on dispersion spectral patterns of individual specimens.

Keywords: Biological systems, Liposcelididae, stored product pests, Vibrational spectroscopy.

1. Introduction

Psocoptera is a relatively small order of insects with 4400 species worldwide (Pascual-Villalobos et al., 2005; Dong et al., 2007); many of these species are associated with stored grains and other food products in many parts of the world (Turner, 1994; Nayak et al., 2003). Psocids used to be considered as only nuisance secondary pests and were often overlooked because of their small size and the presence of more damaging primary pests, especially in cereal grains. Before 1990, psocids were not considered serious pests of stored products; although in some countries such as Australia, they have become the most frequently encountered storage pest in some areas (Rees, 2003). However, their world-wide prevalence and increasing importance as pests contaminating food and agricultural commodities is now documented from all continents (Kucerova et al., 2006; Stejskal et al., 2006; Throne et al., 2006). The recent rise of psocids to prominence can be attributed to their varied response to management tactics that have been developed for coleopteran pests and the resistance of some psocopteran species to residual insecticides and phosphine (Nayak et al., 2003; Nayak, 2006). In Australia, detection of high levels of resistance to phosphine in psocids (Nayak et al., 2003) has elevated their pest status enormously and put them alongside the major coleopteran pests (Nayak et al., 2003). In addition, the importance of psocopteran pests has increased in recent years due to the failure of almost all registered grain protectants to control them (Nayak and Daglish, 2007).

Precise identification of pest species is fundamental to pest control, quality-control of food products and the settlement of legal disputes resulting from insect contamination of goods. However, it is difficult to define and to characterize insect biodiversity, especially in groups of little known insects, such as those in the order Psocoptera. A new promising approach in biodiversity is the science of metabolomics. Metabolomics is an emergent field in the "omics" research area and is related to global characterization of small metabolic molecules in biological systems. Metabolomics is the last expression of the cellular regulation resulting in the visible phenotypes. A research method for metabolomics is spectroscopy in the near infrared (NIRS). The NIRS technology was developed in the mid-1990s by the United States Department of Agriculture. It was first used to quantify protein levels in wheat and fat/oil levels in soybeans in order to facilitate payment of farmers by grain cooperatives and for rapid segregation of wheat in different silos given that conventional methods were time consuming.

The NIRS is a type of vibrational spectroscopy that uses light energy from wavelengths corresponding to 750 to 2500 nm. The interaction of light with matter in those frequencies can provide qualitative and quantitative information at the molecular level. Advantages of this technique are: universal application (works on any molecule containing the connections C-H, N-H, O-H and S-H); it is a fast method (one minute or less per sample); it doesn't generate solid, liquid or gaseous residues; it is a clean technology (environmentally friendly); it uses small samples, in situ or alive, no previous sample treatment is needed and importantly it is neither invasive nor destructive. NIRS data analysis requires statistical software for sample identification, qualification, and quantification of the entities being studied.

Application of NIR as an analysis tool can be found in practically all areas, from astronomy, industry, quality control, environment, taxonomy, and medicine. NIRS has been used to identify several coleopteran species (Dowell et al., 1999), detect parasitized weevils in wheat kernels (Baker et al., 1999), and to detect external and internal insect infestation in wheat (Ridgway & Chambers, 1996; Ghaedian & Wehling, 1997; Dowell et al., 1998).

In Brazil, the application of NIRS was mainly for soil analysis and quality control of medicines, lubricants, and other products. Nowadays, NIRS is broadly used in industry and in scientific research around the world because it is a fast and reliable technique for measurement, quality-control and for analytic process technology. Lazzari et al. (2009) demonstrated patterns of diversity in Psocoptera using near infrared spectroscopy. The objective of the present work is to demonstrate that metabolomics, by spectroscopy in the NIR, is a fast non-destructive and robust strategy to test and organize hypotheses related to sex and species determination and diversity patterns of Psocoptera.

2. Materials and methods

Samples of ten species of Liposcelis (Psocoptera: Liposcelididae) from laboratory cultures were investigated: *L. bostrychophila* Badonnel, 1931; *L. brunnea* Motschulsky, 1852; *L. corrodens* (Heymons, 1909); *L. decolor* (Pearman, 1925); *L. entomophila* (Enderlein, 1907); *L. fusciceps* Badonnel, 1968; *L. granicola* Broadhead & Hobby, 1944; *L. paeta* Pearman, 1942; *L. pearmani* Lienhard, 1990; and *L. rufa* Broadhead, 1950. The previous identification of the species was accomplished based on the key by Opit et al. (2008) for *Psocoptera* of grains and stored products. This identification key is based on the infrageneric classification proposed by Badonnel where the species are placed in a section, group and subgroup using morphological characters (Mockford, 1993). For the NIRS analyses, the reflectance spectra (R) were obtained in a spectrometer of the series Excalibur Bio-Rad FTS 3500GX (Bio-Rad Laboratories, Cambridge, MA, USA); equipped with KBr beam splitter; detector of deuterate triglicerin sulfate (DTGS); radiation source of silicon carbeto; and accessory of diffuse reflectance in the near infrared ranges from 7500 to 4000 cm-1 (1428 to 2500 nm) with a resolution of 1 cm-1.

Each insect was positioned directly on the accessory of diffuse reflectance and a total of 64 readings were obtained for each insect in each spectrum, using ten replicates for each species. Processing of the spectra used the first Savistky-Golay derivative (21 point window and second order polynomial) and smoothing (seven point window, The Unscrambler™ version 9.1, Camo Software AS, Oslo, Norway). Discriminant analysis (DA) was then applied to the spectra to evaluate the discrimination between groups (sex, species, and sex + species) proposed by NIR spectroscopy (JMP™ version 8.0.1, SAS Institute, Cary, NC, USA).

3. Results

Using discriminant analysis, we tested the NIRS data for sexual dimorphism, species discrimination and the combination of species and sex of psocids. The analysis separates the sexes for all individuals independent of species with 100% resolution. There is high probability that a given individual can be correctly placed in a given group based on its sex (Fig. 1). The next analysis was to discriminate each species. The results showed that there was practically no overlapping of patterns between species when their NIR spectral data were analyzed by discriminant analysis (Fig. 2). Finally, the discriminant analysis showed that the NIR spectral data can give sufficient information to discriminate simultaneously sex and species with 100% resolution (Fig. 3).

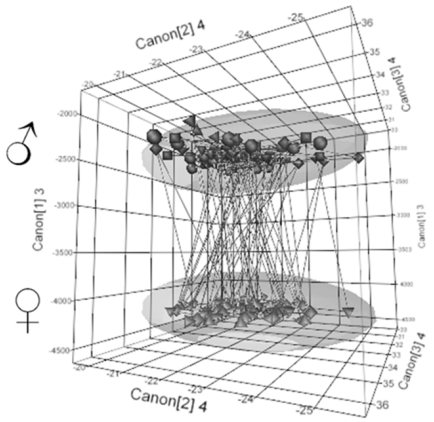

Figure 1 Sexual discrimination based on discriminant analysis of NIRS data for ten species within the genus Liposcelis. Clear spheres represent the males and dark triangles the females. Male and female of each species are connected by lines. FD1, FD2 and FD3 are the discriminant factors 1, 2 and 3, respectively.

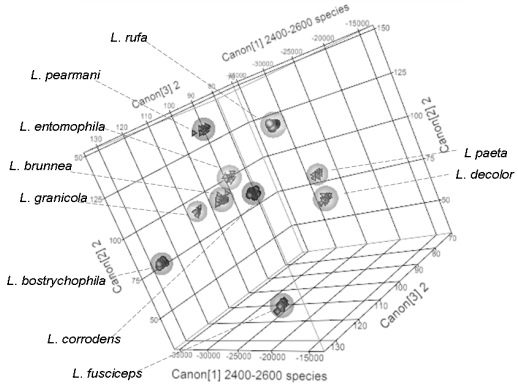

Figure 2 Species discrimination based on discriminant analysis of NIRS data for ten species within the genus Liposcelis. Groups of the same symbols locked by ellipses represent different species. Probabilities of each ellipsis in each species are > 90%. FD1, FD2 and FD3 are the discriminant factors 1, 2 and 3, respectively.

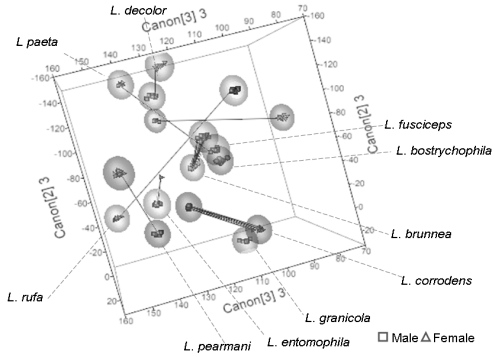

Figure 3 Simultaneous discrimination of sex and species within the genus Liposcelis based on discriminant analysis of NIRS data. Groups of the same symbols locked by ellipses represent different species and sex. Probabilities of each ellipsis for each species are between 82% (larger circular areas - more disperse) and 92% (smaller circles). Male and female of each species are connected by lines. FD1, FD2 and FD3 are the discriminant factors 1, 2 and 3, respectively.

4. Discussion

The order Psocoptera is divided in three suborders: Trogiomorpha, Troctomorpha and Psocomorpha. The suborder Troctomorpha, that contains the family Liposcelididae, consists of two infraorders and eight families. Members of the family Liposcelididae are small, flattened psocids, with enlarged hind femora; when wings are present, venation is reduced; in alate forms both fore and hind wings are present; eyes near vertex; in apterous forms eyes remote from vertex, each consisting of two large elements alone or preceded by six or fewer small ocelloids; pronotum lobed; thoracic sterna broad and bearing cilia. According to the Psocoptera World Catalogue (Lienhard and Smithers, 2002), the species *L. bostrychophila*, *L. fusciceps* and *L. lenkoi* have already been recorded in Brazil along with other 370 Psocoptera species, in 85 genera and 29 families - most of them have been poorly studied.

The integument, based on its composition, is a part of the phenotypic expression of the insect. Thus, it can be considered as a character that is as valuable as other phenotypic or genotypic expressions, such as ethnology, morphology or molecular biology. Entomological literature demonstrates the importance of cuticular hydrocarbons in insects (Lazzari et al., 1991) and other arthropods; these function as a barrier that protects the insect against desiccation, entrance of microorganisms, and other numerous biochemical, physiological, semiochemical, and intraspecific and interspecific ecological functions (Howard and Blomquist, 2005). NIRS does not only detect the cuticular hydrocarbons, but it also analyses other molecules present in small amounts that possess the groups O-H, C-H, N-H and C=O in some part of their structure (Kradjel, 1991).

The great resolution of NIRS to discriminate *Liposcelis* species demonstrated herein by the multivariate analysis is corroborated by studies of other insect taxa (Benedict, 1955; Dowell et al., 1999; Stackebrandt et al., 2002; Cole et al., 2003; Ami et al., 2004; Paliwal et al., 2004; Zhao et al., 2006; Aldrich et al., 2007). Therefore, NIRS can be considered as a viable technique for insect species identification. It is pertinent to mention that the report of the committee for revision of the definition of species in bacteriology (Stackebrandt et al., 2002) includes the use of NIRS techniques for systematic studies of prokaryotes. Considering the literature that reports the application of NIRS in taxonomy and systematics of prokaryotes, we suggest that NIRS has an even greater potential as a novel technique in the systematics of eukaryotes.

The delimitation of species is a topic of constant discussion and refinement (Wiens, 2008). If NIRS has the capacity to recognize the identity of a given species, as shown in this study for the species of Liposcelis, NIRS could also contribute to defining whether the extension of the variability in the spectrum is evidence of the intraspecific variability of a given species, such as sex discrimination. It is already clear that spectrum variability can be considered as evidence of interspecific variation; therefore, NIRS would be a valuable tool for the discovery of cryptic species.

The NIRS technique is a promising alternative for distinguishing between not only genera and species of Psocoptera, but as shown in this study, for discriminating males from females of each species simultaneously (Fig. 3). One of our objectives was simply evaluating sexual variation not other types of intraspecific variation. Although, the intraspecific variation not related to sex may be noticed in the three figures presented by the dispersion of each point (specimen) from the centroid of its group (sex or species). We calibrated the analysis based on regions of the spectrum that would give us the highest possible discrimination between groups (sex, species, and both combined); however, if one wants to investigate ecological or evolutionary relationships among groups, other spectral regions could be analyzed to complement the information on affinities among the groups. Therefore, in this paper, only autapomorphic and synapomorphic characters to discriminate species and sex were evidenced, but much more information can be obtained.

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From RFLP, specific primer to DNA Barcoding: preliminary study on molecular identification of common stored product psocid

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Abstract

Stored product psocids (Insecta: Psocoptera) is one kind of common storage insect pests in the world and is difficult to be identified morphologically. The molecular identification methods, from Restriction Fragment Length Polymorphism (RFLP), specific primer, to DNA Barcoding, were studied gradually in this paper with international collaboration and based on sequence of 16S rDNA gene of the common species of stored psocids such as *Liposcelis. bostrychophila*, *L. brunnea*, *L. corrodens*, *L. decolor*, *L. entomophila*, *L. mendax*, *L. paeta*, *L. pearmani*, *L. rufa*, and *L. tricolor*. The samples of different geographical populations in this study were from Peoples Republic of China, Czech Republic, United States of America, Croatia, Portugal and Denmark respectively. The result presented that it was successful to accomplish the sequencing of 16S rDNA gene from single individual of above psocids with one pair of primers (16Sar and 16Sbr) and our methods of DNA extraction, Polymerase Chain Reaction (PCR) reaction mix and condition. Twenty-two sequences were submitted to GenBank and the accession numbers were allocated (Table 1). According to the related sequences, RFLP method was studied firstly and one restrictive endonuclease (DraI) was selected to discriminate the most common four species such as *L. bostrychophila*, *L. entomophila*, *L. decolor*, and *L. paeta*. The method of specific primer was researched in order to determine *L. corrodens* which species had not occurred in China. Two pairs of specific primers were designed and selected for the identification of *L. corrodens* with specific bands from other species of common stored psocids. As an applied example, the samples of *Liposcelis captured* from imported graze seeds of Denmark by Chinese plant quarantine agency were identified with method of DNA Barcoding. The results showed that Denmark sample shared 98.94% sequence similarity with *L. corrodens*, and the maximum-likelihood (ML), neighbor-joining (NJ), and maximum parsimonious (MP) phylogenetic analysis indicated that Denmark sample and *L. corrodens* were in the same subgroup in the phylogenetic relationship tree. For further development, we are researching the DNA Barcoding with mtDNA COI (mitochondrial cytochrome oxidase I) gene of stored psocids as the prototype of other stored pests. More collaboration from the world is regarded as the key point for the successful research of molecular identification of stored insect pests.

Keywords: Stored product psocid, Molecular identification, RFLP, Specific primer, DNA Barcoding

Table 1 Liposcelis species and populations sequenced in this study (populations were coded by combining species names with acronyms of collection countries and sites).

Population	Location collected	GenBank Accession No.
*Liposcelis*_DK	Denmark, 2008	FJ418874
*L. corrodens*_P-CZ	Central Bohemia, CZ, 2007	EU863792
*L. corrodens*_Port.	Portugal, 2008	GU563531
*L. corrodens*_U.S.A	USA, 2008	FJ865400
*L. brunnea*_P-CZ	Central Bohemia, CZ, 2007	FJ439564
*L. brunnea*_U.S.A	USA, 2008	FJ865401
*L. mendax*_JS-P. R. China	Jiangsu, P. R.China, 2006	EU872216
*L. bostrychophila*_GX-P. R. China	Guangxi, P. R.China, 2006	EU863796
*L. bostrychophila*_P-CZ	Central Bohemia, CZ, 2007	EU863798
*L. bostrychophila*_U.S.A	USA, 2008	GU563532
*L. entomophila*_P-CZ	Central Bohemia, CZ, 2007	EU863795
*L. entomophila*_CRO	Croatia, 2009	GU563529

Population	Location collected	GenBank Accession No.
*L. entomophila*_CQ-P. R. China	Chongqing, P. R.China, 2006	EU863794
*L. entomophila*_U.S.A	USA, 2008	FJ865402
*L. decolor*_CQ-P. R. China	Chongqing, P. R.China, 2006	EU 878400
*L. decolor*_P-CZ	Central Bohemia, CZ, 2007	EU 878398
*L. paeta*_ZJ-P. R. China	Zhejiang, P. R.China, 2006	EU 878399
*L. paeta*_P-CZ	Central Bohemia, CZ, 2007	EU 863800
*L. paeta*_U.S.A	USA, 2008	GU563533
*L. pearmani*_U.S.A	USA, 2008	GU563530
*L. rufa*_U.S.A	USA, 2008	GU563527

Egg hatching at different temperatures and relative humidities in *Idaea inquinata* (Scopoli) (Lepidoptera: Geometridae)

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Abstract

Idaea inquinata (Scopoli) feeds mainly on dried plants, nevertheless, it is also a potential pest of stored products as it is able to develop on cereal products. The few references on the biology of this species do not deal with the influence of temperature and relative humidity on egg hatching. To fill this gap, groups of 100 eggs, 24-48 hours old, were exposed to five constant temperatures (17, 21, 26, 29 and 34±1°C), two relative humidities (35 and 70±5%) and a photoperiod of 0:24 (light:dark); ten tests were carried out. Each test was replicated four times and egg hatching was observed daily. The highest mean number of hatched eggs was observed at 26 and 29±1°C, 70±5% r.h. with 91.5 and 91.0 eggs, respectively. The lowest mean number of hatched eggs was 61.5 observed at 17°C and 70±5% r.h. The mean numbers of hatched eggs, 83.5, 77.5, 78.5 and 79.8 were similar at 21, 26, 29 and 34±1°C, 35±5% r.h., respectively. Eggs hatched between the sixth and the eighth day at all the temperatures tested, except for 17±1°C and 35±5% r.h., where hatching started on the twelfth day. At this temperature, the duration of the hatching period increased with increasing humidity: 11 d at 35% r.h. and 15 d at 70% r.h.

Keywords: Egg, Hatching, Temperature, Relative humidity, Rusty wave moth

1. Introduction

The rusty wave moth, *Idaea inquinata* (Scopoli) (Lepidopitera: Geometridae) develops on dried plants with a preference for medicinal species that can be heavily damaged and made unsuitable to essential oil extraction. In Southern Italy, Candura (1931a, b) studied I. inquinata from April to October in a warehouse. He observed two generations on hay, and three generations on chamomile and leguminous plants. On average, each female laid one hundred eggs within a week. Oviposition started on the fourth and lasted until the eleventh day after eclosion. Eggs were laid singly or in pairs, and hatching occurred between 4-15 d, depending on season and weather, in a temperature range of 19-28°C. The length of larval development time varied between 60 to 333 d, dependent on the dried plant species.

In the last few years, *I. inquinata* has proven to be a potential pest of stored products, as it can also develop on bran, maize meal, wheat kernels and rice (Locatelli et al., 2005). This species can be a serious pest in warehouses where spices, dehydrated plants and cereals are stored. Since larvae penetrate the substrate and adults have low activity, it is difficult to detect infestations in warehouses.

There are few references to the biology of this species. The purpose of the study is to determine the incubation and hatching time of eggs of I. inquinata at different temperatures and relative humidities.

2. Materials and methods

Idaea inquinata has been reared continuously for 5 years, on an artificial diet consisting of 62 g bran, 8 g corn flour, 7 g wheat flour, 4 g wheat germ, 3 g dried yeast, 9 g glycerine and 7 g honey (Stampini and Locatelli, 2007). The diet was stored in polyethylene bags at 6°C until needed. Insect cultures were maintained in a thermostatic chamber at 26±1°C, 70±5% r.h. and photoperiod of 16:8 (light:dark).

Groups of 100 eggs, 24-48 h old, were placed in 6-cm diameter Petri dishes at different temperatures and relative humidities. Tests were carried out at 17, 21, 26, 29 and 34±1°C with two relative humidities (35 and 70±5%) and a photoperiod of 0:24 (light:dark). Each combination of temperature and humidity was replicated four times and egg hatching was observed daily. Eggs were considered hatched when the young larvae successfully chewed emergence holes in the chorion and left the egg shell. Data were submitted to Duncan's multiple range test, Student's t-test and two-way ANOVA (SPSS 17.0 for Windows and Microsoft Excel 2003).

3. Results

The lowest mean number of hatched eggs was 64.7 observed at 17°C and 35% r.h. ($F_{4,15}=8.275$, $P<0.01$) during a long hatching period of 12-23 d (Table 1). The mean numbers of hatched eggs (83.5, 77.5, 78.5 and 79.8) were not significantly different at 21, 26, 29 and 34±1°C, 35±5% r.h., and the hatching periods were shorter and similar.

Table 1 Mean number (SD) of eggs of Idaea inquinata (Scopoli) hatched at 17, 21, 26, 29, 34°C and 35% r.h. and hatching period

Temperature(°C)	35% r.h.		
	Mean number of eggs (SD)	Number of eggs min-max	Hatching period (days)
17	64.7 (7.8) a	57-72	12-23
21	83.5 (3.9) b	79-88	8-10
26	77.5 (3.9) b	72-81	8-12
29	78.5 (3.5) b	75-82	6-8
34	79.8 (4.3) b	74-83	6-9

Values followed by the same letter in a given column are not significantly different (Duncan multiple range test, $F_{4,15}=8.275$, $P<0.01$).

At 70% r.h., the lowest number of hatched eggs 61.5 was observed at 17°C (Table 2) ($F_{4,15}=12.3$, $P<0.01$). The highest numbers of hatched eggs 91.5 and 91.0 were observed at 26±1°C and 29±1°C, 70±5% r.h., respectively. Eggs hatching started between 6-8 d, the hatching period was 3-4 d at 26, 29 and 34°C, while at 17 and 21°C egg hatching lasted longer at 15 and 7 d, respectively.

Table 2 Mean number (SD) of eggs of Idaea inquinata (Scopoli) hatched at 17, 21, 26, 29, 34°C and 70% r.h. and hatching period

Temperature (°C)	70% r.h.		
	Mean number of eggs (SD)	Number of eggs min-max	Hatching period (days)
17	61.5 (11.1) a	51-73	6-20
21	73.7 (7.2) b	63-79	8-14
26	91.5 (2.4) c	89-94	7-9
29	91.0 (6.5) c	84-97	6-8
34	81.7 (5.9) bc	73-86	6-9

Values followed by the same letter in a given column are not significantly different (Duncan multiple range test, $F_{4,15}=12.3$, $P<0.01$).

The mean numbers of hatched eggs at the two relative humidities were compared for each temperature considered (t-Student test). At 17, 21 and 34°C, there were no significant differences between the effects of the two relative humidities (t-values of 0.47, 2.36, 0.54, df=5, NS, respectively), while at 26°C (t-value of 6.16, df=5, P=0.001) and 29°C (t-value of 3.39, df=5, P=0.01) the differences were significant.

The results were confirmed by two-way ANOVA where temperature effects were significant ($F=16.59$, df=4, $P<0.01$), but relative humidities were not ($F=2.52$, df=1, P=0.122); however, the interaction of the two variables was significant ($F=5.42$, df=4, $P<0.01$).

4. Discussion and conclusions

Idaea inquinata tolerates low relative humidity and high temperatures. In fact, high percentages of eggs hatched even at 34°C at both 35 and 70% r.h. This is in contrast to many other insects that require high relative humidity with high temperatures. In the laboratory, Lee (1988) observed lower numbers of hatching eggs of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) at 22 and 27°C with 35% and 55% than with 75% r.h. A significantly higher number of hatched eggs was observed at 26° and 29°C at 70% r.h. In contrast, Kamel and Hassanein (1967) observed that relative humidity in the range 40-80% did not influence egg hatch in a temperature range of 24-32°C for *Corcyra cephalonica* (Stainton) (Lepidoptera: Galleridae), but relative humidity lower than 20% inhibited hatching.

With 35% and 70% r.h., the hatching period was similar at 29 and 34°C but shorter at 17, 21, and 26°C. In the case of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), Jacob and Cox (1977) found that "humidity has little influence on egg development and developmental periods increase only at very low relative humidities". Other authors observed a gradual decrease in time with an increase of temperature. The incubation period of eggs of *Dasyses rugosella* Stainton (Lepidoptera: Tineidae) decreased with an increase of temperature in the range 25-35°C (Iheagwam and Ezike, 1989; Ashamo and Odeyemi, 2004). At 17°C and both 35 and 70% r.h., a lower egg hatch and longer hatching period was observed. Comparison of model predictions made by Subramanyam and Hagstrum (1993) "indicated that temperature greatly influenced development times, followed by relative humidity, and diet".

Further research on *I. inquinata* is required with determining egg hatching at lower and higher temperatures in order to identify the thermal limits of this species. These results will be useful for the safe storage of foods.

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How methodical approaches affects results of pest sampling in stores and counting in laboratory

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Abstract

In this study we analyzed impact of the method of sampling of pests in stores on the subsequent counting of pests in the laboratory. First, we compared results obtained from two methods of sampling a highly infested flat grain store (surface sampling with cup-sampler vs. subsurface sampling with spear-sampler) and two extraction methods (Tullgren apparatus vs. automated sieving machine). For samples collected from the same store and sampling date, we found that pest population density and spatial distribution differed significantly between methods. Sampling method had a significant influence on number of trapped arthropods: surface sampling recovered significantly more arthropods than use of spear-sampler (LR chi-squared test: $\chi 2 = 4.46$, d.f. = 1, P = 0.034). Number of identified arthropod species was not influenced by sampling method (LR chi-squared test: $\chi 2 = 1.91$, d.f. = 1, P = 0.167). The Tullgren apparatus extracted consistently more arthropods from samples than the automated sieving machine, but the difference was not significant (LR chi-squared test: $\chi 2 = 3.55$, d.f. = 1, P = 0.059). The extraction method also did not influence the number of arthropod species (LR chi-squared test: $\chi 2 = 9.5–09$, d.f. = 1, P = 0.99). Different combinations of sampling/extracting methods led to different estimations of infestation levels and their location. The most sensitive approach to estimate arthropod pests' abundance and spatial distribution revealed to be a combination of surface sampling and Tullgren extraction. Using the sieving machine to extract arthropod individuals from sampling spear samples gave the poorest picture of pests' status in the inspected store (Stejskal et al., 2008).

Second, we compared results of counting mites and psocids in samples obtained either by digital image analysis (DIA) or traditional visual direct counting (VDC) under a binocular microscope. Our DIA method estimated the number of arthropod individuals using distinguishing features of size and shape. The accuracy and time required were similar to those of the traditional direct visual counting approach when samples comprise fewer than 100 individuals. However, as the true sample size increased above 100 individuals, the DIA method was significantly more precise and quicker than visual counting. The direct visual counting method always underestimated the number of individuals per sample. As expected, the time required for direct visual counting increased with sample size, while the time required for DIA-based counting remained the same. Thus, the DIA method is 10 times quicker than the direct visual counting method with a sample of 500 mites (Lukas et al., 2009).

Our work shows that different sampling, extraction and counting methods may convey different infestation "pictures" for the same store. The methodological approach used profoundly affects the evaluation of population density and interpretation of obtained results.

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Some biological characteristics of the *Batrachedra amydraula* Meyrick (Lepidoptera: Batrachedridae) on main varieties of dry and semi-dry date palm of Iran

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Abstract

Although Iran is the leading producer of dates in the world, only 10% of its product is exported. One of the factors which limits exportation is postharvest pests. Lesser date moth (*Batrachedra amydraula*) is one of these postharvest pests. This pest is found in all date palm plantations of Iran. The biology of this pest has not been studied under laboratory conditions on main varieties of stored date in Iran. In this research some biological characteristics including survivorship, developmental time, fecundity, oviposition and postoviposition period, and longevity of adults on date palm varieties Zahedi, Ghasb, Rabbi, Deyri and a semi-artificial diet were studied. All experiments carried out at constant temperature room (30 ± 0.5 °C and 55 ± 5% r.h.). Highest survivorship of egg and combined larval and pupal stages were 86 and 85% respectively on semi-artificial diet. Shortest total development time from egg to adult was 43 days for males and 43 days for females on semi-artificial diet. Longest total development time was 69 days for male and 65 days for female on Ghasb variety. The highest (45 eggs per female) and lowest (25 eggs per female) fecundity occurred on the Deyri variety and semi-artificial diet, respectively. Results of this study would be useful for making pest management decisions in date palm storage.

Keywords: Lesser date moth, *Batrachedra amydraula*, Biology, Date palm

1. Introduction

Iran is the leading producer of date palm, *Phoenix dactylifera* L. (Arecales; Arecaceae) in the world, but only 10% of its production is exported. Some factors such as postharvest pests limit exportation. Because of the length of storage period in stores (almost one year), damage caused by stored-products pests to semi-dry and dry stored date palm is considerable. Also most stores of date palm in Iran are located in regions where climatic conditions (temperature and humidity) are suitable for development of stored-products pests. Since according to Codex standards for date palm, maximum allowance for defects shall be a total of six percent by count of date palms (Barreveld, 1993), a major concern after harvesting of date palm is to prevent or control insect infestation.

One of the pests of date palm is lesser date moth, *Batrachedra amydraula* Meyrick (Lepidoptera: Batrachedridae). It starts its activity in date palm plantations, is then transmitted into storage with infested dates and can go through multiple generations within stored dates. B. amydraula has existed in date palm plantations within Iran for many years (Gharib, 1968), but was reported as a stored date palm pest in Iran for the first time in 1998 (Shayegan et al., 1998). Wiltshire (1957) reported that B. amydraula attacked stored date palm and he collected it from a warehouse in Baghdad. Based on Dowson (1982), this pest damages immature date fruits and stored date palm. *Batrachedra amydraula* is distributed from Pakistan to North of Africa and Middle Eastern countries such as Iran, Iraq, Saudi Arabia, Bahrain, Yemen, Egypt, Tunisia and Libya (Martin, 1972; Hussain, 1974; Abdul-Jabar, 1982; Rohani, 1988; Riedl, 1990; Howard et al., 2001). This pest is distributed in all date palm plantations of Iran (Gharib, 1968, 1991; Modarres-Awal, 1994).

The biological characteristics of this pest vary among regions with different climatic conditions in which they are distributed. *B. amydraula* can produce several generations per year in storage (Shayegan et al., 1998). Howard et al. (2001) reported two generations per year for this pest, but based on Hussain (1974) and Damghani (1998) this pest has three generations in date palm plantations of Iraq and Iran per year. Collected samples of infected dry date palm (Ghasb variety) from traditional stores and date palm

plantations in Kerman (shahdad region) and other varieties from Busherhr, Khuzestan, Jiroft, and Bam showed that *B. amydraula* can produce continuous generations when temperature is above 20°C (Shayegan et al., 1998).

Since there is a little information about the biology of B. amydraula on dry and semi-dry date palm varieties in Iran and other date growing countries, the main objective of this study was to compare life history parameters and fecundity of *B. amydraula* on dry and semi-dry date palm varieties of Iran. The information provided by this study could help us in developing IPM programming for pests of stored date palms.

2. Materials and methods

2.1. Collection and identification of B. amydraula

Originally, infected dry date palms (Ghasb variety) were collected from traditional stores in Shahdad region (30° 25' N, 57° 42' E) in Kerman province. Infected date palms were kept under controlled conditions at 30±5°C, 55±5% (r.h.) and photoperiod of 14:10 (L:D) h in a constant temperature room. Adult moths were anesthetized with CO2 and B. amydraula specimens were separated based on morphological characteristics. One pair (male+female) of adult moths was transferred to a plexiglass box (14×8×4 cm) containing dry date palm. Adults of F1 were used in identification of species (*B. amydraula*). Earlier method offered by Badr et al. (1990) was used for this purpose.

2.2 Mass rearing of B. amydraula in laboratory

Mass rearing of B. amydraula was carried out on introduced semi-artificial diet (400 g powder of dry date Ghasb variety, 400 g whole wheat flour, 150 g honey, 25 g yeast and 120 mL glycerin) developed by Marouf et al. (2004). Fifty unsexed adult moths were released on 35 g semi-artificial diet in a plexiglass box (14×8×4 cm) and the boxes were kept in a constant temperature room as described above. Before starting experiments, insects had been reared for one generation on date palm varieties.

2.3. Biology of B. amydraula on different varieties of date palms

Biology of *B. amydraula* was studied on four dry and semi-dry date palm varieties (Ghasb, Zahedi, Deyri, Rabbi) and on semi-artificial diet. Half of one date palm fruit or 1.5 g of semi-artificial diet were placed in plastic Petri dishes (diameter, 6 cm; depth, 1 cm) with a hole in the lid covered with a fine mesh net for ventilation. Then 80 one day old eggs (each egg was considered as a replication) were transferred individually into the plastic Petri dishes containing date palm or semi-artificial diet.

All Petri dishes were placed in a constant temperature room under environmental conditions described above. The Petri dishes were checked every day and egg incubation period and the duration of other developmental stages were recorded. Last instar (fifth instar) *B. amydraula* larvae before start of pupal stage produce a yellow silk cocoon around their bodies so determination of the start of pupal stage was very difficult. Therefore, in this study, duration of larval and pupal stages were recorded together. The ratio between the percentage of individuals completing development and the average time required to complete development was taken as the development index (Singh and Rembold, 1988). All Petri dishes were checked daily and after adult emergence, a pair of female and male moths were introduced into plastic Petri dishes (diameter, 6 cm; depth, 1 cm) on same date or semi-artificial diet as they had developed on. Oviposition period, daily fecundity, total fecundity and adult longevity were recorded daily until the death of the last female in the cohort.

One-way ANOVA was used to variance analysis and compare of means performed by Duncan's multiple range tests.

3. Results

3.1. Development time and longevity

Mean development times and adult longevity of *B. amydraula* on different varieties of date palm and semi-artificial diet is shown in Table 1. Incubation period showed significant differences among the date palm varieties and semi-artificial diet. Incubation period was longest on Ghasb, Zahedi, and Rabbi varieties and shortest on semi-artificial diet. No significant difference was observed for larval+pupal period among Zahedi, Deyri and Rabbi varieties. Longest and shortest larval+pupal period was on Ghasb variety and semi-artificial diet, respectively. Total development time on Ghasb variety and semi-artificial

diet showed significant differences with other date palm varieties. Total development time was longest on Ghasb variety and shortest on semi-artificial diet.

As showed in Table 1, the total development time of B. amydraula was generally shortest on semi-artificial diet and longest on Ghasb. The whole lifespan of B. amydraula significantly affected by variety of date palms. The whole lifespan of males and females was longest when the larvae were reared on Ghasb, Zahedi, and Deyri varieties and Ghasb, and Rabbi varieties, respectively, and shortest on semi-artificial diet.

Table 1 The mean (±SE) development stages and adult longevity of Batrachedra amydraula on different date varieties

Varieties	Incubation period (days)	Larval + Pupal period (days)	Development time (days)	Adult longevity (days)		Whole lifespan (days)	
				Female	Male	Female	Male
Ghasb	7.77±0.07a	59.65±2.05a	67.35±2.04a	9.92±0.55ab	9.71±0.39ab	75.31±2.96a	78.89±2.73a
Zahedi	7.60±0.12a	51.91±1.99b	59.56±2.04b	8.67±0.64bc	10.07±0.47a	64.60±2.15bc	71.57±3.10ab
Deyri	6.30±0.15b	53.03±2.03b	59.28±2.04b	10.70±0.55a	9.60±0.49ab	63.25±3.23c	71.67±3.72ab
Rabbi	7.82±0.14a	53.28±1.43b	61.02±1.42b	9.86±0.33ab	8.67±0.66bc	71.09±2.27ab	69.44±1.91b
Semi-artificial diet	5.84±0.07c	37.25±.39c	49.09±0.39c	8.06±0.34c	7.89±0.26c	51.09±0.68d	51.03±0.72c

Means with same letter(s) in each column are not significantly different at P>0.05

3.2. Oviposition period and fecundity

The oviposition period and fecundity of *B. amydraula* adults from larvae reared on different varieties of date palm are summarized in Table 2. Significant differences were observed in the pre-oviposition period of *B. amydraula* on different date palm varieties. The preoviposition period was shortest on Zahedi and longest on semi-artificial diet. The oviposition period was significantly shorter on semi-artificial diet than on any of the date varieties, which did not differ from each other. Also the shortest post-oviposition period was observed on semi-artificial diet, although the Rabbi variety was not different from the semi-artificial diet.

The daily and total fecundities per *B. amydraula* individual are given in Table 2. There were no significant differences among the different varieties of date palms as larval food on the daily number of eggs laid per female. However, the total fecundity of females was significantly higher on Deyri and Rabii than on Ghasb or semi-artificial diet.

Table 2 The mean (±SE) pre– and post-oviposition and oviposition periods and fecundity of *Batrachedra amydraula*

Varieties	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Fecundity	
				Daily	Total
Ghasb	2.53±0.26 (15)b	4.13±0.32 (15)a	2.80±0.47 (15)a	8.35±0.58a	28.33±4.03b
Zahedi	1.77±0.23 (13)b	4.62±0.18 (13)a	2.69±0.48 (13)a	8.53±0.80a	36.07±4.37ab
Deyri	2.31±0.21 (13)b	4.62±0.35 (13)a	3.31±0.59 (13)a	9.78±1.91a	45.23±5.78a
Rabbi	2.50±0.31(14)b	4.36±0.31 (14)a	2.43±0.26 (14)ab	10.09±1.10a	41.43±3.59a
Semi-artificial diet	3.43±0.25 (28)a	2.39±0.23 (28)b	1.43±0.20 (28)b	8.70±0.99a	24.97±2.75b

Means with same letter(s) in each column are not significantly different at P>0.05; Numerals in parentheses are the number of Petri dishes for each varieties including one pair of male and female

3.3 Mortality and development index

The percent mortality and development indices of *B. amydraula* on some varieties of date palm are given in Table 3. The lowest percentage of egg incubation period mortality was on Ghasb variety and semi-artificial diet (14%) and the highest was on Deyri variety (34%). In larval+pupal development stage, the lowest (1.3%) and the highest (20%) percent mortality were on semi-artificial diet and Rabbi variety, respectively. The lowest percent mortality during total development time (egg to adult) was on semi-artificial diet (15%) and the highest was on Deyri and Rabbi varieties (50%).

Among the different varieties of date palm used in this study, the highest development indices for egg, larval+pupal and overall immature stages of *B. amydraula* were on semi-artificial diet. The lowest development indices of egg incubation, larval+pupal and overall immature stages of *B. amydraula* were on Rabbi, Ghasb and Rabbi, respectively (Table 3).

Table 3 Percentage of mortality (±SE) and development index (D.I.) (±SE) of lesser date moth

Varieties	Egg		Larvae + Pupae		Overall immature	
	Mortality(%)	D.I.	Mortality(%)	D.I.	Mortality(%)	D.I.
Ghasb	13.75±4.19b	11.35±0.38b	18.75±6.39a	1.36±0.11c	32.50±5.20b	1.00±0.08bc
Zahedi	18.75±3.23ab	10.46±1.13b	8.75±3.50ab	1.76±0.10b	27.50±3.13b	1.22±0.07b
Deyri	33.75±6.79a	10.52±1.31b	16.25±3.23a	1.58±0.09bc	50.00±7.07a	0.84±0.11c
Rabbi	30.00±8.23ab	8.95±1.02b	20.00±5.34a	1.50±0.08bc	50.00±5.66a	0.82±0.09c
Semi-artificial diet	13.75±3.23b	14.77±0.59a	1.25±0.25b	2.65±0.04a	15.00±3.27c	1.97±0.07a

Means with same letter(s) in each column are not significantly different at P>0.05

4. Discussion

According to results, the length of incubation period was the shortest (5.84 days) on semi-artificial diet. Rahmani et al. (2008) and Marouf et al. (2004) reported the length of incubation period of this pest on semi-artificial diet as 6.49 and 5.04 days respectively. The mean length of total development time (egg to adult) on semi-artificial diet was 49.09 days which is close to Rahmani et al. (2008) who reported this value as 51.91 days for males and 53.3 days for females. Also it is close to the value reported by Marouf et al. (2004) (52.91 days). Total development time was longest on Ghasb variety (67.35 days). Since one of the main ingredients of semi-artificial diet was powder of dried Ghasb variety, the marked difference between total development time on semi-artificial diet and Ghasb variety shows that other ingredients of semi-artificial diet like honey, yeast, wheat flour, and glycerin increase its nutritional value for *B. amydraula*.

Our results for longevity of males and females on semi-artificial diet (7.89 and 8.06 days) agree with those reported by Rahmani et al. (2008) (7.04 and 8.71 days). Shayegan (1997), however, reported longevity for males and females on semi-artificial diet consisting of baked Ghasb, agar, and date palm pollen as, 3.5 days and 6.67 days, respectively. This variation may be due to the difference between ingredients of two kinds of semi-artificial diet. The difference also can be related to differences in the photoperiod and relative humidity conditions under which the two studies were carried out. Shayegan (1997) did the experiments under complete dark and 40±5% relative humidity conditions.

Among date palms varieties, shortest development time was observed on Deyri (59.28 days) and Zahedi (59.56 days) varieties. It might indicate that these varieties are preferred by *B. amydraula*. Latifian et al. (2004), also, reported fresh Deyri variety as the most infested and fresh Zahedi as the second most infested among date palm varieties of Khuzestan province.

Mortality of different *B. amydraula* developmental stages on Zahedi, and Ghasb (without considering of semi-artificial diet) was lower than Deyri and Rabbi varieties, and as a result the development index on Zahedi and Ghasb was higher than the other two varieties (Table 3). Higher percent moisture of Deyri and Rabbi varieties in postharvest period comparing to semi-artificial diet, Zahedi, and Ghasb might be one of the reasons of their increased mortality. It was observed that the nectar of Deyri and Rabbi varieties coats eggs and suffocates them. Also some of the larvae of the *B. amydraula* were observed to become trapped on nectar and die. Such mortality was not seen on semi-artificial diet, Zahedi, and Ghasb varieties because of their lower moisture. The highest total development index among date palm varieties was on Zahedi (without considering of semi-artificial diet) (Table 3). Zahedi has a higher exportation value than the other varieties and it also makes up a large amount of the exported dry date palm of Iran, so the results of this study may help in programming measured control and reducing the damage of stored products pest to date palm.

The other point is that no comprehensive study about the suitability of date palm varieties for insect pests of date palm has been done yet, and in Iran the only related study is susceptibility of native fresh fruit date palm varieties of Khuzestan province carried out by Latifian et al. (2004). Considering all these,

more similar studies about susceptibility of main date palm varieties of Iran to date palm pests are needed to design a comprehensive scheme for an IPM program of date palm pests.

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The influence of a DDGS diet on the development and oviposition rate of *Tribolium castaneum* (Herbst)

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Abstract

Dried Distillers Grains with Solubles (DDGS) is used in livestock feeds and finishing diets to replace maize or other grains. As more of this product is available to the feed industry, the effect it might have on the vulnerability of animal feed to insect infestation is unknown. This research focused on the influence of old generation DDGS as food and oviposition resource of red flour beetle *Tribolium castaneum* in contrast with a traditional a flour (90%)/yeast (10%) diet. Larval development was significantly faster ($P<0.05$) on a flour/yeast diet (19.15±0.16 d) compared to the DDGS (45.44±0.72 d). Both DDGS and the flour/yeast diet had no significant influence on egg hatch or pupation time. These results indicate that this type of DDGS is not as suitable a developmental diet compared to the standard laboratory diet and that the addition of DDGS to animal feeds should not increase feed vulnerability to flour beetle infestation. Additionally, in a no-choice situation, oviposition rate was significantly lower ($P<0.05$) on DDGS compared to the flour/yeast diet. In conclusion, old generation DDGS is not a good substrate for red flour beetle and thus vulnerability of products to red flour beetle development is not increased with DDGS as an ingredient.

Keywords: DDGS, Red flour beetle, *Tribolium castaneum*, Oviposition, Development

1. Introduction

Dried Distillers Grains with Solubles (DDGS) is a byproduct when maize or other distiller grains are dry milled. The fractionized starch portion is fermented and then alcohol is removed by distillation for the production of ethanol as fuel and alcohol for beverage (Cromwell et al., 1993; Ileleji et al., 2007). The remaining solid (DDGS) contains protein, fiber, starch, oil and ash with approximately 2-3 times higher concentration of nutritional components than the raw grain (corn) which DDGS is originally derived from except starch, which is lower in DDGS (Shurson et al., 2003). Studies show that different types of DDGS vary in color, odor, concentration of nutritional elements and digestibility. DDGS have been used in livestock feeds, especially pigs and cattle, or finishing diets in all of types of feed production to replace maize or other grains (Shurson et al., 2003; Stein et al., 2009). As more of this product is available to the feed industry, understanding the effect it might have on the vulnerability of animal feed to insect infestation is important. The objective of this study was to determine the vulnerability of old generation DDGS to infestation by red flour beetle by examining the development and oviposition rate of the red flour beetle, *Tribolium castaneum* (Herbst), on DDGS in contrast with a standard laboratory diet.

2. Materials and methods

2.1. Insects and diets

Red flour beetle colonies were maintained in environmental chambers at 27±1°C in the Department of Entomology at Purdue University. A diet of wheat flour (90%) and brewer's yeast (10%) was used for the colony maintenance and this diet was used as the control for comparison to a diet of DDGS. The DDGS diet used in this experiment was obtained from an "old" generation dry-grind fuel ethanol process plant. The approximate percentage of components (w/w% dry basis) in the bulk composite was crude protein (26.55); crude fat (10.56); crude fiber (6.1), and ash (4.19) (Ileleji et al., 2007).

2.2. Developmental rate

Eggs were obtained by placing about 100 adult red flour beetles on a thin layer of wheat flour (90%) and brewers' yeast (10%) in a jar (400 mL) for 24 h at 32.5°C in the environmental chamber. One day old eggs were sifted from the laboratory diet using a No. 80 sieve (Seedburo Equipment Company (Des Plaines, IL, USA)/180µm hole size). Eggs were then placed singly in the wells of a 16-well plate half filled (2 mL) with one of the test diets to determine the developmental period. Wells were check twice a day until larval emergence. Once larvae emerged, and for the duration of larval and pupal stage, well plates were checked on a daily basis until adult emergence. Sixty-four wells were used for each test diet.

2.3. Oviposition rate

Pupae were sexed and kept in separate containers until adult emergence. One pair of 3-5 d-old adults was placed in a Petri dish half-filled (20 mL) with either test diet and held at 32.5°C in the environmental chamber. Preliminary experiments indicated the unsuitability of DDGS, thus more DDGS plates were prepared (thirty two dishes of flour/yeast diet and seventy dishes of DDGS). After a 3 wk oviposition period, both adults were removed from the Petri dish. Since it was difficult to separate eggs from the DDGS diet, the numbers of larvae alive after two additional weeks were counted. Thus oviposition numbers recorded reflect the number of eggs laid, less those eggs that did not hatch and those that did not survive the first two weeks of life.

3. Results and discussion

3.1. Developmental rate

The larval stage, in contrast with other stages of development, was significantly elongated when fed a diet of DDGS ($P<0.05$). Development on flour/yeast diet averaged 19.15±0.16 d compared to 45.44±0.72 d when larvae fed a diet of DDGS (Table 1). Developmental times for the flour and yeast diet were comparable to other published rates for this insect on similar diets (Good, 1936; Howe, 1956). As expected there was no effect of diet on the length of the egg or pupal stage since these are non-feeding life stages (Table 1). Egg hatch on both diets demonstrated higher rates than Howe's results, but mortality rates were similar to published rates (Howe, 1956). This elongated development period for larvae on DDGS is good news for grain handlers, feed processors and millers who store DDGS, especially during the warmer storage periods. Infestations by storage insects may grow significantly slower, resulting in less damage over the storage period.

Table 1 Influence of diet on average developmental periods and mortality rates with comparable published data.

Diet	Egg		Larva		Pupa	
	X±S.E.(d)	Hatch (%)	X±S.E.(d)	Mortality (%)	X±S.E.(d)	Mortality (%)
Flour and Yeast	3.76± 0.03	96.29	19.15± 0.16	7.69	4.75± 0.07	5.9
DDGS	3.79± 0.02	97.91	45.44± 0.72	11.2	4.81± 0.05	2.9
Wheat feed (Howe, 1956)	2.9	75	14.6	7	4.6	2.5

The difference in development time for red flour beetle on these two diets may be related to nutritional content of the diet. We are still investigation this possibility. A proximate analysis of DDGS particles in old generation DDGS by Illeleji et al. (2007) indicated protein levels in the acceptable range for insect development. Shurson et al. (2003) examined the amino acid composition of old generation DDGS and found they contained (dry matter basis, % of diet) 0.92% arginine, 0.61% histidine, 1.00% isoleucine, and 2.97% leucine. These amounts are within the acceptable minimum requirements of red flour beetle (Taylor and Medici, 1966). They found that red flour beetle require arginine (0.4%), histidine (0.2%), isoleucine (0.3%) and leucine (0.6%) for survival, but that these were just minimal levels. Higher levels found in their confused flour beetle (Tribolium confusum Jacquelin du Val) control diet (histidine (0.6%); isoleucine (1.2%); leucine (1.4%); phenylalanine (0.8%); and tryptophan (0.2%)) improved larval growth (measured by insect weight) significantly. They did not measure developmental time. This weight gain difference could also be true for red flour beetle since it is closely related to confused flour beetle. The elongated larval development period on DDGS that we found might be related to the amino acid profile. We are currently examining the amino acid profile of our control diet to determine if the

levels are acceptable, but do not anticipated that it contains below minimum levels of important amino acids.

3.2. Oviposition rate

Twenty-six of the 70 red flour beetle females on the DDGS diet laid no eggs, and in 12 instances one of the pair died with no oviposition occurring compared to the flour/yeast dishes, in which all females laid eggs and none died (Fig. 1). The number of eggs that hatched and survived two weeks was significantly lower ($P<0.05$) on DDGS (17.37+1.22 eggs per female (n=32)) compared to a flour/yeast diet (204.7+10.99 eggs per female (n=32)). The DDGS rate decreased to an average of 9.59±1.33 eggs per female (n=58) when females that survived but did not lay eggs are included. Thus oviposition rate in the control diet was 12-21 times higher when compared to DDGS as an oviposition substrate.

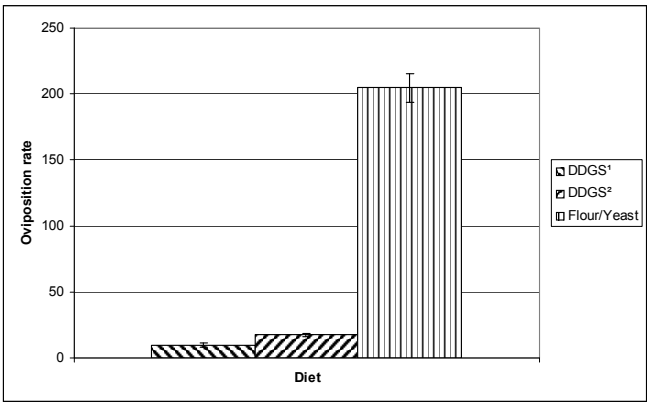

Figure 1 Red flour beetle oviposition rate during a 3-week period on flour/yeast (n=32) or DDGS with (n=58) or without (n=32) females that did not lay eggs.

Howe (1962) found that the mean number of eggs per female laid in 7 wks on finely divided wheat feed at 32.5°C and 70% r.h. was 539.2 eggs. This is equal to 77 eggs per week which is comparable to our average of 68 eggs per week at 32.5°C on the control diet. Although these averages do not take into consideration a cyclic nature of oviposition, the numbers are within a reasonable approximation. Additionally, our numbers could be a slight under estimation of oviposition due to the mortality of larvae in the first few weeks. However, it would be an under estimation for both the control and DDGS diet and thus the relative magnitude of the difference would not change and DDGS would still be considered a relatively unsuitable diet for red flour beetle. Thus in a no-choice situation, red flour beetle females will choose to either not lay eggs or lay very few eggs when presented with a DDGS diet. We are currently investigating this situation. Oviposition data combined with developmental data indicates that even if eggs are laid on DDGS, the development time is greatly increased, resulting in a significantly extended life cycle. Therefore the feed industry is not at greater risk for red flour beetle infestation when using old generation DDGS in feeds.

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Mating disruption field trials on *Plodia interpunctella* (Hubner)

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Abstract

After four decades of using pheromone-baited traps for monitoring populations of stored product insects, a new method to control stored product moths using mating disruption with pheromones is being implemented into pest management programs. The pheromone for Indianmeal moth, Mediterranean moth (*Ephestia kuehniella)*, Tobacco moth (*Ephestia ellutella*), and the Almond moth (*Ephestia cautella*), Z, E- 9, 12 tetradecadienyl acetate was registered with the US EPA as a bio-insecticide in 2007. This pheromone-based product is exempt from food tolerance for residues if the dose does not exceed 3.5 grams active ingredient (ai) 30m^2/year. This MD study examines the use of Allure MD on Indianmeal moths (*Plodia interpunctella*) in five field studies: Food pantry, Seed warehouse, Nut processing building, Organic food storage and processing, and a home. The various stored product storage areas were monitored before, during, and after the MD treatments. Female stored product moths were captured to determine if their eggs were fertilized by examining the spermatophores in bursa sacs. The male Indianmeal moths behavior was dramatically changed with the MD treatments and the pheromone trap captures of male moths was reduced significantly. The results of these five field studies, cost comparisons with traditional insecticide treatments, female fertility, and the changed behavior of male Indianmeal moths will be discussed.

Keywords: *Plodia interpunctella*, *Ephestia kuehniella*, Pheromone, Mating disruption (MD)

The chemical composition of egg plugs deposited by *Sitophilus granarius* L. females on grain

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Abstract

Over 20,000 egg plugs collected from infested wheat grain were subjected to chemical analysis. Elemental analysis showed a relatively high content of nitrogen (about 9%). It suggested that the predominant constituent of egg plugs is a protein. Spectrum obtained in ESI-MS analysis showed a series of peaks characteristic corresponding to protein of molecular weight 30073 Da. The appearance of other peaks in this spectrum suggests that studied protein is not homogenous. A sample of egg plugs incubated with pepsin yielded a complex mixture of peptides. The most abundant peak in the ESI-MS spectrum of enzymatic hydrolysis products corresponds to peptide Mw 4560.76 Da. Chemical analysis indicated that the main component of egg plugs is protein.

Keywords: egg plugs, chemical composition, *Sitophilus granarius*

1. Introduction

Egg deposition by insect females is considered a complicated behavioural process, depending on numerous factors. Selection of place for egg deposition definitely affects the population development rate and survival of a given species in the biocenosis. On the basis of chemical stimuli perceived by receptors of smell, taste and contact chemoreception senses, each female has to find a food source for newly hatched larvae and simultaneously to estimate its abundance and nutritional value. The following step of the behaviour sequence is cognition of whether another female from the same species deposited eggs earlier in the same product. For this purpose, marking pheromones, epideictic pheromones and kairomones left on eggs are used by females. Aquatic insects usually produce a gelatinous secretion covering deposited eggs. In cases of depositing eggs inside a product, females can perceive sounds sent by feeding larvae (Nufio and Papaj, 2001).

Substances covering individual insect eggs or egg clusters are produced by accessory reproductive glands (*Glandulae accessoriae*), also called sebaceous glands (*Glandulae sebaceae*). These glands are twin or non-twin structures, symmetrical or asymmetrical, and their ends are placed in a common oviduct (*Oviductus*). Their secretions contain: poisons, pheromones, special glue substances for attaching eggs to the substrate, and substances for covering the eggs (Chapman, 1971; Gillot, 2002, 2005). In the case of cockroaches (Blatodea) and praying mantides (Mantodea) gland secretions are used for cocoon formation, while tsetse flies (*Glossinia morsitans*) use them for larvae' feeding.

Granary weevil (*Sitophilus granarius* L.) females deposit eggs inside cereal grains (wheat, barley and oat) performing the following routines:

1. boring a hole in grains with their snouts,
2. inserting an ovipositor into the hole and placing one egg in it,
3. closing a grain hole with gelatinous hardening substance (egg-plugs).

Egg deposition process was best described in older German literature (Müller, 1928; Pavlakos, 1931; Kllnike, 1936; Andersen, 1938). Colleteral glands' structure of granary weevil is well known (Krautwig, 1930; Khan and Musgrave, 1969; Müller, 1928). However, chemical composition of gland secretions is only partially recognized (Cox et aI., 2000; Niewiada et aI., 2005). Kunike (1928) provided the first data on egg-plug chemistry: it is not soluble in water, alcohol or acetone, but is slightly soluble in alkalies and reacts in Molish reaction. Egg-plug are stained cherry red with fuchsin (Frankenfeld, 1948), purple with gentian violet (Goossens, 1949), and greenish yellow with barberine sulphate (Milner et al., 1950).

Egg-plugs may provide protection of to deposited eggs from predators and parasitoids, mechanical stimuli, and drying. It would be useful to determine whether they can be perceived by insect contact chemoreceptors and/or whether they contain volatile epideictic pheromones which influence oviposition behavior. Grain weevil females usually deposit one egg inside a cereal kernel, however, under certain conditions numerous egg-plugs are observed on the surface of grain without deposited eggs under them.

Volatile pheromones marking egg depositions have already been described for numerous insect species. Their main role is to warn that a given product has been already occupied and it is an insufficient resource for further oviposition. These compounds are called epideictic pheromones or pheromones hampering egg deposition. The evidence of marking pheromones was revealed for the following Curculionidae species: boll weevil (*Anthonomus grandis* Boheman.) (Hedin et aI., 1974), pine weevil (*Hylobius abietis* L.) (Nordlander et aI., 1977; Borg-Karlson et aI., 2006), cabbage seed weevil (*Ceutorrynchus assimilis* (Paykull.)) (Kozłowski et aI.,1983), and cherry weevil (*Furcipes rectirostris* L) (Kozłowski and Borkowski, 1990).

This paper reports the first chemical analysis of egg plugs collected from infested wheat grain. We believe that a knowledge of their chemical composition may be useful for further studies of the biological activity of the egg plugs in oviposition process.

2. Material and methods

2.1. Biological material

Substance covering deposited eggs was collected from grain with the use of preparation needle. Ten mg of the product (over 20,000 plugs) was subjected to chemical analysis.

2.2. Chemical analysis

The elemental analysis of the sample of egg plugs was performed at Laboratory of Elemental Analysis and Environmental Studies (University of Wroclaw). The mass spectrometric analysis was performed using an Apex-Qe 7T instrument (Bruker Daltonics, Germany) equipped with dual electrospray ionization (ESI) source. Spectra were recorded using aqueous solutions of acetonitrile (50%) and formic acid (1%). The potential between the spray needle and the orifice was set to 4.5 kV. In MS/MS mode, the quadruple was used to select the precursor ions, which were fragmented in the hexapole collision cell applying argon as a collision gas. The product ions were subsequently analyzed by the ICR mass analyzer. For CID MS/MS measurements, the voltage 20 V over the hexapole collision cell was applied. The spectra were analysed using Data Analysis™ and Biotools™ software (Bruker). Two mg of egg plugs were subjected to elemental analysis. The sample was not purified or fractionated before analysis. The analysis revealed relatively high content of nitrogen (9 ± 1%). Sample was not soluble in methanol. The egg plugs were sonicated in formic acid and then diluted with water formed a cloudy solution.

After filtration this solution was separated into three parts. Part 1 was treated with pepsin. The solution resulting from the enzymatic hydrolysis was analyzed by ESI-MS. Part 2 was diluted with acetonitrile (1:1) and directly analyzed by ESI-MS. Finally, Part 3 was freeze-dried. The product obtained by lyophilization has the appearance of white powder. The samples of hydrolyzed and unhydrolyzed extract were subjected to biological activity tests.

2.3. Biological activity tests

The lyophilized products (hydrolyzed and unhydrolyzed extracts) were dissolved in water and deposited on wheat kernels at the amount of 1 µL. Two experiments were carried out simultaneously: a choice test (KE) (l0 treated grains with the extract and 10 grains treated with the same amount of water in one Petri dish) and no-choice test (EE) (20 grains treated with extract in one Petri dish). The control treatment was 20 wheat kernels treated with water in one Petri dish (KK). Twenty granary weevils (l0 females and 10 males, 1 - 5 days old) were placed on previously prepared grains. After 10 days of feeding the number of egg-plugs was recorded on all experimental kernels. Each experiment was carried out in 5 replications.

3. Results and discussion

3.1. Chemical analysis

The average content of nitrogen in purified, dry protein is about 16%, but real, air dried samples (Chibnall, 1948) of various proteins contained 8.1 (amandin) to 13.3% (egg albumin, native). The decreased content of nitrogen in these samples is a result of water content and mineral compounds. The relatively high content of nitrogen in our sample suggests, that the main component of egg plugs is a protein.

The result of ESI-MS measurement of soluble egg plug fraction presented in Fig. 1 demonstrated very characteristic pattern consisting of a series of multiply charged positive ions (the charge varies from +20 to +36). The spectrum was deconvoluted by maximum entropy algorithm (Fig. 2), revealing a predominant component with molecular mass of 30,073 ± 5. The ESI-MS spectrum confirmed that protein is the main component of studied material.

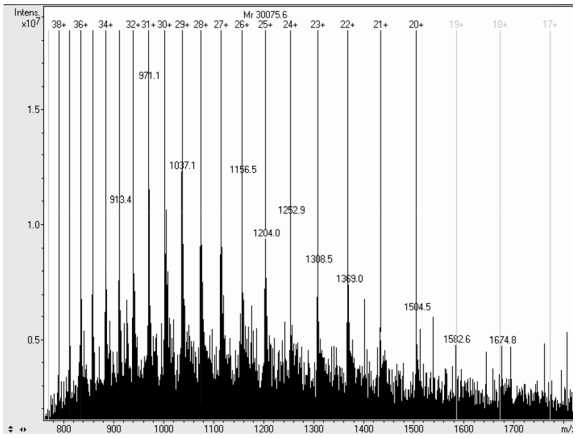

Figure 1 The ESI-MS spectrum of the protein extracted from egg plugs, with the peaks labeled with charges and m/z (mass to charge ratio).

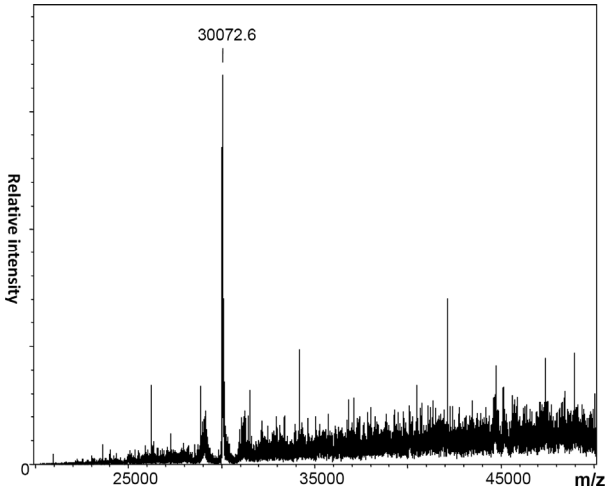

Figure 2 The deconvoluted ESI-MS spectrum of the protein extracted from eggs plugs.

The sample incubated with pepsin demonstrated a completely different spectrum character. Instead of a series of peaks with the charge ranging from +36 to +20, a series of relatively short peptides (the highest charge +7) was observed. The spectrum showed in Fig. 3 presented numerous fragments presumably formed by enzymatic hydrolysis of extracted protein. The susceptibility to the pepsin catalyzed hydrolysis is an another piece of evidence confirming proteic character of main egg plugs component.

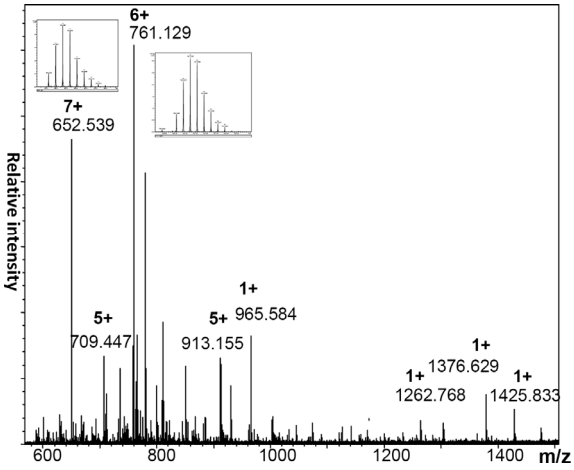

Figure 3 The ESI-MS spectrum of products obtained by pepsin catalyzed hydrolysis of egg plugs protein, with the isotopic distribution for two peaks of the highest abundances shown as inserts.

Selected peptide (m/z = 761.127) was subjected for MS/MS experiment. The fragmentation was performed by two methods: CID (collision induced dissociation) and ECD (electron capture dissociation). The obtained, CID fragmentation spectrum is presented in Fig. 4. The fragmentation pattern was interpreted using Biotools program (Bruker Daltonics, Germany). Although the sequence coverage was not sufficient to determine the whole sequence of the peptide, the program found many fragments , including: ANVVR; VGVV, VAPS. The analysis of fragmentation of peptide at m/z 761.127 suggested a high content of Val in investigated peptide.

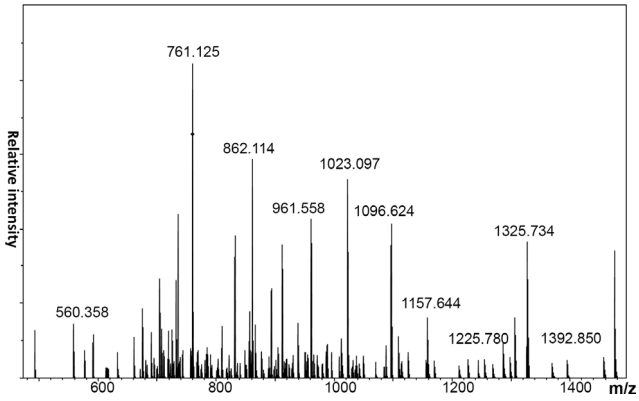

Figure 4 The ESI-MS/MS spectrum of most abundant component of peptic hydrolysis of egg plug protein.

3.2. Biological activity test

Results on biological activity of plug extracts are presented in Table 1. The numbers of egg plugs deposited on treated kernels was similar to that in control. Probably the applied amount of substance was not sufficient to be detected by S. granarius females. Further experiments should be performed with peptide derivatives synthesized on the basis the partial sequences of studied proteins.

Table 1 Number of egg plugs deposited by one female during 10 days on wheat kernels treated with hydrolyzed and unhydrolyzed extracts of plugs (SD in parenthesis)

	Number of egg plugs			
	Choice test		**No-choice test**	
	K	**E**	**KK**	**EE**
Hydrolyzed extract	15.5 (±2.1)	16.6 (±2.4)	15.0(±1.8)	14.7 (±1.3)
Unhydrolyzed extract	13.1 (±2.3)	10.8 (±2.8)	13.7 (±3.1)	11.6 (±2.0)

4. Conclusions

The chemical analysis revealed that the basic component of egg plugs produced by *S. granarius* is a protein with molecular mass 30,075. The mass spectrometry allowed us to find the partial sequences of the isolated protein.

The biological activity of egg plugs in oviposition process will be studied on the basis of knowledge their chemical composition.

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Resistance of strains of rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) to pirimiphos methyl

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Abstract

Insecticidal resistance of five strains of *Sitophilus oryzae* (L.) to pirimiphos methyl (Actellic®) was investigated in the laboratory at 28 ± 2oC and 89 ± 5% relative humidity. Rice infested by *S. oryzae* was sampled from Nigerian stores in Akure (Ondo state), Ibadan (Oyo state), Ikotun (Lagos state) and Ilesha (Osun state) and a laboratory culture (in Akure). Twenty adult weevils from each location were exposed to filter papers impregnated with liquid pirimiphos methyl at one of seven concentrations ranging from 0.0 to 5.0%. Mortality rates of the rice weevils were observed at 3, 6 and 24 h after treatment. The mortality of weevils increased with increasing concentration and period of exposure. Irrespective of the strain, 100% mortality was observed in all the treatments at 24 h after exposure to pirimiphos methyl. Weevils sampled from Ibadan were the most resistant, while laboratory strains were the least resistant at 2 and 5% concentrations.

Keywords: Strains, insecticide, Pirimiphos methyl, Resistance, Sitophilus oryzae.

1. Introduction

Rice is an essential component of the diet of many people. It has been estimated that half the world's population subsists wholly or partly on rice (Grist, 1986). The bran of rice can be mixed with skimmed milk and used for feeding domestic animals. Broken rice grains are used for distillation of alcohols and in the production of laundry starch. Traditionally, rice grains are stored in granaries either in the threshed or unthreshed forms. These traditional methods expose stored grains to pest infestation.

Pest infestation in the field and during storage has greatly reduced the production of rice. In Nigeria, pest infestation plays a key role in keeping cereal production below the qualities and quantities demanded by an increasing human population and rapidly expanding livestock industry (Ogunwolu and Nwosu, 1987). The rice weevil, *Sitophilus oryzae* (L) (Coleloptera: Curculionidae) is a major cosmopolitan pest affecting stored rice in the world. A survey on the incidence of major pests of rice in different ecological areas of Nigeria showed, in general, that the amount of damage caused by *S. oryzae* was high (Soto et al., 1976). Both the larval and adult stages of this insect devour the kernel, causing weight losses.

Different methods (orthodox and traditional) have been developed and used to control infestation by rice weevil. Planting of resistant varieties have been reported to be effective in the control of this insect pest (Stoll, 1986). Enobakhare and Wey (1996) reported that resistant rice varieties ITA 306 and American Lamount inhibited to S. oryzae infestation. Treatment of grains with wood ash or rice husk ash have been suggested and used with some degrees of success (Stoll, 1986). Also, oils from plant sources have been reported to be toxic to S. oryzae, in addition to adversely affecting oviposition and weevil development (Su et al., 1972, Sighamony, 1986).

The most important and commonly used control measure is chemical control. This involves the use of synthetic chemicals such as pirimiphos methyl (Actellic®), permethrin (Coopex®), etc. which have been found effective in controlling S. oryzae (Wilkin et al., 1999). Although the use of synthetic chemical insecticides is a powerful tool against insect pests, indiscriminate use of different conventional insecticides by farmers and marketers has led to the development of resistance and a resurgence in some insect pests. For example, it was documented that S. oryzae showed resistance to deltamethrin (Ceruti and Lazzari, 2003). Ecological variations in the resistance status of different insect pests to diverse insecticides have been observed by various researchers (Jermannaud, 1994; Shelton et al., 2000; Pereira et al., 2006). Insecticide resistance and the consequent losses of food arising from failure of chemicals to

control pests, have caused economic losses of several billion dollars worldwide each year (Elzen and Hardee, 2003).

This study was carried out to determine how geographical variation has influenced the resistance of *S. oryzae* to liquid pirimiphos methyl (Actellic®) in south western Nigeria.

2. Materials and methods

2.1. Insect culture

Sitophilus oryzae was originally obtained from naturally infested rice sampled from retail stores in different locations such as Akure (Ondo state), Ibadan (Oyo state), Ikotun, (Lagos state) and Ilesha (Osun state), Nigeria. All insects used were reared and studied in the laboratory at 28 ± 2oC, 70-75% relative humidity and a photoperiod of 14h:10h (L:D).those used as a standard were also obtained from established laboratory cultures. The laboratory cultures of S. oryzae had been maintained for more than nine generations on whole rice grain and these weevils were not exposed to insecticides in the laboratory. The weevils were reared to F1 generation on clean disinfested rice grain. The rice was disinfested by placing it inside a deep freezer for 72 h, then warming to ambient temperature before use. The insects were reared inside plastic containers (0.5 L) covered with muslin cloth and kept inside insect cages.

2.2. Synthetic chemical insecticide

The insecticide, pirimiphos methyl (Actellic® 25EC) used for the study was bought from a chemical store in Arakale, Akure. Graded acetone was used as diluent for the insecticide. Six different concentrations of pirimiphos methyl were prepared: 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0% while acetone only (0.0%) served as the control treatment. To prevent evaporation of acetone, each concentration was prepared in a tightly covered reagent bottle.

2.3. Experimental procedure

An impregnated filter paper technique was used for the bioassay. Filter papers of 11-cm diameter were treated with varying concentrations of the insecticides described earlier and placed in the middle of a 9 cm-diameter Petri dish. For each treatment, there were three replicates. 0.5 mL of each concentration was applied to a filter paper and allowed to air-dry for 4 min. Twenty unsexed adult *S. oryzae* were released onto the treated filter paper and the Petri dish was covered. Weevil mortality was observed at 3, 6 and 24 h after treatment. Weevils were considered dead when they did not move after their abdomen was pricked with a sharp object.

2.4. Morphometrics of S. oryzae

Adult *S. oryzae* obtained from different locations were identified following Halstead (1963). Ten pairs of adult weevils were randomly selected from each location together with those from the laboratory and their length was measured under a microscope with a graticule in one eyepiece. A further 10 female weevils was obtained as described above and weighed to two decimal places. This was replicated three times.

2.5. Statistical analysis

Data obtained from morphometric evaluations were subjected to one-way analysis of variance (ANOVA) while mortality was subjected to two-way ANOVA using dose and location as factors to test for differences of mortality rates within and between locations. Where ANOVA results were significant means were separated using the Tukey's test.

3. Results

3.1. Response of S. oryzae obtained from different locations to different concentrations of pirimiphos methyl

Tables 1-3 show that the percentage mortality of S. oryzae obtained from the study varied with respect to the concentrations of pirimiphos methyl and the duration of exposure. At 3 h after treatment, there was no significant difference ($F_{6, 14} = 2.047$, $P = 0.073$) in the mortality of S. oryzae at the various insecticide concentrations. At 1.0% concentration there was significant difference ($F_{6, 14} = 167.72$, $P<0.001$) from the control in the mortality of *S. oryzae* from Ikotun and laboratory cultures (Table 1). And at highest concentration (5.0%) of insecticide after 3 h of exposure, mortality of *S. oryzae* from

laboratory cultures was highest (51.65%), while the lowest mortality (26.65%) at this concentration and exposure period was observed in weevils from Ibadan.

Table 1 Effect of pirimiphos methyl at 3 h after treatment on the percentage mortality (Mean ± S.E.) of *Sitophilus oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures.

Conc (%)	Akure	Ibadan	Ikotun	Ilesha	Lab. culture
0.0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
0.1	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
0.2	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	1.65 ± 0.33^{aA}
0.5	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	6.65 ± 0.33^{aB}
1.0	1.65 ± 0.33^{aA}	5.00 ± 0.58^{aA}	18.35 ± 0.33^{bB}	1.65 ± 0.33^{aA}	25.00 ± 0.58^{bB}
2.0	35.00 ± 0.58^{bB}	25.00 ± 0.58^{bA}	21.65 ± 0.33^{bA}	35.00 ± 0.58^{bB}	35.00 ± 0.58^{cB}
5.0	41.65 ± 0.33^{cC}	26.65 ± 0.33^{bA}	31.65 ± 0.33^{cA}	40.00 ± 0.58^{bBC}	51.65 ± 0.58^{dD}

Each value is a percentage mean of triplicate samples ± standard error of the mean. Mean values followed by the same lower-case letter within a column are not significantly different at $P<0.05$ by Tukey's multiple range test. Mean values followed by the same upper-case letter within a row are not significantly different at $P<0.05$ by Tukey's multiple range test.

Pirimiphos methyl on *S. oryzae* mortality after 6 h after exposure is shown in Table 2. Except for weevils from laboratory cultures with 16.65% mortality at 0.1% concentration, the mortality of all weevils at this concentration was not significantly different ($F6, 14 = 2.194$, $P=0.169$) from the control. However, at 1.0% concentration, 100% and 70% mortality was observed in weevils from laboratory cultures and Ibadan, respectively. There was no significant difference between the mortality of *S. oryzae* treated with 2.0% and 5.0% insecticide concentration.

Table 2 Effect of pirimiphos methyl at 6 h after treatment on the percentage mortality (Mean ± S.E.) of *Sitophilus oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures.

Conc (%)	Akure	Ibadan	Ikotun	Ilesha	Lab. culture
0.0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
0.1	6.65 ± 0.88^{aA}	3.35 ± 0.33^{aA}	1.65 ± 0.33^{aA}	1.65 ± 0.33^{aA}	16.65 ± 0.33^{bB}
0.2	33.35 ± 0.33^{bAB}	26.65 ± 0.88^{bA}	36.65 ± 0.67^{bB}	31.65 ± 0.33^{bAB}	45.35 ± 0.33^{cC}
0.5	66.65 ± 0.67^{cB}	51.65 ± 0.67^{cA}	75.00 ± 0.58^{cC}	66.65 ± 0.58^{cB}	81.65 ± 0.33^{dC}
1.0	75.00 ± 1.00^{cB}	70.00 ± 0.88^{dA}	90.00 ± 0.58^{dC}	76.65 ± 0.33^{dB}	100.00 ± 0.00^{eD}
2.0	98.35 ± 0.33^{dB}	83.35 ± 0.67^{dA}	100.00 ± 0.00^{eB}	95.00 ± 0.58^{eB}	100.00 ± 0.00^{eB}
5.0	100.00 ± 0.00^{dB}	86.65 ± 0.33^{dA}	100.00 ± 0.00^{eB}	100.00 ± 0.00^{eB}	100.00 ± 0.00^{eB}

Each value is a percentage mean of triplicate samples ± standard error of the mean. Mean values followed by the same lower-case letter within a column are not significantly different at $P<0.05$ by Tukey's test. Mean values followed by the same upper-case letter within a row are not significantly different at $P<0.05$ by Tukey's test.

After 24-h exposure periods to pirimiphos methyl at the varying concentrations tested, there was 100% mortality of S. oryzae irrespective of their source and this was significantly different ($P>0.05$) from the control where no mortality was recorded (Table 3).

Table 3 Effect of pirimiphos methyl at 24 h after treatment on the percentage mortality (Mean ± S.E.) of *Sitophilus oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures.

Conc (%)	Akure	Ibadan	Ikotun	Ilesha	Lab. culture
0.0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
0.1	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}
0.2	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}
0.5	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}
1.0	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}
2.0	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}
5.0	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bA}

Each value is a percentage mean of triplicate samples ± standard error of the mean. Mean values followed by the same lower-case letter within a column are not significantly different at $P<0.05$ by Tukey's test. Mean values followed by the same upper-case letter within a row are not significantly different at $P<0.05$ by Tukey's test.

3.2. Comparison of the mortality of S. oryzae from different locations

There was no mortality of *S. oryzae* obtained from all locations under study (except laboratory samples) after 3 h of exposure to pirimiphos methyl at 0.1-0.5% concentration. However, at the highest concentration (5%) tested, the mortality rate of S. oryzae from Ibadan was the lowest and significantly different ($F_{5, 10}$= 62.89, $P<0.001$) from that of other locations (Table 1). After 6 h of exposure to 0.1% pirimiphos methyl, mortality rate of S oryzae from laboratory cultures was significantly different from other locations. And at 2 and 5% concentration, mortality rates (83.35 ± 0.67 and 86.65 ± 0.33, respectively) observed in S. oryzae from Ibadan was significantly different ($F_{5, 10}$ =114.38, $P<0.001$) from other locations (Table 2). At 24 h after treatment, 100% mortality rate was observed in weevils across all locations (Table 3).

3.3. Morphometrics of S. oryzae obtained from four geographical locations

The morphometrics of *S. oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures is shown in Table 4. Result showed that there was no significant difference ($F_{4,10}$ = 2.874, $P=0.251$) in the length and weight of weevils from all locations sampled. Although both male and female weevils from the laboratory cultures appeared to be slightly longer, this apparent difference was not statistically significant.

Table 4 The morphometrics of *Sitophilus oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures.

Source of weevil	Length (mm)		Weight (g)	
	Male	Female	Male	Female
Akure	2.88 ± 0.34	3.20 ± 0.35	0.01 ± 0.00	0.02 ± 0.00
Ibadan	2.75 ± 0.35	3.15 ± 0.41	0.01 ± 0.00	0.02 ± 0.00
Ikotun	2.85 ± 0.41	3.20 ± 0.48	0.01 ± 0.00	0.02 ± 0.00
Ilesha	2.80 ± 0.35	3.20 ± 0.42	0.01 ± 0.00	0.02 ± 0.00
Lab. culture	2.90 ± 0.39	3.25 ± 0.26	0.01 ± 0.00	0.02 ± 0.00

Each value is a percentage mean of triplicate samples ± standard error of the mean.

4. Discussion

The results from this study showed that adult *S. oryzae* mortality was modulated by various concentrations of pirimiphos methyl and by the period of exposure. Rice weevils from Ibadan are the most resistant to pirimiphos methyl at higher concentration and longer exposure time. Those from Akure and Ilesha are the least resistant while weevils from the laboratory cultures are the most susceptible.

Insecticide resistance of some storage pests to certain synthetic chemical insecticides has been reported. Rahim and Ong (1991), from a survey on resistance carried out in Malaysia in 1985 reported that *Sitophilus* spp., *Tribolium castaneum* (Herbst) (Coleloptera: Tenebrionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) had shown resistance to malathion and methyl bromide. In addition, it was reported by Suleiman et al (1994) that four major grain beetles: *S. oryzae*, *S. zeamais* (Motschulsky), *T. castaneum*, and *R. dominica* collected from 30 locations in Malaysia showed some levels of resistance to phosphine and methyl bromide, with resistance more pronounced in *S. oryzae* and *T. castaneum* than other insects evaluated. Ceruti and Lazzari (2003) when using molecular markers to detect insecticide resistance in stored-product beetles reported that *S. oryzae* was more resistant compared to other beetles tested.

The differences in the resistant status of *S. oryzae* are influenced by different factors. Ecological differences in the resistance status of *S. oryzae* to pirimiphos methyl in this study was in agreement with the ecological response reported for some field and stored-product insect pests to insecticides. Jermannaud (1994) observed that different levels of resistance exist among *S. zeamais* obtained from five different locations in Ghana. He also reported that S. zeamais from seven different locations in Zimbabwe show high resistance to pirimiphos methyl. Fragoso et al. (2002) also reported that insect population of the coffee leaf miner *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) obtained from 10 sites show different levels of resistance to chemical insecticides. Perez et al. (2000) tested the resistance of field population of *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae),

Plutella xylostella (L.) (Lepidoptera: Plutellidae), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), *Helicoverpa* spp. (Lepidoptera: Novtuidae), and *Bemisia tabaci* (Gemadius) (Homoptera: Aleyrodidae) obtained from six different sites from Nicaragua to different insecticides and observed that these insects show different levels of resistance to different insecticides. Perez-Mendoza (1999) observed that *S. zeamais* collected from field had moderate resistance to DDT, moderate to high level of resistance to lindane, low to high level of resistance to primiphos methyl.

Rice weevils obtained from Ibadan proved to have the highest level of insecticide resistance to pirimiphos methyl. Ibadan has a tropical climate and falls within savannah forest, which is characterized by high temperature throughout the year. Temperature affects the activity of insects, therefore, the population growth rate of the pest is always high throughout the year (Lale and Ofuya, 2001). Ibadan also has a large human population and there are many farms and markets within and around the metropolis. More insecticides might be used in the region to treat produce. Although pirimiphos methyl is not normally used directly on rice, the walls and storage pallets in rice stores are treated. It is also used to treat produce like wheat, cowpea and maize which are marketed together with rice and S. oryzae can cross infest from treated produce and develop resistance to pirimiphos methyl. Fragoso et al. (2002) and Pereira et al. (2006) attributed the variation in the resistance of insect from different locations to greater use of insecticides and usage pattern in those locations.

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Ecological studies of the Psocids *Liposcelis brunnea*, *L. rufa*, *L. pearmani*, and *Lepinotus reticulatus*

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Abstract

Psocids (*Psocoptera*) are an emerging problem in grain storages, grain processing facilities, and product warehouses in the United States and many other countries. Development of effective pest management programs for psocids is dependent on having sound knowledge of their ecology. Given the limited information available on the ecology of psocids, we conducted ecological studies of four psocid species namely, *Liposcelis brunnea* (Liposcelididae), *Liposcelis rufa*, *Liposcelis pearmani* and *Lepinotus reticulatus* (Trogiidae). We conducted population growth studies of these four psocid species at different temperatures and relative humidities; development studies of *L. brunnea*, *L. rufa*, and *L. reticulatus* at different temperatures; and investigated the effects of temperature on reproductive parameters of *L. reticulatus*. Our studies provide important data on life history and reproductive parameters of four stored-product psocid pests. Because these parameters affect population dynamics, these data can be used in simulation models to predict psocid population dynamics and thereby aid in the development of more effective management strategies.

1. Introduction

Psocids are an emerging problem in stored grain and in food facilities such as mills, processing plants, and warehouses (Rees and Walker, 1990). Before the 1990s in Australia and China and 2000s in the United States, psocids were not considered serious pests of stored products (Phillips and Throne, 2010). However, in some countries such as Australia, they have now become the most frequently encountered stored-product pest in some areas (Rees, 2003). In other countries such as Britain, Denmark, and The Netherlands, psocids (*Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae)) are major household pests of farinaceous products (Turner, 1986).

Prior to 2008, many detailed studies had been conducted on management of psocid pests of stored products (e.g., Leong and Ho, 1994; Ho and Winks, 1995; Santoso et al., 1996; Wang et al., 1999a; Ding et al., 2002), but few detailed studies had been conducted on their biology (Fahy, 1971; Khalafalla, 1990; Wang et al., 1999b; Wang et al., 2000; Wang et al., 2001). Wang et al. (1999b; 2000) conducted life history studies on *L. bostrychophila* and developed predictive models. Sinha (1988) determined temporospatial distribution of psocids in stored wheat in Canada. The recognition of psocids as pests of stored grain and grain processing facilities in the United States (Phillips and Throne, 2010) and the limited amount of published information on their biology prior to the 2000s led to the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS, USA initiating ecological studies of psocids infesting stored commodities in the United States in 2004. The Stored-Product Entomology Laboratory at Oklahoma State University, Stillwater, OK, USA has since joined CGAHR in conducting psocid research in the United States.

The fact that psocids were considered mere nuisance pests until the 1990s was probably partly responsible for the limited amount of published information available on their biology. The small size of psocids (1 mm) and the difficulty handling and identifying them could also be other contributing factors. Furthermore, the techniques used to conduct biological studies on psocids prior to 2004 were laborious, imprecise, and not user friendly. Therefore, to facilitate ecological studies of stored-product psocids, new techniques for studying their biology had to be developed. This paper provides information on the new, faster, and more user-friendly techniques developed for studying psocid biology and presents data on ecological studies conducted on the psocids *Liposcelis brunnea* Motschulsky, *L. rufa* Broadhead,

L. pearmani Lienhard, and *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae) using these techniques.

2. Materials and methods

2.1. Effects of Temperature and Relative Humidity on Population Growth

We determined effects of temperature and relative humidity on increase in number of *L. reticulatus* over a 46-d period at six temperatures (22.5, 25.0, 27.5, 30.0, 32.5, and 35.0°C) and four relative humidities (32, 43, 55, and 75%); *L. brunnea* and *L. pearmani* over a 30-d period at six temperatures (22.5, 25.0, 27.5, 30.0, 32.5, and 35.0°C) and four relative humidities (43, 55, 63, and 75%); and *L. rufa* over a 30-d period at eight temperatures (22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, and 40.0°C) and four relative humidities (43, 55, 63, and 75%). The top third of the inner surface of vials was coated with Fluon® (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent psocids from escaping, and 5 g of psocid diet were placed in each vial. Psocid diet used for *L. reticulatus* was a mixture of 97% cracked hard red winter wheat (*Triticum aestivum* L.), 2% rice krispies, and 1% brewer's yeast and for the other three species was cracked hard red winter wheat. A screen (US #40 mesh) was placed in the snap-cap lid to allow air movement. For *L. reticulatus*, vials were randomly placed in each of four plastic boxes (40 x 27.5 x 16 cm high) containing saturated solutions of MgCl2, K2CO3, NaBr, and NaCl below perforated false floors to maintain r.h. of 32, 43, 55, and 75% (Greenspan 1977), respectively, and the diet in the vials was equilibrated at room temperature for 4 wk. For *L. brunnea*, *L. rufa*, and *L. pearmani*, saturated solutions of K2CO3, NaBr, NaNO2, and NaCl were used to maintain r.h. of 43, 55, 63, and 75%, respectively, during equilibration.

Five 1 to 2-wk-old adult females, obtained using the protocol described in Opit and Throne (2008, 2009), were added to vials containing equilibrated diet, which were then incubated at each temperature-r.h. combination. Incubators were set at desired temperatures, and four plastic boxes (20 x 12.5 x 10 cm high) containing saturated solutions of the desired relative humidities were placed into each incubator. Six vials containing diet equilibrated at room temperature and each relative humidity were randomly assigned to the corresponding relative humidity box in each incubator. Four locations were established in each incubator for the boxes to occupy. Every 7 d (11 d for L. reticulatus), the boxes in each incubator were shuffled so that each box spent a total of at least 7 or 11 d in each location during the course of the experiment to counteract any temperature variability that may have existed in the incubators. During shuffling, the boxes were also checked to ensure that the salt solutions were still saturated. Environmental conditions in each incubator were monitored using a HOBO temperature and r.h. sensor. Live insects in each vial were counted after 30 or 46 d, depending on the species, by spreading a portion of the contents of a vial on a 9-cm Petri dish, which had a coat of Fluon® on the walls, and removing motile psocids using a moist brush under a dissecting microscope. For all species, the experimental design was a randomized complete block (RCBD) with subsampling.

2.2. Effects of Temperature on Development

Three species were investigated; *L. brunnea*, *L. rufa*, and *L. reticulatus*. The procedures for obtaining and setting up eggs for the experiment were similar to those used by Opit and Throne (2008, 2009). Thirty centrifuge caps containing eggs were then randomly placed in each of 6 or 8 plastic boxes (30 x 23 x 9 cm high) that were painted black and contained saturated NaCl solution to maintain 75% r.h. The number of plastic boxes used depended on the number of temperatures that were under investigation (six for *L. brunnea* and *L. reticulatus* and eight for *L. rufa*). One box was placed in each of six or eight incubators set to maintain the desired treatment temperatures. The procedures for monitoring egg and nymphal development were similar to those used by Opit and Throne (2008) where psocids were marked using fluorescent powder. The experiment for each species had three temporal replications.

In the determination of the effects of temperature on the duration of development for each of the psocid species, data for male and female psocids were analyzed separately (*L. reticulatus* has only females). For both data sets, the design used for analysis was a RCBD with subsampling.

2.3. Effects of Temperature on Reproductive Parameters

Only *L. reticulatus* was investigated. One newly emerged adult female was placed in each 35-mm Petri dish containing five pieces of cracked wheat and 20 mg of colored diet. Twenty Petri dishes were placed in each of six plastic boxes (28 x 19 x 9 cm high) containing saturated NaCl below their false floors to maintain 75% r.h.; the plastic boxes, containing newly emerged adults and diet, were randomly assigned to one of six incubators set at temperatures of 22.5, 25.0, 27.5, 30.0, 32.5, and 35.0°C. Each Petri dish was checked daily until the adult female in it died, and any eggs found were counted and removed each day. When the amount of colored diet in Petri dishes was depleted to 30% of the original amount present (due to egg removal), 20 mg were added. Replenishment occurred only once. During the checking of Petri dishes for eggs, psocid excrement was also removed using a moist brush in order to keep the Petri dishes clean.

This experiment had four temporal replications. The experimental design was a randomized complete block (RCBD) with subsampling. PROC GLM was used for analysis of variance (ANOVA) to determine the effects of temperature on preoviposition period, oviposition period, postoviposition period, longevity, fecundity, and the percentage of total life span spent in oviposition.

3. Results and discussion

3.1. Effects of Temperature and Relative Humidity on Population Growth.

Liposcelis brunnea, *L. rufa*, *L. pearmani*, and *L. reticulatus* will not survive at 43% r.h. (Fig. 1) At 55% r.h., *L. reticulatus* will not survive; *L. rufa* and *L. pearmani* will survive between 22.5 and 30°C; and *L. brunnea* between 22.5 and 35°C (Fig. 1).

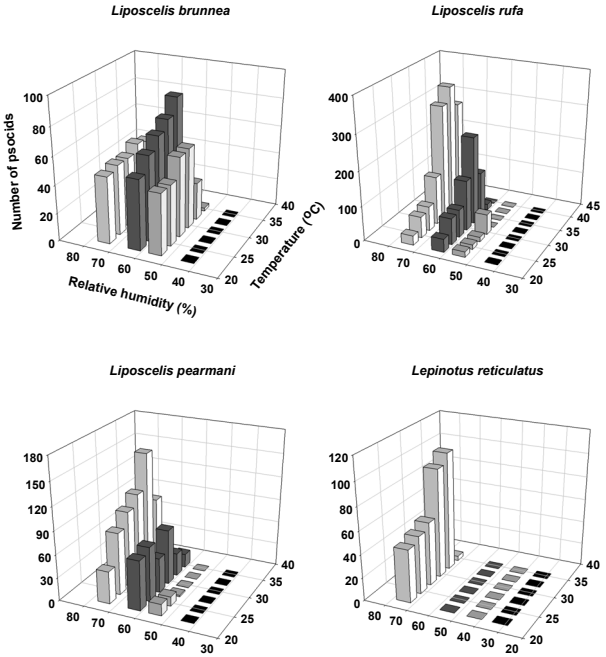

Figure 1 Numbers of psocids found at different relative humidity and temperature combinations. *L. brunnea*, *L. rufa*, and *L. pearmani* populations were allowed to increase over a 30-day period; L. reticulatus over a 46-day period.

At 63% and 75% r.h., *L. pearmani* and *L. brunnea* will not survive above 35°C. *L. rufa* will not survive at 40°C and 63% r.h. but will survive at 40°C and 75% r.h. Of these four species, *L. reticulatus* appears to survive under the narrowest set of conditions and appears suited to cooler and more humid conditions. *L. pearmani* and *L. brunnea* appear capable of surviving under a much wider range of conditions ranging

from 22.5 to 35°C and 55% to 75% r.h. The ability of *L. rufa* to multiply at 55% r.h., at temperatures of 22.5, 25, 27.5, and 30.0°C may allow it to thrive under conditions of low relative humidity where other Liposcelis species may not.

In addition, its ability to multiply rapidly at high temperatures of 35 and 37.5°C and 75% r.h. may allow it to thrive at temperatures too high for most Liposcelis species. Therefore, *L. rufa* appears to have the widest distribution of these four species. Optimal breeding conditions for *L. brunnea*, *L. rufa*, *L. pearmani*, and *L. reticulatus* were 32.5°C and 63% r.h.; 35°C and 75% r.h.; 32.5°C and 75% r.h.; and 30 and 32.5°C and 75% r.h., respectively. Starting from an initial population of five females each, populations of these psocid species grew by 17-, 73-, 31-, and 21-fold, repectively, under optimal conditions (*L. reticulatus* populations increased over a 46-d period whereas those of the other three species was over a 30-d period). Among these species, *L. rufa* populations grew fastest. Based on our data, the predicted size of the range of the four species in declining order would be: *L. rufa*, *L. brunnea* and *L. pearmani*, and *L. reticulatus*.

3.2. Effects of Temperature on Development.

Temperature had no effect on egg viability of *L. rufa*, *L. brunnea*, and *L. reticulatus*. Average percentages of viable eggs across all temperatures were 90, 87, and 80%, respectively. Nymphal survivorship averaged 78, 63, and 36%, respectively. The low nymphal survivorship for *L. brunnea* and *L. reticulatus* may be due to the susceptibility of nymphs to handling or that the relative humidity in which the development studies were conducted (75%) was not the optimal r.h. for these species. The fact that *L. brunnea* populations were higher at 63% r.h. than at 75% r.h. appears to support this explanation. The shortest development times for the egg and combined nymphal and combined immature stages of *L. rufa* females generally occurred at higher temperatures compared to *L. brunnea* and *L. reticulatus* (Table 1). This observation further shows that *L. rufa* is adapted to surviving in relatively high temperature environments. Prior to our ecological studies on L. rufa, the only two *Liposcelis* species that were known to survive well under relatively high temperature were *Liposcelis paeta* (Wang et al., 2009) and *L. decolor* (Tang et al., 2008).

Table 1 Shortest development times for the egg and combined nymphal and combined immature stages of female psocids, and the corresponding temperatures at which they occurred.

Species	**Development time (d) [temperature (°C)]**		
Egg	**Combined nymphal**	**Combined immature stage**	**stage**
Liposcelis rufa	4.5 (40.0)	15.9 (32.5)	21.6 (37.5)
Liposcelis brunnea	6.0 (32.5)	15.9 (32.5)	21.6 (35.0)
Lepinotus reticulatus	6.4 (32.5)	16.6 (32.5)	22.9 (32.5)

In its development from egg to adult, *L. reticulatus*, which is a parthenogenetic species, has four instars (Table 2). We found exuviae consumption after molting to be quite common in *L. reticulatus*. In the case of *L. rufa* and *L. brunnea*, which are described from both males and females, there were at least three different numbers of instars for each sex (Table 2); exuviae consumption in these species was not as common as in *L. reticulatus*. In *L. reticulatus* where exuviae consumption was common, the general trend was that development was slower for psocids that did not eat exuviae. The possible reason for this could lie in the nutritional status of exuviae. Lipids and nitrogenous compounds (protein and chitin) can account for as much as 4.4% (Nelson and Sukkestad, 1975) and 87% (Mira, 2000), respectively, of the total weight of insect exuvia. Therefore, eating exuviae would be beneficial to psocids.

Table 2 Mortality of N1 and N2 instars as a percentage of total instar mortality and variation by gender of the total number of instars and the observed percentage of each number.

Species	**N1 and N2 Mortality (%)**	**Variation in total instar number (observed % of each number)**	
		Males	**Females**
Liposcelis rufa	64	2, 3, and 4 (31, 54, and 15, respectively)	2, 3, 4, and 5 (2, 44, 42, and 12, respectively)
Liposcelis brunnea	94	2, 3, and 4 (13, 82, and 5, respectively)	3, 4, and 5 (18, 78, and 4, respectively)
Lepinotus reticulatus	83		4 (100)

3.3. Effects of Temperature on Reproductive Parameters.

All reproductive parameters varied with temperature (Table 3). Intrinsic rate of population increase for *L. reticulatus* increased with temperature until 32.5°C (0.128) and then declined. If the intrinsic rate of increase at 32.5°C is considered the optimal fitness of 1, then the fitness of *L. reticulatus* at 22.5, 25, 27.5, 30, and 35°C equals 0.52, 0.70, 0.84, 0.87, and 0.84, respectively. Highest intrinsic rates of increase for *Liposcelis badia* Wang, Wang, and Lienhard (Jiang et al., 2008), *L. bostrychophila* (Wang et al., 2000), *L. decolor* (Pearman) (Tang et al., 2008), *L. paeta* Pearman (Wang et al., 2009), and *Liposcelis tricolor* Badonnel (Dong et al., 2007) occurred at 27.5, 30, 32.5, 32.5, and 30°C, respectively. Intrinsic rates of increase at these temperatures were 0.0455, 0.0946, 0.0609, 0.0542, and 0.0367, respectively. At optimal temperatures for intrinsic rate of increase, *L. reticulatus* has the highest potential for population growth among these psocid species.

Table 3 Life table parameters (mean ± SE) of *Lepinotus reticulatus*.

Temperature (°C)	N	r	Ro	T (d)	t (d)
22.5	73	0.066 ± 0.002	26.07 ± 2.50	49.0	10.5
25.0	73	0.090 ± 0.005	33.68 ± 5.76	38.7	7.7
27.5	77	0.108 ± 0.005	35.67 ± 5.37	32.7	6.4
30.0	76	0.111 ± 0.004	27.09 ± 3.18	29.6	6.3
32.5	77	0.128 ± 0.007	28.39 ± 4.75	25.7	5.4
35.0	77	0.107 ± 0.004	16.26 ± 1.47	26.1	6.5

N, number of females in the analysis; r, intrinsic rate of population increase; Ro, net reproductive rate; T, generation time (d); and t, population doubling time (d).

All reproductive parameters varied with temperature (Tables 4 and 5). Regression equations described the relationship between temperature and each of the parameters preoviposition period, oviposition period, postoviposition period, oviposition rate (eggs/female/wk), and longevity quite well (Table 5). We found that *L. reticulatus* oviposition period and longevity declined with increasing temperature (Tables 4 and 5). A possible explanation for this may be that the higher egg maturation rates that occur at higher temperatures are associated with an overall higher metabolism which could reduce the life span (Papaj, 2000; Jervis et al., 2005). At higher temperatures, they may also be allocating significantly more energy resources to egg production than maintenance of body functions thereby resulting in reduced performance and survival (Papaj, 2000; Carey, 2001; Jervis et al., 2005, 2007). It is plausible that at 22.5 and 35°C, *L. reticulatus* has a proportionately shorter egg laying period than at optimal temperatures because of the diversion of resources from egg production and maturation that may occur at these suboptimal temperatures, for example, resources could be diverted to maintenance of body functions other than reproduction.

Table 4 Effects of constant temperatures on *Lepinotus reticulatus* preoviposition period (mean ± SE), oviposition period, postoviposition period, longevity, fecundity, and the percentage of adult lifespan spent in oviposition.

Temperature (°C)	Preoviposition period (d)	Oviposition period (d)	Postoviposition period (d)	Longevity (d)	Fecundity (eggs/♀)	Percentage of lifespan spent in oviposition
22.5	4.4 ± 0.25	65.5 ± 2.3	13.1 ± 1.24	82.9 ± 2.3	31.7 ± 2.1b	78.5 ± 1.79bc
25.0	4.4 ± 0.25	48.8 ± 2.3	8.4 ± 1.24	61.6 ± 2.4	38.9 ± 2.1ab	79.4 ± 1.80bc
27.5	3.6 ± 0.23	40.6 ± 2.1	4.4 ± 1.16	48.7 ± 2.2	40.7 ± 2.0a	82.7 ± 1.68ab
30.0	3.3 ± 0.23	33.2 ± 2.2	3.0 ± 1.17	39.4 ± 2.2	36.4 ± 2.0ab	83.0 ± 1.70ab
32.5	2.8 ± 0.24	30.9 ± 2.2	2.0 ± 1.20	35.6 ± 2.3	39.2 ± 2.0ab	85.0 ± 1.74a
35.0	2.7 ± 0.23	18.4 ± 2.1	2.8 ± 0.02	23.8 ± 2.2	21.3 ± 2.0c	75.2 ± 1.70c

ANOVA results were $F = 5.7$; df = 5,15; $P = 0.004$ for the preoviposition period; $F = 47.5$; df = 5,15; $P < 0.001$ for oviposition period; $F = 12.6$; df = 5,15; $P < 0.001$ for the postoviposition period; $F = 34.5$; df = 5,15; $P < 0.001$ for longevity; $F = 8.9$; df = 5,15; $P = <0.001$ for fecundity; and $F = 4.4$; df = 5,15; $P = 0.012$ for percentage of lifespan spent in oviposition. We were unable to quantify the relationships between fecundity and percentages of life span spent in oviposition at different temperatures using a biologically meaningful equation, so means within these two columns followed by different letters are significantly different using a least significant difference (LSD) test.

Table 5 Parameters describing the effects of constant temperatures on *Lepinotus reticulatus* preoviposition period, oviposition period, postoviposition period, oviposition rate (eggs/female/wk), and longevity.

Subject	Maximum R2	R2	F	a	b	c
Preoviposition period	0.43	0.38	13.7	1.39 ± 0.60	1646.19 ± 444.30	--
Oviposition period	0.90	0.87	152.7	-8.33 ± 4.05	36971.45 ± 2992.18	--
Postoviposition period*	0.56	0.49	13.1	120.36 ± 40.19	-7.30 ± 2.84	0.11 ± 0.05
Oviposition rate	0.49	0.45	17.7	8.14 ± 0.95	-2951.27 ± 701.70	--
Longevity	0.87	0.86	134.1	-13.76 ± 5.64	48203.12 ± 4162.70	--

In the case with an asterisk (*), df = 2,21; equation is of the type y = a + bx + cx2 with an adjusted R2 value. In all other cases, df = 1,22; equation is of the type y = a + b/x2. In all cases P < 0.01. Lack-of-fit P-values for preoviposition period, oviposition period, postoviposition period, oviposition rate, and longevity were 0.42, 0.79, 0.99, 0.86, and 0.86, respectively.

We found post-oviposition periods for temperatures of 22.5, 25, and 27.5°C to be longer than pre-oviposition periods; at temperatures of 30, 32.5, and 35°C they were either similar or shorter (Table 4). Pre-oviposition period declined with temperature most probably due to already stated reasons related to resource allocation, egg production, and egg maturation. Post-oviposition also showed the same trend except there was an increase in the post-oviposition period at 35°C for the same reasons.

Our studies provide important data on life history and reproductive parameters of four stored-product psocid pests. Because these parameters affect population dynamics, these data can be used in simulation models to predict psocid population dynamics and thereby aid in the development of more effective management strategies.

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Applications of semiochemicals for managing stored-product insects: research and product development

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Abstract

Pheromones have been identified for over forty species of stored-product insect and commercial formulations are available for nearly 20 species. Food-based attractants have been identified that can act alone to attract pests or provide additive or synergistic increase to pheromone response. Recent research on semiochemical-based pest management methods on three pest groups will be presented. Experimental studies on the use of synthetic sex pheromone for mating disruption of storage moths and also for the "attract-and-kill" method will be reported. Initial studies indicate the mating disruption of the cigarette beetle, Lasioderma serricorne is possible using its synthetic sex pheromone. Lastly, studies on the response of the lesser grain borer, *Rhyzopertha dominica* (F.), to its aggregation pheromone suggest that this behavior can be manipulated with other semiochemicals. Semiochemical-based pest management can go beyond the effective use of traps in monitoring and control decision-making to actual population suppression with these behavioral compounds as alternatives to traditional insecticides.

Regulation of the pheromone biosynthetic pathway in the Indian meal moth

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Abstract

The circadian regulation by Pheromone-Biosynthesis-Activating-Neuropeptide (PBAN) of the main diene pheromone component of *Plodia interpunctella*, (Z,E)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac) has been demonstrated (Rafaeli and Gileadi, 1995). In *P. interpunctella*, nine different desaturase encoding transcripts have been isolated (Knipple et al., 2002) as potential desaturase genes but their functionality has not been demonstrated. Here we combine the use of labeled precursors with enzyme inhibitors to decipher the rate-limiting step that is regulated in the biosynthetic pathway (Choi et al., 2003). Incorporation of label from the 13C sodium acetate precursor is activated by PBAN, whereas no stimulatory action is observed in the incorporation of the precursors: 13C malonyl coenzyme A; hexadecanoic 16,16,16-2H3 or tetradecanoic 14,14,14-2H3 acids. The Acetyl Coenzyme A Carboxylase (ACCase) inhibitor, tralkoxydim, inhibits the PBAN-stimulation of incorporation of stable isotope. These results (Tsfadia et al., 2008) provide irrefutable support for the hypothesis that PBAN affects the synthesis of malonyl coenzyme A from acetate by the action of ACCase in the pheromone glands of this moth species. The study showed that *P. interpunctella* utilizes hexadecanoic acid, and to a lesser extent tetradecanoic acid, for the biosynthesis of Z9,E12-14:Ac with the involvement of mainly D11 desaturase, chain shortening, followed by D12 desaturase. Nine different female specific desaturase encoding transcripts are detected by specific primers using PCR but only two desaturase genes need to be functional to produce the diene pheromone component. Relative gene expression (Real Time Quantitative PCR, RT-qPCR) of these gene transcripts in the pheromone glands showed high expression levels in only two genes accession numbers: AF482923 and AF482924). The other seven genes are at least 105 fold lower or undetectable. Future characterization of full gene sequences of the two highly expressed genes and appropriate functional expression studies will define the functional genes.

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Are the volatile chemicals from wheat and grain insect (*Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Sitophilus granarius* (L.)) related with inter-communication between insects and host?

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Abstract

We assume that selection pressure can act on both the biochemical and the physiological regulation of the signal and on the morphological and neurophysiologic filter properties of the receiver. Communication is implied when signal and receiver evolves toward more and more specific matching, culminating. In other cases, receivers respond to portions of a body odour bouquet that is released to the environment not as a (intentional) signal but as an unavoidable consequence of metabolic activity or tissue damage. Breath, sweat, urine, faeces, their aquatic equivalents, and their bacterial and other symbiotic embellishments all can serve as identifiers for chemoreceptive insects interested in finding food or hosts. Understanding the biological and chemical bases for these signals could lead to new approaches to the diagnosis and bio-treatment of insect pests. The principal of this research is based on the volatiles released from both commodities and insects after harvest and during storage. The contents of volatiles are related with history of grain (pre and post harvest conditions and treatment). Therefore, the volatiles can be monitored as indicators for diagnostic of grain qualities (insects at this stage). The headspace Solid Phase Mico-extraction Gas Chromatography (HS-SPME-GC) method was used for analysis of volatiles from host wheat, three species of stored grain pests *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Sitophilus granarius* (L.), and wheat plus each insect species, respectively. The primarily GC results showed that chemical signals or GC spectra maps are different between the different combinations, such as wheat or insect only or wheat plus insects. The primarily models were explored which could partly illustrate stored grain insect evolution from the point of view of inter-communication between insects and host wheat with the volatile chemicals.

Efficacy of pheromone-based control system, Exosex™ SPTab, against moth pests in European food processing facilities

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Abstract

The Exosex™ SPTab auto-confusion system is a novel pheromone-based method for control of stored product moth pests in both food and tobacco processing and storage facilities. The method uses a female-produced sex pheromone, (Z,E)-9,12-tetradecadienyl acetate, combined with a patented electrostatic powder delivery system known as Entostat™ to disrupt mating and interrupt the lifecycle of several important moth pests: *Plodia interpunctella*, *Ephestia kuehniella*, *Ephestia cautella* and *Ephestia elutella*. Male moths are attracted to a compressed tablet of the powder, which contains the sex pheromone. The powder releases pheromone at a slow enough rate to attract males to make contact with the tablet. The powder adheres to the moth cuticle via electrostatic attraction and the moth leaves the tablet coated in female sex pheromone. Flight tunnel studies have shown that this disrupts their ability to locate female moths and they become attractive sources for other males.

Here we will present findings from full scale, long term trials that were conducted under real conditions at commercial food processing facilities across Europe. Populations of target moth species were monitored alongside deployment of the SPTab system and compared with untreated control areas and historical data from the test areas in the years prior to deployment. In all cases populations were reduced compared to the same area in the previous year and compared to untreated control areas under local pest control practices.

The SPTab auto-confusion system could offer the opportunity to actively reduce the use of pesticides and its use as an integrated pest management tool within the food and tobacco processing and storage industry is discussed.

Keywords: Mating disruption, *Plodia interpunctella*, *Ephestia kuehniella*, SPTab, Sex pheromone.

1. Introduction

Concerns over worker safety and residues in our food and the environment have led to a withdrawal of many previously available chemicals to treat stored and processed food environments. Stored and processed food, and tobacco, is subject to attack by a variety of moth pests from the family Pyralidae. Several of these species share a common female sex pheromone component, (Z,E)-9,12-Tetradecadienyl acetate, also known as ZETA (Kuwahara and Casida, 1973).

Sex pheromones have considerable potential in indoor pest management (Phillips, 1997; Cox, 2004), particularly for monitoring moth populations but also for direct control via disruption of mating behaviour. Mating disruption with sex pheromones has achieved widespread use in agricultural settings such as top fruit and vine protection (Cardé and Minks, 1996) but could have great potential for use indoors where airflow is restricted and potential for mated female immigration is less. ZETA has the additional benefit of being a very potent male attractant for the most important stored product moths: *Plodia interpunctella* (Hübner), *Ephestia kuehniella* (Zeller), *Ephestia cautella* (Walker) and *Ephestia elutella* (Hübner) (Kuwahara and Casida, 1973). Thus, there is the potential to control all species with one type of pheromone system. Some experimental assessments of the mating disruption technique by releasing high concentrations of ZETA into the air using pheromone dispensers have shown the potential to reduce moth populations (Hodges et al., 1984; Suss and Trematerra, 1986; Prevett et al., 1989; Fadamiro and Baker, 2002; Ryne et al., 2006).

Instead of relying on pheromone saturation of the air through emission from a fixed number of point sources, a system, Exosex™ SPTab, has been developed that delivers a carrier powder containing ZETA directly to the male moth cuticle. The proprietary carrier powder, Entostat™ (Exosect Ltd, Winchester, UK) is electrostatically chargeable and adheres to insect cuticles via electrostatic attraction (Armsworth et al., 2006; 2008; Nansen et al., 2007a: b). Male moths are attracted to dispensers containing a compressed Entostat tablet, become contaminated with Entostat-containing ZETA (Baxter et al., 2008) and after leaving male moths show reduced responses to calling females and become attractive to conspecific males, spreading the confusion effect (Huggett et al., 2010). The tablets contain less pheromone (10 mg) and have lower release rates than traditional mating disruption systems; for example at recommended application rates, some commonly used dispensers for *Cydia pomonella* result in 7.5-410 g of ai/ha/yr (OECD Environment Directorate, 2001) and SPTab deployment results in 2.4 g of ai/ha/yr, well below the recommended threshold of 375 g ai/ha/yr set by the US EPA for experimental use and waiving residue data. In enclosed indoor environments, lower pheromone rates may be beneficial due to the likely higher expected exposure levels for workers. Traditional mating disruption dispensers work by a combination of factors, including saturation of the air with a fog of pheromone so that males cannot detect the calling female pheromone plumes against the background level in the air (Cardé and Minks, 1996). SPTab has a release rate in the range between a female moth and a typical monitoring lure (Storm, unpublished data) and releases pheromone at a rate to attract males to land on the dispensers. Because the dispensers release pheromone at a lower rate, trap shutdown does not occur and pheromone traps may still be used to monitor populations.

We monitored three separate food processing facilities (one spice processing plant and two flour mills) for one year before and one year after deployment of SPTab using pheromone-based monitoring systems. In addition we collected monitoring data for control areas at each site, which did not receive an application of SPTab and which were separated by at least a closed door or stairwell. Historical data were consulted to ensure control areas had measurable population levels of target moths, similar if possible to the treated area. By obtaining control data, the percent efficacy achieved by SPTab in the treated areas of each location could be calculated using the Henderson-Tilton formula (Henderson and Tilton, 1955). We discuss the merits and limitations of such an approach to evaluate a pheromone control product for storage and processing environments. The potential for the SPTab system for stored product moth control in fully commercial settings are also discussed.

2. Materials and methods

2.1. Test sites and pest species

Three independent experiments were conducted at commercial food production and processing sites and run for one year. Experiment A) was conducted at a flour mill in the UK between July 2007 and June 2008. An entire area containing the packing lines and ground floor processing area measuring approximately 2000 m2 was treated with the test product. Experiment B) was conducted in a spice factory in the Netherlands between March 2008 and March 2009. The treated area was approximately 1730 m2 containing both process and packing lines. Experiment C) was conducted at a flour mill in the UK between January 2009 and January 2010. Two floors containing milling and processing lines and measuring approximately 4200 m2 in total were treated.

Untreated control areas were monitored at all three sites. At site A the second, third and fourth floor of the processing area, connected to the test area by staircase, were used. At site B the adjoining warehouse area, separated by a plastic-covered doorway, was used as an untreated comparison. At site C three separate untreated areas were monitored. All three areas were separated from the treated area by at least a corridor and a door.

At sites A & C the pest species, typical of low humidity flour milling facilities, was *E. kuehniella*. At the spice factory, site B, the pest species was the more commonly encountered *P. interpunctella*.

2.2. SPTab dispensers and application process

SPTab dispensers were deployed as per manufacturer's recommendations. Dispensers were placed at a height of between 1.5 – 2.0 m in an approximate grid pattern with a 5 x 5 m spacing to achieve a calculated density per test area of one dispenser per 25 m2. Total dispensers used at sites A, B and C were 80, 69 and 168 respectively. The dispensers were located at even spacing on the walls, on central

structures of the building and where possible on machinery focusing around likely areas of infestation. Pheromone based monitoring trap placement, already established within the test sites, were noted and test dispensers were placed to incorporate them as part of the grid system layout. Once established the pheromone dispensers were maintained at the same location throughout the trial. Tablets were replaced at approximately 60 day intervals to give a total of 6 applications for the one year duration of the trials.

2.3. Monitoring and data collection

Standard funnel traps (Killgerm, Ossett, UK) baited with commercial pheromone lures (Spectrum, Ephestia, Killgerm, Ossett, UK) were used to record numbers of adult male moths in both treated and untreated areas for one year before and one year after product deployment. Monitoring points were consistent throughout both the pre and post-treatment periods. Total adult moths were recorded at approximate one monthly intervals throughout. Pest control operator companies servicing each site collaborated to assist with the provision of historical monitoring data, servicing of traps during deployment and replacement of SPTab at 60 d intervals.

2.4. Data analysis

For each site, the effect of the SPTab treatment on the number of moths in monitoring traps in treated areas relative to the control areas was compared using the Henderson-Tilton formula (Henderson and Tilton, 1955); on an annual basis to account for seasonality in moth capture. The mean number of moths caught per trap per year in treated and control areas was entered into the formula to give the % population suppression achieved by the end of one year of treatment (% moth catch reduction = (1-(C1×T2)/(C2×T1)×100) where C1 = mean trap count total in control area in pre-treatment year, C2 = mean trap count total in control area in post-treatment year, T1 = mean trap count total in treated area in pre-treatment year and T2 = mean trap count total in treated area in post-treatment year). Data presented as mean +/- standard error of the mean.

3. Results

For each site the seasonal male moth catches over the pre and post treatment periods are represented in Figure 1. Because of the variability in trap servicing, at each service interval the catches per trap were converted to daily trap catches (by dividing the trap catch by the number of days since the last service) and the mean of these, for control and treated areas, at each interval, are represented on the graphs.

At site A, the mean annual moth capture per trap in the treated area decreased from 835±94 in the pre treatment year to 340±60 in the post-treatment year (Figure 1A). Conversely, in the untreated control area, annual moth capture actually increased from 276±33 to 769±163. The suppression of male *E. kuehniella* numbers in the treated area was calculated as 85% using the Hendersen *Tilton formula*.

At site B, the mean annual moth capture per trap in the treated area decreased from 18±16 in the pre-treatment year to 2±2 in the post-treatment year. In the untreated control area, annual moth capture also decreased, from 31±12 to 16±4 (Figure 1B). The reduction in male *P. interpunctella* moth numbers was greater in the treated area and calculated as 79% using the Hendersen *Tilton formula*.

At site C, the mean annual moth capture per trap in the treated area decreased from 120±31) in the year prior to treatment to 30±12) in the year of treatment (Fig. 1C). Conversely, in the untreated control area, annual moth capture was very similar in both years: 72±21 in the pre-treatment year and 74±16 in the post-treatment year. The suppression of male *E. kuehniella* numbers in the treated area was calculated as 76% using the Hendersen *Tilton formula*.

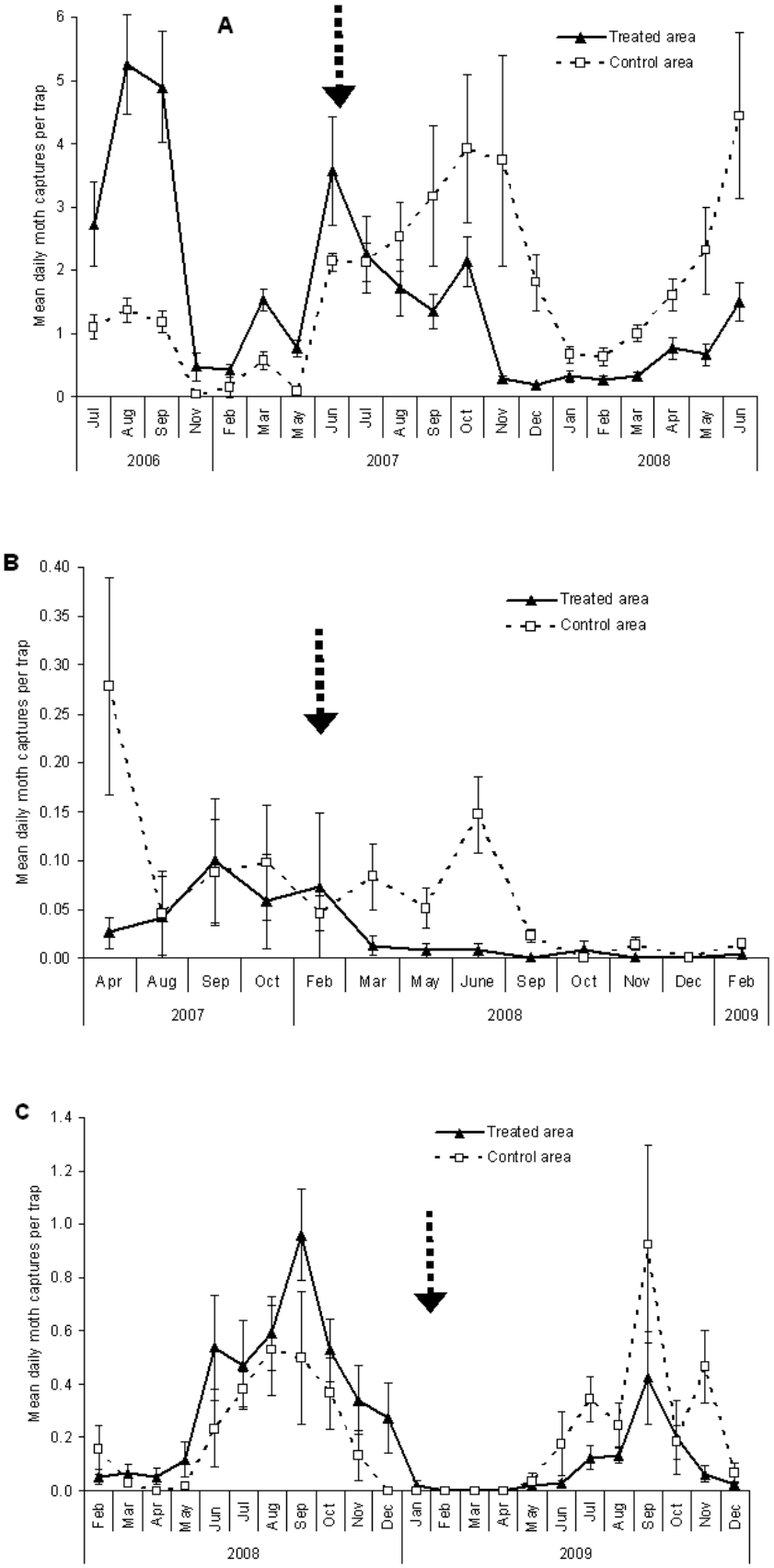

Figure 1 Mean number (+ SE) of *Ephestia kuehniella* (A and C) or *Plodia interpunctella* (B) per trap in each monthly monitoring period for control and treated areas over a one year pre-treatment period and a one year post-treatment period. A = site A, B = site B and C = site C. Closed bars = standard errors. Black dashed arrow indicates time of Exosex SPTab deployment.

4. Discussion

The decrease in moth captures in all three trials potentially shows that population reductions have been achieved. It is likely that the reduction in moth capture was caused by a combination of population reduction and competition with SPTab point sources leading to reduced monitoring trap efficacy. With the current experimental design it is not possible to determine the proportion contribution of each factor to the trap capture reductions. Additional methods of sampling the population should be introduced in future trials to quantify the effects.

The three trials have also shown the ease and safety with which the SPTab system, which utilises comparatively small quantities of pheromone, can be used in enclosed environments where both human health and quality of food produce is of great importance. The feedback we received from the Pest Control Operators from each site was that the SPTab system was both quick and easy to deploy with minimal training and did not require removal of people and produce or interruption to normal operating practices whilst being used.

Whilst large mean reductions were recorded in all three trials over the course of a full year compared to both control and historical records, there was large variation between individual monitoring traps both spatially and temporally. This was often found to be correlated with known hotspots of infestation, as reported by facility pest control technicians during the trial and related to historical trap counts. Other trials (Pease, unpublished data) have also shown that under high levels of infestation it may also be possible to detect such hotspot areas within commercial facilities with increased clarity following deployment with this system in conjunction with standard pheromone monitoring traps. Where large populations have often been visible in several traps over greater areas prior to deployment, unequal reductions post-deployment of SPTab, by reducing the overall population level, have drawn attention to smaller more defined areas where key harbourage sites have existed. When used as part of an integrated pest management (IPM) system in conjunction with deep cleaning and hygiene this may allow for a more targeted approach. It was noted that the expectations and interpretation of results by both pest controllers and facility personnel would require management where resulting reductions in trap catches were not instant in comparison to expectations with traditional pesticide use. SPTab and other pheromone-based control systems work by long-term reduction of populations, and they do not provide the instant knockdown of mainly adult life-stage seen with current fogging and ULV type treatments.

It has been acknowledged that there are limitations when measuring population reduction success with the use of pheromone baited monitoring traps in trials that test mating disruption products (Ryne et al., 2006). With increased point source release of pheromone after installing such a system there may be interference competition with monitoring traps and hence apparent reduction within the population may not be wholly assigned to the effect of the product on lifecycle disruption. To minimise these effects the trials were conducted over long time periods to see the long-term effects, and the product placement took into account the positioning of monitoring traps within the deployment grids. It has also been observed that point source competition can be largely off-set by increased flight activity of adult male moths following SPTab deployment. Large initial increases in trap catch have been recorded in pheromone monitoring traps in other trials (Pease, unpublished data), perhaps due to the pheromone drawing males out of harbourage sites. This was always followed by significant reductions at two to three months post-deployment, coinciding with a single full life-cycle of the pest and indicating that disruption of mating and not interference with monitoring traps is the more likely observed effect.

It is also acknowledged that designing and conducting full scale field trials of this type has additional limitations. Mating disruption control systems require large areas over long-time periods to determine efficacy. The complexity at such fully commercial sites, in terms of both pest population dynamics and commercial activities makes it difficult to establish a robust comparative control area for the duration of the trial (Ryne et al., 2006. Pease, unpublished data). Likelihood of adult moth migration and re-infestation via contaminated material is also possible and un-quantifiable. This has been minimised by using long-term historical data in addition to untreated control areas within these trials in order to determine population reduction over a full one year period. Sites were also selected according to likelihood of re-infestation. Processing and packing areas within the flour mills and the spice processing plant were tested as resident populations are more likely the result of inherent breeding and not due to continual re-infestation from an external source.

Future trials should attempt to incorporate other methods of population monitoring, such as water traps (Chow et al., 1977) and measurement of oviposition (Nansen et al., 2006), which could be used to offset the problem of pheromone point source competition. However, unlike traditional mating disruption systems that rely on environmental flooding with pheromone, immediate trap shut-down is neither expected nor desired with SPTab. Volatile capture analysis of pheromone release from SPTab has indicated that release rates are slightly below the rate of standard monitoring lures whilst much greater than that reported for calling females (Storm, unpublished data). We hypothesise that this explains why male moths are still able to locate and travel up the pheromone plume from monitoring traps. Valuable interpretation of data using the industry standard pheromone monitoring traps is therefore still possible, which is considered advantageous in commercial working sites where methods such as water trapping may not be practically possible.

In conclusion, the SPTab system has demonstrated good efficacy against *P. interpunctella* and *E. kuehniella*, and offers an alternative to pesticidal treatment of stored product moths, of which few alternatives are available. SPTab could offer an environmentally safer alternative by reducing risk to pest control operators and workers and by reducing residue exposure to the fabric and produce within such facilities. In addition to this is an indication that by not causing trap shut-down this system may also be used in conjunction with current industry standard monitoring traps as part of an efficient and effective IPM approach to stored product pest control.

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Monitoring of arthropod infestations on high quality hard wheat in southern Italy

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Abstract

The results of a survey in 2006-2009 on high quality hard wheat, coming from 31 different storage centers located in Southern Italy, are reported. About 300 samples were analyzed by visual test, while 891 by both sieving and biological test. For the three different kinds of test the infesting species are listed and their relative incidence on the samples is reported. The most widespread species in the samples belonged to the order Coleoptera, i.e. *Sitophilus granarius*, *Rhyzopertha dominica* and *Oryzaephilus* spp., while Lepidoptera were less abundant. The results are discussed with the aim of providing the storage centre operators with helpful information on the correct monitoring strategies to adopt in case of arthropod infestations in high-quality hard-wheat warehouses.

Keywords: Stored grain, Insects, Sampling, Coleoptera, Italy

1. Introduction

Durum wheat (*Triticum durum* Desf.) in Italy represents one of the main crops. In Sicily, in particular it is an important resource both in terms of contribution to the agricultural income and for the preservation of the landscape and rural traditions. This region produces 17% of the total hard wheat and together with Marche, Molise, Apulia and Basilicata regions, it provides more than 60% of the national production (ISTAT, 2008). Preservation and promotion of this important sector is possible with product or system certification, and with technological and innovatory systems to monitor the quality of the product in all the steps of its production, transformation and distribution (Sgrulletta et al., 2002).

The grain crop value is strictly influenced by the insect infestation that causes quantity and quality losses of the product. Intense insect activity not only consumes grain affecting quality through depletion of specific nutrients, but it is also responsible of different levels of contamination due to the presence of their metabolic by-products and body parts. Infestation often causes the development of hotspots and growth of microflora, making the products unsuitable for human consumption. Worldwide every year, about 10–30% of produced grains are lost due to insect damage (White and Leesch, 1995). Several cosmopolitan insect species are reported as pests of stored wheat, and their presence is highly influenced by different parameters like the storage duration, grain temperature, and grain moisture content, while observation of their presence depends on the grain sampling method used for detection. In the current study, we report the results of a survey carried out in different storage centers, with the aim of documenting the levels of arthropod fauna infesting high quality hard wheat.

2. Materials and methods

Monitoring surveys were carried out in 2006-2009 on high quality hard wheat coming from 31 different storage centers located in Southern Italy. The investigations were conducted using conventional survey methods which consisted of visual, sieving and biological testing of 297 cereal lots. Visual testing was carried out on 5 kg samples of hard wheat collected from each single lot (n=297), while sieving and biological tests were conducted on three different 50 g sub-samples (n=891) from each sample.

The visual test was carried out distributing the sample upon an illuminated table and observing it using magnifying glasses. All the arthropods present were collected and successively identified with the aid of a stereo-microscope. The sieving analysis was conducted using a series of sieves placed in decreasing size (2.30, 1.80 and 0.30 mm), from top to bottom, in a mechanical sieve shaker. Each sample was sieved for 10 minutes with 150 vibrations per minute. After the sieved material reached the pan, the arthropods retained in each sieve were then collected in Petri dishes and subsequently counted and identified. In

order to perform the biological test, samples were placed in jars at 26 ± 1°C and 75% relative humidity (r.h.) to allow the development of immature stages and then sieved at regular intervals (3-4 days) during an incubation period of 60 days, sufficient to complete the life cycle of the most common wheat insect pests (Süss and Locatelli, 2001). The data on the infestation levels obtained, were processed using the classes proposed by Gelosi & Süss (1991) based on the quantity of living arthropods present for each kilogram of hard wheat examined.

3. Results

The highest infestation rate was revealed by the visual test (87.54%) compared with the biological (58.36%) and the sieving tests (47.03%). According to the classification of the infestation level (Gelosi and Süss, 1991) (Fig. 1), only 13% of the total samples examined were not infested while 23% and 37% presented relevant and heavy infestation levels containing 4-10/kg, or >10/kg of live arthropods respectively.

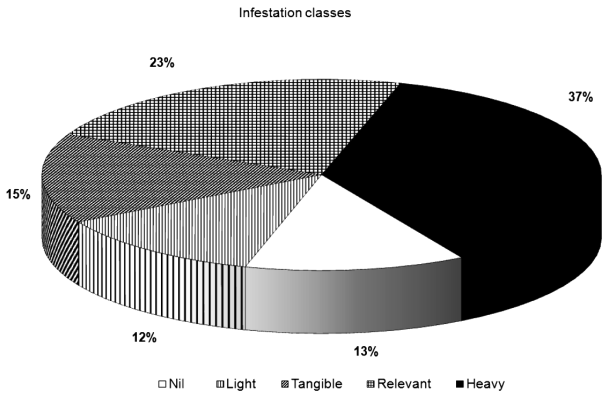

Figure 1 Infestation levels of the grain samples coming from 31 different storage centres, processed using the classes proposed by Gelosi and Süss (1991).

A total of 8 insect pests species were found (Fig. 2), mainly Coleoptera, all of them commonly reported as primary and secondary phytophagous pests typically associated with stored products. The most common internal grain feeders found were the granary weevil *Sitophilus granarius* (L.) and the lesser grain borer, *Rhyzopertha dominica* (F.) Regarding the external grain feeding insects, the main species that were found were Coleoptera; *Tribolium castaneum* (Herbst), *Tribolium confusum* Jaquelin du Val, *Tenebroides mauritanicus* (L.), *Oryzaephilus surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens) and in few samples Lepidoptera; *Plodia interpunctella* (Hübner).

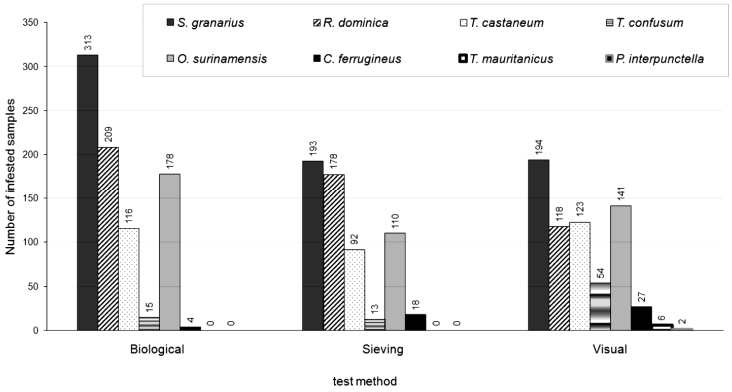

Figure 2 Total number of insect pest species recovered on the grain samples analyzed during the survey in Southern Italy in 2006-2009.

4. Discussion

More data were obtained by the visual test, and this is probably related to the greater sample size used samples (5 kg), which was considerably bigger than that used for the other survey methods (50 g). In relation to the recovered species, the most abundant were granary weevil and lesser grain borer confirming their presence and distribution in warmer areas of Italy, where their numbers increase progressively as a function of the optimal environmental conditions present in Southern Italy (Trematerra and Gentile, 2006). On the other hand, the high number of the saw toothed grain beetle *O. surinamensis* and of *Tribolium* spp., intercepted in the visual test has to be referred not only to the larger sample size, but also to the fast and frequent adult movements that make these species easily visible. The high infestation level recorded in the present study on some of the analyzed samples could probably be due to the longer storage of the product which allowed hatching and subsequent propagation of these insect populations.

In contrast, the total absence of mites as well as beetles belonging to the family of Trogiidae that also feed on mould mycelium, in all the samples examined showed, at the least in terms of moisture presence in the storage centers, that the cereal lots were correctly managed; in fact none of the checked samples, showed mould development that, as well known, represents the main substrate for these arthropods species. In this framework, in order to slow or deter the loss of quality of the grain, some techniques such as the use of extreme temperatures, could represent one important approach to follow for insect pests management. In fact, it has been shown that the use of the high temperatures against the grain insects living outside the kernels does not harm the quality of the grain. However, when this technique is used to control the insects developing inside kernels, it can cause a slight decrease in the germination rate, although this is insignificant for grain to be used as food and animal feed (Mourier & Poulsen, 2000). Moreover, other techniques, some of them actually employed for organic production in Italy, i.e. inert dusts, modified atmosphere based on carbon dioxide (CO2) or nitrogen (N2), could provide a valid support to the control programs of insect pests on grain.

In conclusion, the results indicate that the general hygienic conditions and control measures adopted by these different storage centers against stored-product pests should be revised, at least in terms of preventive interventions, keeping in mind how an early detection and the knowledge of the life histories and habits of the arthropod species present in the storage centers are essential for correct control procedures and for protection of the products involved. The results also indicate the importance of faunistic and bioecological research on arthropods associated with stored durum wheat, considering the economic and sanitary importance of these pests that considerably affect the quality of the product.

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Invasion and threats of *Acanthoscelides obtectus* (Say) (Coleloptera: Bruchidae) to kidney beans in India - a first record

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Abstract

Many pest species have crossed geographical boundaries and become cosmopolitan in distribution through human-mediated migrations and the import and export of food grain consignments. These pest species pose constant threats to our agriculture produce and may be responsible for losses worth billion dollars every year. *Acanthoscelides obtectus* (Say), a serious pest of kidney beans, *Phaseolus vulgaris* L., originated in the neotropics and has been reported from Australia, Europe, United States and a few Asian countries. For the first time it has now been recorded from the Indian subcontinent. The increased geographical distribution and anthropogenic domestication and diffusion of grain legumes have adapted this pest species to climates ranging from temperate to sub tropical. This pest species has invaded the Mid-Himalayan region and is a serious threat to local cultivars of kidney beans. This study on *A. obtectus* examines host range and distribution, invasion and threats, life history on different cultivars of *P. vulgaris*, host susceptibility and resistance, damage and loss and number of annual generations.

Keywords: *Acanthoscelides obtectus*, Kidney beans, Pest, Mid-Himalaya, Life history.

1. Introduction

Bruchids, commonly called pulse weevils, are cosmopolitan in distribution and a major problem affecting edible legumes. These beetles pose a threat to our agriculture produce and cause serious loss to grain legumes both in fields and stores. *Acanthoscelides obtectus* (Say) (Coleloptera: Bruchidae) a bruchid beetle of neotropical origin, is a serious pest of kidney bean Phaseolus Vulgaris L. (Fabaceae) and various other pulses in Africa (Silim and Ambrose, 1993; Msolla and Misangu, 2002), Australia (Keals et al., 1998, 2000), Europe (Nadir et al., 2005; Schmale et al., 2002) and America (Johnson, 1990a; 1990b; Martin and Edmund, 1991). This pest is now reported for the first time from India. However, a congeneric species, *Acanthoscelides pallidipennis* Motschulsky exists in China, Korea and Japan (Tuda et al., 2001). But this is the first time that any species of the genus *Acanthoscelides* has been reported from the Mid-Himalayan range of the Indian subcontinent, where it has been found infesting kidney beans. Thus this species now occupies almost all the continents of the world except Antarctica.

Systematic studies of Indian bruchid species so far have not revealed the existence of this notorious pest species from any part of the country. However, the Quarantine Division, New Delhi, India has warned of the phyotosanitary risk of 13 bruchid species in bulk imports of pulses (Bhalla et al., 2006). This has lead to the compulsory X-ray screening of consignments of pulses, immediately on arrival in India, for hidden infestation by species of Acanthoscelides, Bruchidius and Caryedon (Wadhi and Verma, 1972; Bhalla et al., 2006). *Acanthoscelides* obtectus joins *Zabrotes subfasciatus* (Boheman) as an economically important bruchid pest in the Indian subcontinent. In the Mid-Himalayan ranges *Z. subfasciatus* infests beans in field and continues during storage while *A. obtectus* infests stored beans in plains below 1500 m above mean sea level. The effect of both species is to reduce the quality and quantity of beans, rendering them unfit for human consumption and germination.

2. Materials and methods

Infested cultivars of kidney beans, *P. vulgaris* nurturing different developing stages of *A. obtectus* were collected from different Mid-Himalayan ranges of India. Adults emerging from beans were kept in different Petri-dishes along with seeds of host plants under natural environmental conditions to observe the life history traits of pest. The Mid-Himalayan range of Indian branches off from the Great Himalayan range near Badrinath in Uttrakhand state and extends to the Pir Panjal range in Jammu and Kashmir, passing across the state of Himachal Pradesh in India. The range is wholly mountainous with altitudes ranging between 1500 to 4500 m above sea level. Different cultivars of kidney beans (*P. vulgaris*) are

grown throughout the range. Dead adult insects of both sexes were treated with 10% w/v hot KOH solution in order to dissolve away the soft muscular tissue and treated specimens were dissected to expose their genitalia. Internal structures so exposed were washed repeatedly with fresh and acid water and mounted in DPX after dehydrating in different grades of ethanol. The pest was observed using a stereoscopic zoom-triocular microscope fitted with image capturing devices. Results reported here come from three years of continuous study of the insect. Expert opinions were sought to confirm species identity.

3. Results

Adult insects were 3.25 - 4.50 mm long, 1.50 - 2.50 mm wide and greyish brown in colour. Of the antenna, segments 1-4 are filiform, 5-10 broadened and more serrated and segment 11 is non-serrated and acute apically. Regarding colour variations, segments 1-5 are grey or of the same colour as the body, 6-10 dark blackish and segment 11 red/orange. Bean weevils start to infest beans in the field and continue to develop during storage. The pest is sexually dimorphic, males are active, smaller in size with a vertical pygidium whereas females are more bulky, inactive with a sub-vertical pygidium. Like other bruchids, adult A. obtectus do not feed, they are weak flyers and feign death during disturbance.

Freshly emerged adults copulate at any time from about one hour after their emergence. During copulation the male normally raises its fore and middle legs to hold the female. Copulation lasts for 4-5 min. Gravid females lay eggs on and around the host seeds. Eggs are usually deposited singly and unlike other bruchids this pest does not stick its eggs to pods and seeds but scatters them irregularly among host seeds. Eggs are ellipsoidal in shape and oviposition lasts for 6-9 days and the rate of egg lying is high on first day of oviposition period. Freshly laid eggs are milky white, but become transparent before the larvae hatch. Larvae possess three pairs of legs, are white in colour and are covered with white shining setae of variable size. The first instar larva has a large golden head and white elongated body that can be seen through the transparent egg shell. The incubation period is about 9-14 d and first instar larvae bore into the host seed with the help of an 'H' shaped prothoracic plate. Since the eggs are not glued onto seeds it is essential for freshly hatched first instar larva to find the host seeds for food. Entrance holes into the host seed later become plugged by faecal matter and further development of successive larval instars inside the host seed, under favourable conditions, can be completed in 3-4 wk. Pupal development is completed in 18-26 d during March – September but hibernating larvae and pupae take 4-5 months during winter (October - February) and then emerge as adults during March and April the following year. The pest is multivoltine and completes three to four overlapping generation in a year (Table 1).

Table1 Life history traits of Acanthoscelides obtectus on *Phaseolus vulgaris* (values are mean ± SD).

Mating duration (minutes)	Oviposition period (days)	No. of eggs laid per female	Developmental duration (days)	No. of generations completed in a year	Duration of hibernated stages (months)
4-5	7 ± 0.77	53.2 ±7.91	58.6 ± 1.49	3-4	4-5

Since the first instar larvae bore into the seed and then feed, grow and moult into successive instars entirely within the seed, no evidence of their presence appears except circular windows that are created when the last instar larvae gnaw close to the seed coat in preparation for adult emergence (Fig. 1A). Pulses with such hidden infestations move across the geographical boundaries in import /export consignments and may pose a great phytosanitary threat in new ecological niches due to the absence of natural enemies. This internal mode of life protects them from variations of temperature and humidity and enables them to be carried unnoticed during trade across the international boundaries. All the larval instars are voracious feeders and develop at the cost of legume proteins so that heavily infested beans are often reduced to empty shells (Fig. 1B).

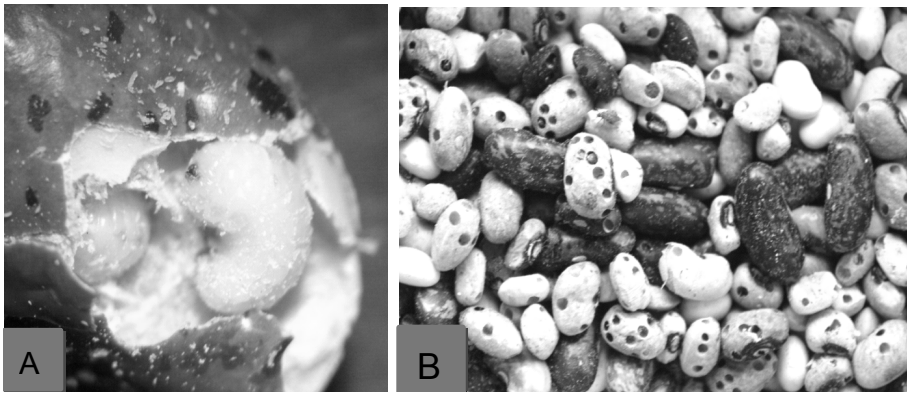

Figure 1 Last instar larvae of *A. obtectus* just below the seed coat of (A) *P. vulgaris* and heavily (B) damaged beans.

4. Discussion

Previous taxonomic revisions and pioneer studies on the bruchids fauna from unexplored areas of the Indian subcontinent have not previously mentioned this notorious pest species (Thakur, 2008). But now *A. obtectus* has been reported from the Mid-Himalayan range which extends across the three major states of the Indian territory and ranging between 1500 to 4500 m above sea level. *Acanthoscelides obtectus* seems well adapted to different cultivars of kidney beans (*P. vulgaris*) throughout this range. Observations to date suggest that the morphology, behaviour and pest status *A. obtectus* in India is no different from the same pest in other countries/continents reported by Masolwa and Nchimbi (1991), Nadir et al., (2006) Parsons and Credland (2003), and Pfaffenberger (1985).

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Seasonal dynamics of three lepidopteran stored grain pests in Slovenia

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Abstract

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.

Keywords: Monitoring, Lepidoptera, Stored grain pest, Pheromones, Slovenia

1. Introduction

Stored-product pests are an important group of pests, and are becoming even more so because of increasingly intensive transportation of plant material and food, together with constantly increasing human population and need for larger and larger storage structures. These structures present a favorable environment for many pest species (Hamel, 2007; Ryne and Bensch, 2008). In Slovenia, storage insect pests have not been given a systematic scientific investigation, but during the last several years some primary and secondary coleopterian and lepidopterian pests have been causing more and more trouble. For an effective control of the pests in question, one first has to know about their bionomics since only adequate knowledge about their ways of life and development in the areas where they cause damage enables the optimal choice of treatment type and timing.

Monitoring of stored lepidopterian pests can be performed using pheromone traps, which have the advantage of species selectivity and can be an economic and effective way of insect determination (Stockel, 1976; Zakladnoi and Saul'kin, 2008). The purpose of our investigation was to study seasonal dynamics of three species of stored pests on three locations close to Ljubljana in order to get information needed to develop a strategy for their control in the warehouses of grain and grain products. Before the investigation we had the information about Mediterranean flour moth (*Ephestia kuehniella* Zeller, Phycitidae), Indianmeal moth (*Plodia interpunctella* [Hübner], Phycitidae) and Angoumois grain moth (*Sitotroga cerealella* Olivier, Gelechiidae) being present in Slovenia, but we had no data on their seasonal dynamics.

2. Materials and methods

In 2004-2006, pheromone traps (VARL+ type, (Csal♀m♂N® Budapest, Hungary) were used to monitor the occurrence of Angoumois grain moth, Indianmeal moth and Mediterranean flour moth.. The pheromone traps were set from March to December. Angoumois grain moth males were monitored during 2005 and 2006 in Obrije, and Indianmeal moth and Mediterranean flour moth males were monitored during 2004 and 2005 in Želimlje and Jable and during 2004-2006 in Obrije.

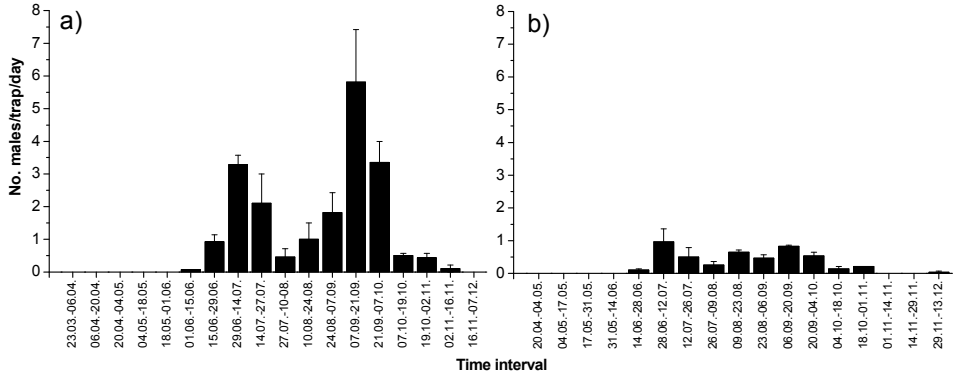

Figure 1 Number of *Sitotroga cerealella* males caught by pheromone traps in Obrije in 2005 (a) and 2006 (b).

In Želimlje, the lepidopterian pests were monitored using two traps hung under the ceiling of a maize open air storage (part of a barn). It was used for storage of maize (cobs) from harvest (the end of September) till the end of July. In the lower part (under this maize open air storage) maize that was ground into flour was kept. In Obrije, an organic farm was monitored using four traps, three in the storage room and one in the mill. In Jable, the traps were placed in the Agricultural Centre, with one trap in the grain storage, a second one in the mill, and two outside the building (in front of the storage). At none of these locations were treatments against stored pests performed.

For Indianmeal moth males and Mediterranean flour moth males the same pheromone lure was used since the manufacturer does not offer a specific pheromone for each species. Following the manufacturer's instructions, the pheromone lures were changed monthly. They were checked in 7-day intervals (Želimlje) or 14-day intervals (Obrije and Jable). The trapped males were stored in the lab at room temperature until identification. The determination was carried out using an Olympus SZ30 (manufacturer: Olympus Europa Gmbh, Hamburg, Germany) stereomicroscope (magnification about 10 times). The number of the trapped moths/day was calculated as the intervals were not the same for all the locations.

3. Results and discussion

In 2004, the first males of Angoumois grain moth were caught in the first half of June, while more substantial numbers (more than 2 males/trap/day) were observed in Obrije in the first half of July. This period may correspond with the peak of first generation of the year. As the study went on, a second peak was observed in the midst of September (6 males/trap/day), which could indicate a second generation. The adults were active till the first half of November. In 2005, the pest was less numerous and the first specimens were found in the traps during the second half of June. As in 2004, two peaks in trap capture were observed, the peaks being 1 male/trap/day at the beginning of July and in the midst of September.

The males of Indianmeal moth were most numerous in Obrije during the three years of the study. The results suggest two peaks of flight activity, perhaps correlated with generations, of this moth, with the first being rather more numerous. The first peak occurred during the second half of July (7-9 males/trap/day). In all three years, the second peak was in the middle of September, with captures of 2 males/trap/day. In Jable, the pest also showed two capture peaks as in Obrije, but captures were less numerous. This can be explained by the fact that two traps were set outside the storage, were only single specimens were captured. The first peak in captures at this location was during the second half of July, and captures were five times more numerous in 2004 (4 males/trap/day) compared to the next year. The second peak in captures was less numerous and appeared at the end of August and in September. In Želimlje, captures of Indianmeal moth were the least numerous, probably because monitoring was in a non-protected open air storage, where maizecobs were stored. There was never more than 1 male/trap/day caught at this location, and adults were active from the beginning of June till the end of

September (both years). These results are opposite to reports of Campbell and Arbogast (2004), who found the greater activity of Indianmeal moth outside wheat flour meal compared to inside of them.

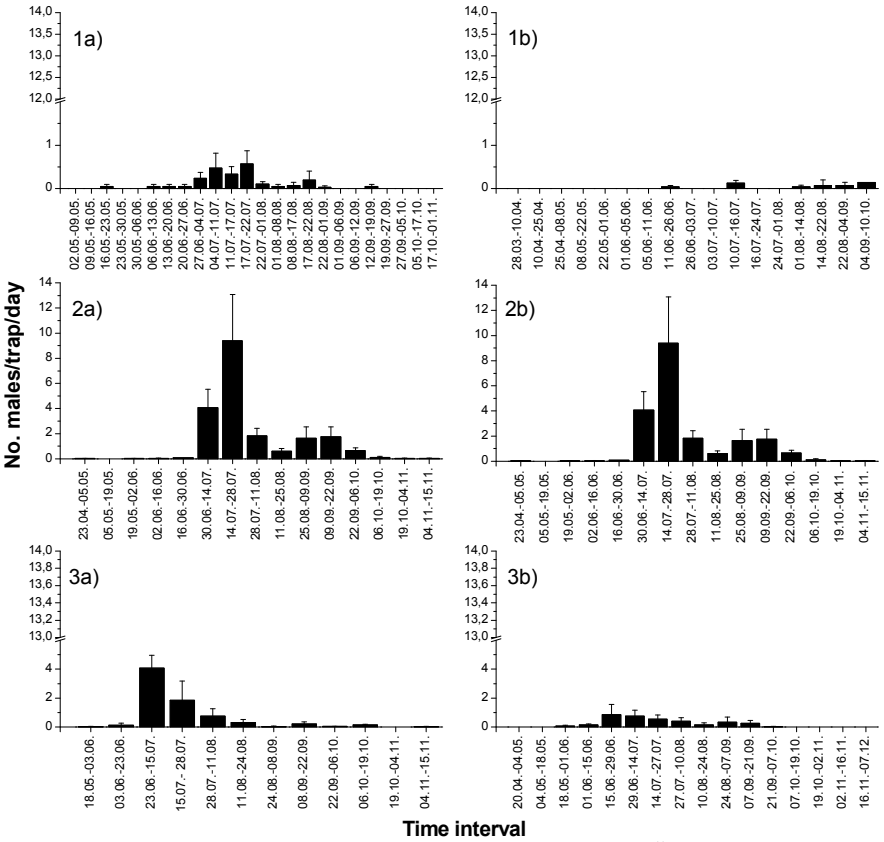

Figure 2 Number of *Plodia interpunctella* males caught by pheromone traps in Želimlje (1), Obrije (2) and Jable (3) in 2004 (a) and 2005 (b).

Mediterranean flour moth captures in the pheromone traps were more numerous than captures of the other species. The captures of this pest, which like the other two species, also appeared to have two peaks in capture, was most numerous in Želimlje, where the first peak was in the beginning of June (both years); 22 to 25 males/trap/day. Later in the year, the abundance of this moth decreased significantly, possibly also due to removal of the stored cobs from the previous season. Single males were found in the traps till the end of the monitoring in October. In closed storage rooms, In Obrije and Jable, captures of Mediterranean flour moth were less numerous, the peak being about 7 males/trap/day in Obrije in the second half of July. The males at these locations were spotted from the beginning of June until October.

The results of our investigation showing a typical two peaks in flight activity, allow the conclusion, that, on the average, the three species under investigation develop two generations under the conditions in Central Slovenia. The Mediterranean flour moth was the most numerous and in future deserves the most attention in developing suitable controlling strategies. In closed room storages and mills in Obrije and Jable the Indianmeal moth was the most numerous, which speaks for its preference for stable environmental conditions. Compared to some other European areas, where this pest develops 3 to 5 generations in closed room storages (Buchelos, 1998), it seems that the conditions in similar storages in Central Slovenia are less suitable for its development. In Obrije, Angoumois grain moth was quite numerous during the first year of study, which makes it another species deserving some attention in future. The two generations yearly which we witnessed in storage rooms during our investigation do

appear in Southern Italy till the wheat harvest, and that the pest continues to appear till September (Trematerra and Gentile, 2002). Compared with some warmer areas in Europe (Italy, Greece), in Slovenia the pests under investigation develop fewer generations and are for the time being a lesser problem for storing grain and grain products. However, the changing climate that we experienced during the last decades, can enable changes in bionomics and potential harm of many pests (Bergant et al., 2005), including stored pests (Estay et al., 2009), so we have every reason to focus also on these for the time being only potentially harmful insects.

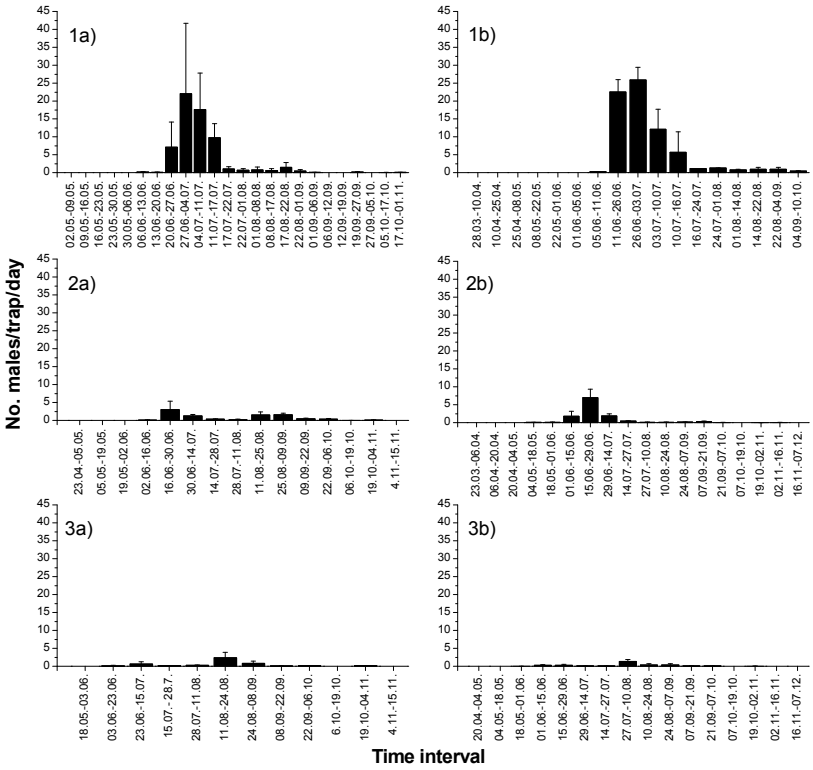

Figure 3 Number of *Ephestia kuehniella* males caught by pheromone traps in Želimlje (1), Obrije (2) and Jable (3) in 2004 (a) and 2005 (b).

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Partial characterization of glutathione S-transferases from different field populations of *Liposcelis bostrychophila*

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Abstract

Glutathione S-transferases (GSTs) from different field populations of *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) were purified by glutathione-agarose affinity chromatography and characterized subsequently by their Michaelis-Menten kinetics toward the artificial substrates 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH). The specific activity of the affinity of purified GST toward CDNB was highest in lab population, 2.7-fold higher than that of Guanghan population with the lowest value observed. GSTs of lab population exhibited higher apparent Michaelis-Menten constants (Km) and higher maximal velocity (Vmax) values than those of Jianyang and Guanghan populations, revealing that the latter two populations exhibited significantly higher affinities to the test substrates. Inhibition kinetics showed that all test compounds (ethacrynic acid, curcumin, diethyl maleate, bromosulfalein, and carbosulfan) possessed significant inhibitory effects on GSTs. Curcumin appeared to be the most effective inhibitor. Compared to the other compounds, diethyl maleate and carbosulfan exhibited their I50s (the concentration required to inhibit 50% of GSTs activity) at higher concentrations.

Keywords: GSTs, purification, Psocids, Xenobiotic compounds, Field populations

1. Introduction

Liposcelis bostrychophila Badonnel is a prevalent insect pest in large grain depots in China. Routine fumigations of warehouses and storage facilities with methyl bromide have failed to control the pest (Ho and Winks, 1995). Moreover, the serious damage by psocids in Australia has increased in recent years, mostly due to the failure of almost all currently registered grain protectants against these pests (Nayak and Daglish, 2006, 2007).

Glutathione S-transferases (GSTs; E.C. 2.5.1.18) are a large family of multifunctional enzymes found ubiquitously in aerobic organisms (Clark et al., 1984; Ranson et al., 2001). The majority of studies on insect GSTs have focused on their role in detoxifying xenobiotic compounds, in particular insecticides and plant allelochemicals and, more recently, their role in mediating oxidative stress responses (Fournier et al., 1992; Ranson et al., 2001; Vontas et al., 2001, 2002; Sawicki et al., 2003). Increased GST activity has been detected in strains of insects resistant to organophosphates and organochlorines (Fournier et al., 1992), and this enzyme family has recently been implicated in resistance to pyrethroid insecticides (Kostaropoulos et al., 2001; Vontas et al., 2001). What's more, GST-based resistance to insecticides was described to be facilitated by the increase in the level of expression of one or more GSTs.

Currently, research on the psocid pest genus *Liposcelis continues* to increase, but knowledge of the pest remains limited compared to other stored-products insect pests. Very recently, GSTs from *L. bostrychophila* and *L. paeta* were purified and their partial characterizations analyzed from the perspective of resistance of different strains (Dou et al., 2009; Wu et al., 2009). In this paper, the partial characterization of purified GSTs were investigated and further comparatively analyzed from different field populations of *L. bostrychophila*.

2. Materials and methods

2.1. Chemicals and insecticides

Reduced glutathione (GSH, Sigma), 1-chloro-2, 4-dinitrobenzene (CDNB, Shanghai Chemical Ltd.), and other biochemical reagents were of analytical grade. The xenobiotic compounds used for the inhibition bioassays were: ethacrynic acid, 94% curcumin, 97% diethyl maleate (all above from Sigma, St. Louis, MO, USA), bromosulfalein (Dow AgroSciences LLC, USA), and 86% carbosulfan (Zhejiang Chemical Co. Ltd., China).

2.2. Test insects

Three field populations of L. paeta were collected from wheat warehouses in Jianyang and Guanghan of Sichuan Province, China in 2004, and Beibei of Chongqing Municipality, China in 1990. The insects were reared on an artificial diet consisting of whole wheat flour, skimmed milk, and yeast powder (10:1:1) in a temperature controlled room at 27 ± 1 ºC, r.h. 75%-80% and a scotoperiod of 24 h. Stock colonies were never exposed to blended gas or insecticides. All experiments were conducted under the conditions described above with three to five day-old adult females of all populations.

2.3. Purification of enzyme

GSTs from different field populations of *L. bostrychophila* were purified employing glutathione-agarose affinity chromatography as described by Fournier et al. (1992).

Psocid samples of 50 mg were homogenized manually on ice in 1.5 m Lsodium phosphate buffer (20 mM, pH 7.2). The homogenate was centrifuged for 5 min at 5000 g and 4ºC. The pellet was discarded and the supernatant was again centrifuged for 15 min at 17,500 g and 4ºC. Finally, the supernatant was filtered through a 0.45 µm Millex-HV filter (Millipore, USA) and loaded on a GSH-reduced agarose gel column (GE Healthcare, USA) with a bed volume of 2 mL. The column was equilibrated with 20 mM sodium phosphate buffer, pH 7.2 and washed with the same buffer. The bound GST enzyme was eluted with 50 mM Tris-HCl buffer, pH 8.0, containing 5 mM reduced GSH. The collected fractions (200 µLeach) containing GST activity were pooled for further analysis.

2.4. Enzyme activity and kinetics

GSTs activities were determined using 1-chloro-2, 4-dinitrobenzene (CDNB) and reduced GSH as substrates according to Habig et al. (1974) with slight modifications. The total reaction volume was 250 µL, consisting of 50 µLeach enzyme solution, CDNB (1% ethanol (v/v) included) and GSH in Tris-HCl buffer, giving final concentrations of 0.24 mM and 2.4 mM of CDNB and GSH, respectively. The non-enzymatic reaction of CDNB with GSH measured without enzyme solution served as control. The change in absorbance was measured continuously for 5 min at 340 nm and 37°C in a Thermomax kinetic microplate reader. Changes in absorbance per minute were converted into nmol CDNB conjugated/min/mg protein using the extinction coefficient of the resulting 2, 4-dinitrophenyl-glutathione: ϵ340nm = 9.6 mM /cm (Habig et al., 1974). Protein concentrations of the enzyme samples were determined at 595 nm and bovine serum albumin (BSA) as standard (Bradford 1976).

Values of the apparent Michaelis-Menten constant (Km) and maximal velocity (Vmax) of purified GSTs from L. bostrychophila were determined for CDNB and GSH, respectively. The activity was recorded toward a range of concentrations of CDNB (0.08-0.96 mM) or GSH (0.8-9.6 mM), while the concentration of the other substrate was kept constant at 2.4 mM or 0.24 mM of GSH or CDNB, respectively. Km and Vmax values were calculated by SPSS 10.0 (SPSS, Inc., USA) using the Michanelis-Menten equation.

2.5. Inhibition studies

The inhibition studies were performed using the standard GST assay conditions but in the absence and presence of various concentrations of inhibitors. Stock solutions of the inhibitors ethacrynic acid, bromosulfalein, curcumin, diethyl maleate, and carbosulfan were prepared in ethanol and diluted with Tris-HCl (50 mM, pH 7.5), thus the highest ethanol concentration was 1% in the test solutions. Twenty five µLof the enzyme source and 25 µL inhibitor solutions with appropriate concentrations were firstly incubated for 5 min at 25oC and then added to the substrate mixture as described above.

3. Results

3.1. Purification of GSTs from different field populations of L. bostrychophila

The chromatography elution profile from GSH-affinity chromatography column was shown in Figure 1. Totally, first 8 tubes (1 mL per tube) were collected and assayed. Both the fourth tubes exhibited highest specific activity of GSTs in Jianyang and Guanghan populations. For lab population, the highest activity of GSTs was recorded in fifth tube. Table 1 presents the results of the purified GSTs activities from different populations. The specific activity of GSTs in lab population was 1.21 μmol min/mg, 2.7-fold higher than that of Guanghan population (lowest value was recorded, 0.47 μmol/min/mg).

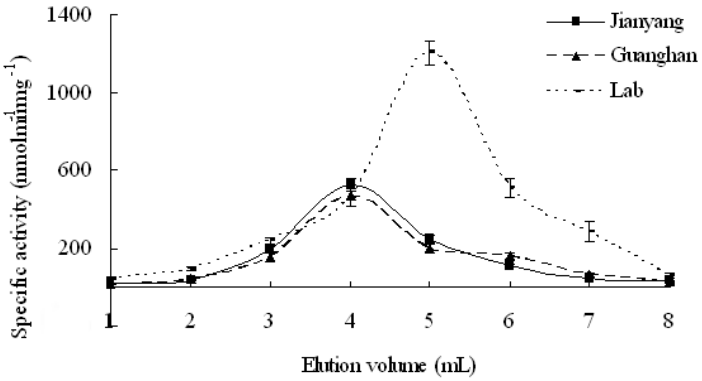

Figure. 1 The elution profile for GSTs activities from different field populations of *Liposcelis bostrychophila*. Each value represents the mean ± SE of three independent experiments.

Table 1 Activity comparison of GSTs purified from different field populations of Liposcelis Bostrychophila.

Population	Total protein (μg)	Total activity (nmol/min)	Specific activity (μmol/min/mg)
Jianyang	6.81 ± 0.37 a	3.57 ± 0.12 a	0.53 ± 0.04 a
Guanghan	7.08 ± 0.37 a	3.33 ± 0.13 a	0.47 ± 0.02 a
Lab	7.94 ± 0.30 b	9.59 ± 0.20 b	1.21 ± 0.06 b

Note: Each value represents the mean (M ± SE) of three replications. Means within the same column followed by different letters are significantly different at $P < 0.05$.

3.2. Kinetics of GSTs

GSTs difference among the different populations was also observed for kinetic parameters (Table 2). The statistical analyses revealed that GSTs in lab population showed a lower affinity to the substrate CDNB or GSH (i.e. higher Km values, 0.33 mM or 6.53 mM, respectively). Meanwhile, GSTs in lab population have higher catalytic activities for CDNB and GSH (i.e. higher Vmax values, 2.37 nmol/min and 3.21 nmol/ min).

Table 2 Kinetic properties of GSTs from different field populations of *Liposcelis bostrychophila*.

	GSH		CDNB	
Population	Km (mM)	Vmax (μmol/ min/mg pro)	Km (μM)	Vmax (μmol/min/ mg pro)
Jianyang	3.4 ± 0.1 a	2.17 ± 0.24 b	289 ± 21 b	1.19 ± 0.12 a
Guanghan	3.5 ± 0.2 a	1.30 ± 0.12 a	138 ± 19 a	1.15 ± 0.13 a
Lab	6.5 ± 0.2 b	3.21 ± 0.29 c	331 ± 13 c	2.37 ± 0.14 b

Note: Each value represents the mean (M ± SE) of three replications. Means within the same column followed by different letters are significantly different at $P < 0.05$.

3.3. In vitro inhibition of GSTs

Based on their I50s (the concentration required to inhibit 50% of GSTs activity), the efficiencies of the tested inhibitors were compared and the corresponding I50s of the tested compounds were listed in Table 3. All compounds exhibited good inhibition effects on GSTs in vitro, and curcumin was considered to be most effective inhibitor. Among the different field populations, the lab population seemed least sensitive to all the test compounds (highest I50s were observed). For Jianyang and Guanghan populations, no significant differences were seen.

Table 3 The I50 inhibition constants of xenobiotic compounds on purified GSTs from different field populations of Liposcelis Bostrychophila.

	I50 (μM)		
Inhibitor	**Jianyang**	**Guanghan**	**Lab**
Ethacrynic acid	0.324 ± 0.057 a	0.278 ± 0.064 a	0.696 ± 0.131 b
Curcumin	0.0080 ± 0.0013 a	0.0073 ± 0.0011 a	0.0096 ± 0.0013 a
Diethyl maleate	2.28 ± 0.34 a	2.13 ± 0.31 a	3.33 ± 0.46 b
Bromosulfalein	0.225 ± 0.046 ab	0.167 ± 0.043 a	0.326 ± 0.057 b
Carbosulfan	6.32 ± 0.73 a	6.19 ± 0.34 a	9.05 ± 1.02 b

Note: Each value represented the mean (M ± SE) of three replications. Means within the same row followed by different letters are significantly different ($P < 0.05$).

4. Discussion

In the current study, the partial characterization of purified GSTs from different field populations of *L. bostrychophila* was compared. Important differences were observed when considering the kinetics assays. For both the tested substrates, the Lab population expressed higher Kms suggesting lower affinities of GSTs toward the substrates in this population.

For *Liposcelis bostrychophila* , a previous study on crude preparations of GSTs has revealed that there was some correlation between resistance development and the quantity of GSTs (Dou et al., 2006). Following GSTs purification and partial characterization analysis for L. bostrychophila and L. paeta, the relationship between GSTs level and resistance of different strains or field populations were further clarified (Dou et al., 2009; Wu et al., 2009). The current results demonstrate that the two populations from Sichuan province possess less specific activities while kinetics analysis reveales that these two populations exhibited significantly higher affinities to the test substrates. GSTs acitivity difference between the two populations may result from local differences insecticide practice.

The CDNB conjugating activity of psocids GSTs was shown to be inhibited by several xenobiotic compounds tested. Among the compounds, curcumin expressed the most effective inhibitory effects with its I50 values at sub-micromolar concentrations. In Anopheles dirus, bromosulfophthalein exhibited its I50 values of 0.805 μM (Prapanthadara et al., 1996). Similarly, some insect GSTs exhibited I50 values for ethacrynic acid at sub-micromolar concentrations, i.e., *Nilaparvata lugens* (40 nM), *Blattella germanica* (350 nM), and *Spodoptera frugiperda* (150nM) (Yu and Huang, 2000; Vontas et al., 2002; Yu, 2002).

Glutathione S-transferases are a large family of multifunctional enzymes involved in the detoxification of a wide range of xenobiotics including insecticides (Enayati et al. 2005). In insect species, many GST enzymes are differentially regulated in response to various inducers or environmental signals or in a tissue- or developmental-specific manner. The current study presented some basic biochemical information of GSTs from different field populations of the psocid, and surely will help in understanding the exact role in detoxification of the various GST isoenzymes and to understand the evolutionary aspects of detoxification related to each insect species.

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Development, relative retention, and fecundity of *Tribolium castaneum* (Herbst) on different starches

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Abstract

The development, relative retention, and fecundity of the red flour beetle, *Tribolium castaneum* (Herbst), on six different types of starches, flour, and flour plus yeast were investigated in the laboratory. The size of 90% of particles among the starches was below 15 to 58 µm, while that of the flour was below 133 µm. Larval length, head capsule width, and weight gain of *T. castaneum* were measured by rearing larvae on starches, flour, and flour plus 5% (by wt) Brewer's yeast diet for 30 d at 28oC and 65% r.h. Larvae reared on flour or flour plus yeast developed normally and showed better survival compared to those reared on starches. Larvae on starches failed to develop beyond second and rarely third instars. Adults of *T. castaneum* did not show any preference to flour over starches in dual-choice tests. *Tribolium castaneum* laid less than 3 eggs/female over a 15 d period on all starches, but laid 97 and 109 eggs/female on flour and flour plus yeast diet, respectively. These initial studies suggest that starches are poor substrates for development, and currently experiments are in progress to improve larval survival and development by incorporating specific nutrients in starches. Starches were as attractive as flour to adults; however, starches do not appear to be a suitable medium for egg-laying. Preliminary experiments by moving adults between starches and flour and vice versa showed that feeding on suitable diets is essential for eliciting oviposition. Although preliminary, these interesting findings suggest that starches may have potential in managing development and reproduction of *T. castaneum*-a pest that is common and severe in food-processing facilities.

Keywords: Nutritional control, *Tribolium castaneum*, Resistant starches, Development, Oviposition, Pest management

1. Introduction

Natural starch is an abundant nutrient carbohydrate, (C6H10O5)n, found mainly in the seeds, fruits, tubers, roots, and stem pith of plants, notably in corn, potatoes, wheat, and rice, and varying widely in appearance according to source but commonly prepared as a white amorphous tasteless powder. Natural starch usually consists of 25% amylose (linear starch polymers) and 75% amylopectin (branched starch polymers). Resistant starches (RS) include starches that are resistant to enzymatic hydrolysis in the small intestines of humans. RS are classified as RS1, RS2, RS3 and RS4. RS1 refers to resistant starch that is physically encased by whole grains. RS2 is a granular resistant starch. RS3 refers to non-granular, retrograded or crystalline resistant starch, and RS4 is a manufactured resistant starch. Whole grains can deliver RS1, green bananas deliver RS2, and RS3 is found in ready-to-eat breakfast cereals, bread crusts, cooked and cooled potatoes and cooked and cooled pasta. RS4 is manufactured from various sources, including wheat, potato and tapioca, and is available from a variety of ingredient suppliers.

Applebaum (1966) used raw and cooked potato starch as the carbohydrate source and evaluated larval development of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleptera: Tenebrionidae), an economically important pest associated with stored grain and food-processing facilities (Hagstrum and Subramanyam, 2009). Applebaum (1966) found that larval mortality reached 100% when larvae fed raw potato starch, while it was 72% on cooked potato starch. Larval weight was 0.44 mg on cooked potato starch compared to 1.84 mg on rice starch (control). Pupation rate was 79% for T. castaneum larvae fed potato starch and it was 90% on corn starch (Pant and Kamlesh, 1965). Applebaum and Konijn (1965) found that larval weight and percentage of larval survival was similar on diet containing rice starch, corn starch, and wheat starch as the carbohydrate source. Baker et al. (1992) observed in vitro and in vivo

digestion of purified wheat starch granules by *T. castaneum* larvae and found a similar digestion pattern– scattered attack initially on the surface and then penetration of the granules by enzyme.

The present investigation was designed to determine development, preference, and oviposition of *T. castaneum* on six different starches with varying amylose content. The goal is to explore the value of starches as a nutritional control method. This method was proposed by Pratt et al. (1972) and involves adding non-nutrients or inert substances to food to render it unsuitable as a source of nutrients.

2. Materials and methods

2.1. Flour and starches

The six types of starches used included waxy corn starch (2% amylose), corn starch (25% amylose), wheat starch (25% amylose), cross-linked wheat starch (70% total dietary fiber), high amylose corn starch (70% amylose), and potato starch (12-20% amylose). In addition to these starches, wheat flour purchased from Heartland Mills, Marienthal, KS, U.S.A., was also used as the control treatment. The particle sizes of all starches and flour were analyzed using a Malvern Mastersizer 2000 by The NanoScale Corporation, located in Manhattan, KS, U.S.A.

2.2. Insects

Cultures of *T. castaneum* have been maintained in the Department of Grain Science and Industry's Stored-Product Entomology Research and Education Laboratory since 1999 on wheat flour plus 5% (by wt) of Brewer's yeast diet at 28oC and 65% r.h. in growth chambers. To collect *T. castaneum* eggs, 50 adults of mixed ages and sexes from cultures were placed on 20 g of flour in separate 150-mL round plastic containers with perforated lids. The flour used was previously sifted through a 250 µm opening sieve. The containers were closed with lids that had perforations which were covered with plastic mesh for ventilation and to prevent adult escape. The containers were held at the rearing conditions for 3 d after which the adults were removed and sifted over two sets of sieves. The top 840 µm sieve retained the adults, while allowing the flour and eggs to pass through. The eggs were retained on the 250 µm sieve.

One hundred eggs were placed in 9-cm glass Petri dishes and held at 28oC and 65% r.h. and examined daily to measure hatchability. There were 10 such dishes (n = 10 replications).

To obtain pupae for fecundity tests, after 3 d the adults were removed using the 850 µm sieve and the flour and eggs were placed back in the 150-mL plastic containers and held at 28oC and 65% r.h. After 30 d the contents were sifted using an 850 µm sieve and pupae retained on the surface of the sieve were collected. After sexing, male and female pupae were placed separately in 9-cm glass Petri dishes and held at 28oC and 65% r.h.

2.3. Procedures for measuring development

About 5 g of each starch, flour, or flour plus yeast diet were transferred separately into 30-mL plastic condiment cups. Fifty *T. castaneum* eggs were added into each cup. The cups were covered with plastic lids. Holes were made in the lids with a pin for ventilation. All cups were placed in a growth chamber at 28°C and 65% r.h. Three cups (n = 3 replications) were removed from the chamber every 3 d, and independent cups were sampled over time. Larvae were sifted using the 250 µm sieve to separate developing larvae. The lengths and head capsule widths of 10 larvae collected from each cup were measured. The lengths and head capsule widths of larvae from a cup were averaged to obtain a single value for that replication. In addition, the number of larvae surviving out of the total (50) was also recorded. To obtain larval weights, all larvae in all three cups were pooled and weighed on a Mettler® balance (Mettler-Toledo, Inc., Columbus, OH, U.S.A.).

2.4. Preference tests

Dual-choice tests were conducted in circular arenas following techniques modified from Subramanyam (1992). Each arena measured 30 cm in diameter and 8 cm in height. Arena floors were cutout from white foam display board into 30 cm diameter circles. Arena walls were made from white mat board and the interior wall of the arena was covered with plastic tapes to close any gaps between the floor-wall junctions. In each arena, 2.5 g of one of the six starches and 2.5 g of flour were placed in the north and south ends of the arena. These locations were randomly selected by a coin toss. Fifty unsexed adults of

T. castaneum were released at the center of the arena. All arenas were covered with round lids made from the same material used for floors.

Arenas were observed at 36, 48, 60 and 72 h after adult introduction to determine number of adults retained in starch or flour. Starch and flour were collected in 9-cm glass Petri dishes and adults in the starch or flour were counted. Each starch and flour comparison and the four observation periods were replicated three times. All tests were conducted at room conditions. Room temperature and humidity during tests were measured using HOBO® data loggers (Onset Computer Corporation, Bourne, MA, U.S.A.), and these environmental variables ranged from 21-27oC and 30-55% r.h.

2.5. Oviposition in starches and flour

Male and female pupae in Petri dishes were examined daily for adult emergence. A pair of adults emerging on the same day were paired and placed on 2.5 g of one of the six starches in 30-mL plastic condiment cups. The control treatments consisted of flour alone and flour plus yeast diet, and each was infested with a pair of adults. The flour was sifted using 840 and 250 µm sieves every 3 d to separate the adults and eggs as explained above. The pair of adults retained on 840 µm sieve was transferred to 2.5 g of fresh diet. The total number of eggs laid in diet were counted every 3 d for 15 d. This experiment was replicated 10 times.

2.6. Data analysis

Particle size data on percentage of particles (10, 50, or 90%) below a certain size among starches and flour were compared using one-way analysis of variance (ANOVA) and Ryan-Einot-Gabriel-Welsch (REGWQ) multiple comparison test (SAS Institute, 2002). Data (x) on adult retention in a starches or flour at each observation time were transformed to log (x + 1) scale and subjected to a paired t-test. Oviposition data were transformed to logarithmic scale and subjected to one-way ANOVA; treatment means were separated using REGWQ test. All statistical differences were considered significant at the $\alpha = 0.05$ level.

3. Results and discussion

Starch and flour particle size distributions are shown with 10, 50, and 90% percentages of particles below a certain size (Table 1). In general, the flour particles were bigger than that of the starches, and differences among flour and starches were significant ($P < 0.05$). Differences were also noted among the starches. Therefore, particle size may not be a limiting factor for consumption of starches by *T. castaneum* larvae.

Table 1 Particle size analysis of flour and starches.

Treatment	Percentage of particles below a certain size (in µm)a,b		
	10%	50%	90%
Flour	13.52 ± 0.12a	60.04 ± 0.02a	132.50 ± 0.39a
Potato starch	24.36 ± 0.04b	37.65 ± 0.04b	57.65 ± 0.04b
Wheat starch	11.81 ± 0.00c	17.88 ± 0.01c	26.74 ± 0.03c
Cross-linked wheat starch	10.69 ± 0.01d	16.98 ± 0.01d	26.44 ± 0.00c
Corn starch	9.14 ± 0.06e	13.32 ± 0.07e	19.21 ± 0.05d
Waxy corn starch	8.13 ± 0.07f	12.97 ± 0.06e	20.38 ± 0.03e
70% Amylose corn starch	5.52 ± 0.01g	9.15 ± 0.16f	14.76 ± 0.46f

a = Each mean is based on n = 2 replications; b = Means followed by different letters are significantly different ($P < 0.05$; REGWQ test).

The mean egg hatchability of *T. castaneum* eggs was 75.4 ± 1.1%. The observed hatchability was 16% less than that observed by Sokoloff (1974).

The development of *T. castaneum* larvae, as determined by larval length, head capsule width, and weight gain, showed that larvae developed normally on flour and flour plus yeast, with the development being better on the latter substrate. On starches, development of larvae was adversely affected, and larvae failed to develop beyond second instars. A few larvae became third instars but failed to molt beyond this stage. Additionally, survival on flour and flour plus yeast was far superior to survival on starches (Fig. 1). The

worst larval survival was on potato starch, followed by cross-linked wheat starch, and 70% amylose corn starch. Potato starch which belongs to RS2 type is resistant to enzyme digestion due to the enriched phosphorus content in large starch granules resulting in a compact structure with crystallinity (Englyst and Cummings, 1987). The cross-linked wheat starch and 70% amylose corn starch are resistant to enzyme digestion, because their tightly packed structure reduces enzyme access to the starch side chains (Woo and Seib, 2002). The larval development results are consistent with this finding. The adverse effects of starches on growth of *T. castaneum* larvae compared to flour or flour plus yeast diet suggest that the starches lack certain essential nutrients for normal development. What these constituents are at this point is unclear, but we speculate that it could be protein components which these starches lack.

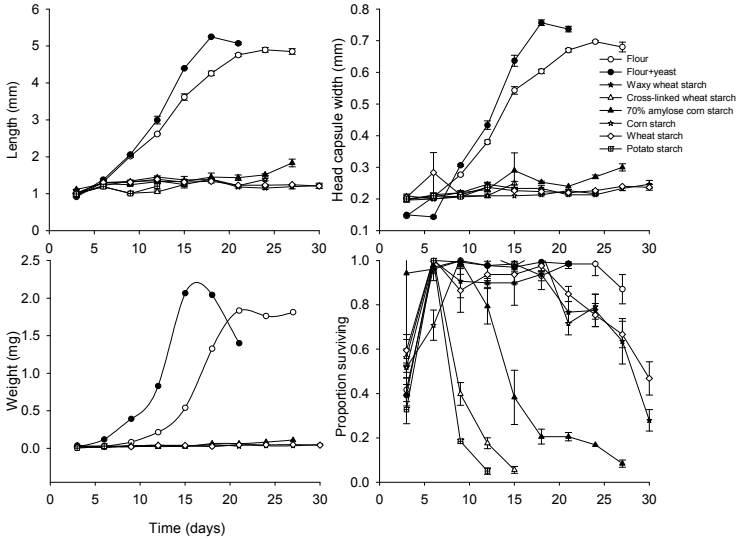

Figure 1 Development and survival of *T. castaneum* larvae on the six starches, flour, and flour plus yeast.

In arena tests, about 48-80% of the released adults were retained in the starch and/or flour substrates. There was no significant difference between the number of adults retained on each of the starches and flour ($P > 0.05$) at each observation time (t, among observation times = -3.67 – 1.79; df = 2; $P > 0.05$). Therefore, only data for 72 h is shown in Table 2. Adults of T. castaneum evaluate suitable media by visual, tactile, and chemical cues (Campbell and Runnion, 2003). Commercial starches are usually odor-free materials. However, lack of significant differences between starches and flour suggest that they are all equally attractive to *T. castaneum*.

Table 2 Relative retention of T. castaneum adults on a specific starch and flour in dual-choice testsa.

Time	Flour vs. starch/ starch type	No. adults retained in:		t (df=2)	P-value*
		Starch	Flour		
72 h	Potato starch	8.3 ± 1.2	16.7 ± 6.3	-1.86	0.20
	70% Amylose corn starch	12.0 ± 4.9	21.7 ± 9.4	-0.25	0.82
	Corn starch	14.0 ± 5.7	13.0 ± 1.0	-0.05	0.97
	Cross-linked wheat starch	15.3 ± 1.8	17.0 ± 5.6	-0.43	0.71
	Wheat starch	13.0 ± 4.7	15.3 ± 1.4	-0.90	0.46
	Waxy corn starch	10.0 ± 4.4	17.0 ± 4.5	-1.61	0.20

n = 3 replications; data (x) transformed to log10 (x) scale to normalize variances; a = Data for the other observation times were similar to data presented for 72 h; *All P-values are not significant ($P > 0.05$; paired t-test).

Very few eggs were laid on all starches (<3 eggs/female) during the 15 d test period compared with 97 to 109 on flour and flour plus yeast diet (Table 3). The low number of eggs laid suggests that triggers for egg laying are absent in starches. A simple test in the laboratory showed that *T. castaneum* adults placed

on a sieve above flour do not lay eggs and need to be in contact with the flour. This suggested that feeding may stimulate egg-laying. In additional tests not reported here, we observed that *T. castaneum* placed on flour and then transferred to starches after 3 d laid eggs. Similarly, when beetles that failed to lay eggs on starches after 3 d when placed on flour laid eggs during the next 3 d. Currently, tests are underway with different percentages of wheat gluten admixed with wheat starch to determine whether protein plays an important role in development, survival, and oviposition of *T. castaneum*.

Table 3 Oviposition of *T. castaneum* on flour plus yeast, flour, and starches over a 15-d period.

Treatment	No. eggs/female (mean ± SE)a
Flour + yeast	108.7 ± 5.7a
Flour	97.3 ± 6.9a
Potato starch	2.6 ± 0.5b
Cross linked wheat starch	1.0 ± 0.2c
Corn starch	1.3 ± 0.5c
Wheat starch	0.8 ± 0.2c
70% Amylose corn starch	0.6 ± 0.3c
Waxy corn starch	0.1 ± 0.1c

n = 10 replications, except for flour and waxy corn starch where there were 9 replications;
a = Means followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

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Mention of trade or proprietary names in this publication does not imply an endorsement by Kansas State University or the USDA.

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National investigations of stored grain arthropods in China

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Abstract

In the 4th national investigation of storage arthropods (2004 to 2005), 270 species of insects and arachnids were collected and identified (including 44 species of natural enemies). The stored grain insects belonged to 2 classes, 12 orders and 54 families, including many families in each order - Coleoptera (31), Lepidoptera (6), Hymenoptera (5), Blattaria (2), Hemiptera (2), Diptera (2), Corrodentia (1), Thysanura (1), Isoptera (1), Dermaptera (1). The arachnids were Chernetidae of Chelonethida and Urocteidae of Araneida. Of the 270 arthropod species, 56 were found in all four national investigations. Four species were new records on this occasion and for the first time the list of species includes some arachnids, although these are all predatory species not pests. The occurrence of some stored grain insects with potential threats and several major types of stored grain insects are noted.

Keywords: Investigation, Stored grain, Arthropods, China

1. Introduction

From 1955 to 2005, there have been four investigations of stored grain arthropods in China. The fourth investigation was lead by CSR (Chengdu Grain Storage Science and Research Institute), and included the Henan University of Technology, Guangdong Institute for Cereal Science Research and Anhui Grain Depot of Chinese Grains and Oils Group with support from the State Administration of Grain (SAG) (Yan Xiaoping et al., 2005, Liu Guolin et al., 1998, Shen Zonhghai et al., 2007). The fourth investigation demonstrated a greater variety of insects than previous surveys (Table 1). It can therefore be regarded as a new base line.

Table 1 Numbers of stored grain insect species and natural enemies observed in four national stored grain insect investigations in China.

Sort	First* (1955-1958)	Second (1974-1975)	Third (1980-1982)	Fourth (2004-2005)
Stored grain insects	109	126	161	226
Natural enemy of stored grain insects	2	6	10	44
Total	**111**	**132**	**171**	**270**

Note: In 1955-1958, in some reports the first investigation on insects had been confirmed in 1955, the second investigation on insects had been confirmed in 1957 and the third investigation on insects had been confirmed from 1956 to 1958. In order to avoid the overlapping of investigation time and to facilitate the work in future, the investigations on stored grain insects from 1955 to 1958 is viewed as the first large scale investigation in this article.

2. Lists of Stored-Grain Insects in China

2.1. Stored-grain insects in China (excluding natural enemies)

Insecta

Coleoptera
Curculionidae
(1) *Sitophilus zeamais* (Motschulsky)
(2) *Sitophilus oryzae* (L.)
(3) *Sitophilus granarius* (L.)
(4)*Caulophilus oryzae* (Gyllenhal)
(5) *Rhyncolus chinensis* Voss
(6) *Euphryum rufum* (Broun)
Cyladidae
(7) *Cylas formicarius* F.
(122) *Thylodrias contractus* Motschulsky
(123) *Orphinus fulvipes*(Guerin-Meneville)Thoractidae
(124) *Thorictodes heydeni* Reitter
Ipidae
(125) *Coccotrypes dactyliperda*(F.)
Lathridiidae
(126) *Lathridius minutus* (L.)
(127) *Migneauxia orientalis* Reitter
(128) *Enicmus histrio* Joy et Tomlin
(129) *Cartodere argus* Reitter

Insecta

(8) *Araecerus fasciculatus* (Degeer)
Lyctidae
(9) *Lyctus linearis* (Goeze)
(10) *Lyctus brunneus* (Stephens)
(11) *Lyctus sinensis* Lesne
(12) *Lyctus* sp. (not denominated)
(13) *Lyctus* sp. (not denominated)
(14) *Lyctoxylon japonum* Reitter
(15) *Minthea rugicollis* (Walker)
Monotomidae
(16) *Montoma picipes* Herbst
(17) *Montoma bicolor* Villa
Bostrychidae
(18) *Rhyzopertha dominica* (F.)
(19) *Dinoderus minutus* (F.)
(20) *Dinoderus japonicus* Lesne
(21) *Sinoxylon anale* Lesne
(22) *Sinoxylon japonicum* Lesne
(23) *Bostrychus capucinus* (L.)
(24) *Bostrychus capucinus* var. rubrirenttis Zouf
Ostomatidae
(25) *Tenebroides mauritanicus* (L.)
(26) *Lophocateres pusillus* (Klug)
Silvanidae
(27) *Oryzaephilus surinamensis* (L.)
(28) *Oryzaephilus mercator* (Fauvel)
(29) *Monotoma picipes* Herbst
(30) *Ahasverus advena* (Waltl)
(31) *Silvanus bidentatus* (F.)
(32) *Silvanoprus cephalotes* (Reitter)
(33) *Silvanoprus angusticollis* (Reitter)
(34)*Silvanoprus scuticollis* (Walker)
Cucujidae
(35)*Cryptolestes pusillus* (Schönherr)
(36)*Cryptolestes ferrugineus* (Stephens)
(37)*Cryptolestes turcicus* (Grouvelle)
Tenebrionidae
(38) *Tribolium castaneum* (Herbst)
(39) *Tribolium confusum* Jacquelin du Val
(40) *Tribolium madens* (Charpentier)
(41) *Tenebrio obscurus* F.
(42) *Tenebrio molitor* L.
(43) *Palorus cerylonoides* (Pascoe)
(44) *Palorus beesoni* Blair
(45) *Palorus ratzeburgi* (Wissmann)
(46) *Palorus subdepressus* (Wollaston)
(47) *Gnathocerus cornutus* (F.)
(48) *Latheticus oryzae* Waterhouse
(49) *Coelopalorus foveicollis* (Blair)
(50) *Alphitobius diaperinus* (Panzer)
(51) *Alphitobius laevigatus* (F.)
(52) *Martianus dermestoides* Chevrolat
(53) *Alphitophagus bifasciatus* Say
(54) *Microdera elegans* Reitter
(55) *Microcrypticus scriptum* (Lewis)
(56) *Crypticus latiusculus* Menetries
(57) *Opatrum subaratum* Faldermann
(58) *Mesomorphus villiger* Blanchard
(59)*Gonocephalum reticulatum* Motschulsky
(60)*Opatrum sabulosum* (L.)
(61)*Blaps rugosa* Gebler
(62)*Blaps japonensis* Marseul
(63)*Lyprops sinensis* Marseul
(64)*Leptodes chinensis* Kaszab
Nitidulidae
(65)*Carpophilus dimidiatus* (F.)
(130) *Cartodere constricta* (Gyllenhal)
(131) *Dienerellaruficollis* (Marsham)
(132) *Dienerella beloni* Reitter
(133) *Dienerella costulata* (Reitter)
(134) *Dienerella costipennis* (Reitter)
(135) *Dienerella filiformis* (Gyllenhal)
(136) *Corticaria japonica* Reitter
(137) *Thes bergrothi* (Reitter)
(138) *Coninomus* sp.
Merophyssidae
(139) *Holoparamecus depressus* Curtis
(140) *Holoparamecus ellipticus* Wollaston
(141) *Holoparamecus signatus* Wollaston
(142) *Holoparamecus* sp.
Mycetophagidae
(143) *Typhaea stercorea* (L.)
(144) *Mycetophagus hillerianus* Reitter
(145) *Mycetophagus antennatus* (Reitter)
(146) *Mycetophagus quadriquttatus* Müller
Biphyllidae
(147) *Cryptophilus integer* (Heer)
(148) *Cryptophilus obliteratus* Reitter
Erotylidae
(149) *Dacne japonica* Crotch
Ciidae
(150) *Cis mikagensis* Nobuchi & Wada
(151) *Ennearthron* sp.
Cerylonidae
(152) *Murmidius ovalis* (Beck)
(153) *Murmidius stoicus* Hinton
(154) *Murmidius* sp.
Histeridae
(155) *Carcinops pumilio* (Erichson)
(156) *Carcinops mayeti* Marseul
(157) *Saprinus tenuistrius* Marseul
(158) *Dendrophillus xavieri* Marseul
(159) *Atholus pirithous* (Marseul)
Cleridae
(160) *Necrobia violacea* (L.)
(161) *Necrobia rufipes* (Degeer)
(162) *Necrobia ruficollis* (F.)
(163) *Tarsostenus univittatus* (Rossi)
(164) *Thaneroclerus buquet* Lefebvre
(165) *Tilloedea notata* (Klug)
Bruchidae
(166) *Callosobruchus chinensis* (L.)
(167) *Callosobruchus maculatus* (F.)
(168) *Callosobruchus* sp. (not denominated)
(169) *Callosobruchus* sp. (not denominated)
(170) *Callosobruchus* sp. (not denominated)
(171) *Bruchus rufimanus* Boheman
(172) *Bruchus pisorum* (L.)
(173) *Bruchidiusdorsalis* (Fahraeus)
(174) *Caryedon serratus* (Olivier)
(175) *Spesmophagus sericeus* (Geoffrey)
Cryptophagidae
(176)*Atomaria lewisi* Reitter
(177) *Cryptophagus dentatus* (Herbst)
(178) *Cryptophagus acutangulus* Gyllenhal
(179) *Cryptophagus cellaris* (Scopoli)
(180) *Cryptophagus affinis* Sturm
(181) *Cryptophagus quadrimaculatus* Reitter
(182) *Cryptophagus pilosus* Gyllenhal
(183) *Cryptophagus distinguendus* Sturm
(184) *Cryptophagus scutellatus* Newman
(185) *Cryptophagus badius* Sturm

Insecta

(66)*Carpophilus mutilates* Erichson
(67)*Carpophilus obsoletus* Erichson
(68)*Carpophilus flavipes* Murray
(69)*Carpophilus pilosellus* Motschulshy
(70)*Carpophilus marginellus* Motschulsky
(71)*Carpophilus delkeskampi* Hisamatsu
(72) *Carpophilus davidsoni* Dobson
(73) *Carpophilus* sp.
(74) *Carpophilus* sp.
(75) *Haptoncus luzonensis* Gillogly
(76) *Nitidula bipunctata* L.
(77) *Nitidula carnaria* (Schaller)
(78) *Urophorus humeralis* (F.)
(79) *Omosita colon* (L.)
Ptinidae
(80) *Ptinus japonicus* (Reitter)
(81) *Ptinus tectus* Boieldieu
(82) *Mezioniptus impressicollis* Pic
(83)*Niptus holoeucus* (Faldermann)
(84)*Pseudeurostus hilleri* (Reitter)
(85)*Mezium affine* Boieldieu
(86)*Gibbium psylloides* (Czenpinski)
(87)*Gibbium aequinoctiale* Boieldieu
(88)*Tipnus unicoler* Piller & Mitterpacher
Anobiidae
(89) *Stegobium paniceum* (L.)
(90) *Lasioderma serricorne* (F.)
(91) *Ptilineurus marmoratus* (Reitter)
(92) *Nicobium castaneum* (Olivier)
(93) *Falsogastrallus sauteri* Pic
(94) *Ptilinus fuscus* Geoffrey
(95) *Mizodoractoma sp.* (not denominated)
(96) *Nicobium* sp.
Dermestidae
(97) *Dermestes maculatus* Degeer
(98) *Dermestes frischii* Kugelann
(99) *Dermestes undulatus* Brahm
(100) *Dermestes tessellatocollis* Motschulsky
(101) *Dermestes ater* Degeer
(102) *Dermestes ater domesticus* Germar
(103) *Dermesteslardarius* L.
(104) *Dermestes carnivorus* F.
(105) *Dermestes vorax* Motschulsky
(106) *Attagenus piceus* (Olivier)
(107) *Attagenus brunneus* Faldermann
(108) *Attagenus vagepictus* Fairmaire
(109) *Attagenus alfireii* Pic
(110) *Attagenus cyphonoides* Reitter
(111) *Attagenus unicolorjaponicus* Reitter
(112) *Attagenus undulates* (Motschulsky)
(113) *Attagenus pellio* L.
(114) *Attagenus unicolor simulans* Solskij
(115) *Attagenus augustatus* Ballion
(116) *Attagenus shaefferi* (Herbst)
(117) *Anthrenus verbasci* L.
(118) *Anthrenus scrophulariae* (L.)
(119) *Anthrenus nipponensis* Kalik et N. Ohbayashi
(120) *Evorinea hisamatsui* N. Ohbayashi
(121) *Trogoderma variabile* Ballion
(186)*Cryptophagus pseudoschmidti* Woodroff
(187) *Micrambe nigricollis* Reitter
Anthicidae
(188)*Anthicus floralis* (L.)
(189)*Anthicus* sp.
Cerambycidae
(190) *Purpuricenus temminckii* (Guerin-Meneville)
(191) *Trichoferus campestris*(Faldermann)
(192) *Purpuricenus* sp. (not denominated)
(193) *Purpuricenus* sp. (not denominated)
Lepidoptera
Pyralidae
(194) *Pyralis farinalis* L.
(195) *Pyralis manihotalis* Guenee
(196) *Pyralis* sp.
(197) *Pyralis lienigialis* Zeller
(198) *Aglossa dimidiata* Haworth
(199) *Aglossa pinguinalis* L.
(200) *Aglossa caprealis* Hübner
Phycitidae
(201) *Plodia interpunctella* (Hübner)
(202) *Ephestia kuehniella* (Zeller)
(203) *Ephestia cautella* (Walker)
(204) *Ephestia elutella* (Hübner)
Galleriidae
(205) *Aphomia gularis* (Zeller)
(206)*Corcyra cephalonica* (Stainton)
Gelechiidae
(207)*Sitotroga cerealella* (Olivier)
Tineidae
(208) *Tinea tugurialis* Meyrick
(209) *Tinea metonella* Pierce & Metcalfe
(210) *Tineola bisselliella* Hummel
(211) *Monopis monachella* Hübner
(212) *Homalopsycha agglutinata* Meyrick
(213) *Setomorpha rutella* Zeller
Oecophoridae
(214) *Anchonoma xeraula* Meyrick
Corrodentia
Liposcelidae
(215) *Liposcelis entomophilus* Enderlein
(216) *Liposcelis bostrychophillus* Badonnel
(217) *Liposcelis decolor* (Pearman)
(218) *Liposcelis* sp.
Thysanura
Lepismatidae
(219)*Ctenolepisma villosa* F.
Blattaria
Blattidae
(220) *Periplanetaamericana* L.
(221) *Periplaneta australasiae* (F.)
(222) *Periplaneta emarginata* Karny
(223) *Periplaneta japonica* Karny
Phyllodromiidae
(224) *Blattella germanica* L.
Isoptera
Rhinotermitidae
(225) *Coptotermes formosanus* Shiraki
Hymenoptera
Formicidae
(226) *Monomorium pharaonis* L.

2.2. Natural enemies of stored grain insects

Insecta

Hemiptera
Anthocoridae
(1) *Xylocoris flavipes* (Reutes)
(2) *Xylocoris* sp.
(3) *Xylocoris galactinus* (Fieber)
(4) *Xylocoris* sp.
Reduviidae
(5) *Peregrinator biannulipes* (Montrouzier& Signoret)
(6) *Myiophanes* sp.
Hymenoptera
Braconidae
(7) *Microbracon hebetor* Say
Pteromalidae
(8) *Lariophagus distinguendus* Förster
(9) *Aplastomorpha calandrae* Howard
(10) *Pteromalus puparum* (L.)
(11) *Pteromalus* sp.
Ichneumonidae
(12) *Venturia* sp.
Evaniidae
(13)*Evania appendigaster* L.
Coleoptera
Carabidae
(14) *Harpalus rufipes* De Geer
(15) *Harpalus amplicollis* Menetries
(16) *Lachnocrepis prolixa* Bates
(17) *Chlaenius micans* (F.)
(18) *Chlaenius virgulifer* Chaudoir
(19) *Chlaenius* sp. (not denominated)
(20) *Chlaenius* sp.(not denominated)
(21) *Bembidion pogonides* Bates
(22) *Bembidion niloticum* Dejean
(23)*Scarites terricola* Bonelli
(24) *Hololeius ceylonicus* Nietner
(25)*Pheropsophous jessoensis*Morawitz
(26) *Pheropsophous* sp. (not denominated)
(27) *Pheropsophous* sp. (not denominated)
Staphylinidae
(28) *Paederus fuscipes* Curtis
(29) *Philonthus nigriventris* Thomson
(30) *Leptacinus parumpunctatus* Gyllonhyl
(31) *Anotylus* sp.
(32) *Philonthus* sp.
(33) *Omaluim huomerale* Cameron
(34)*Falagria* sp.
Diptera
Scenopinidae
(35)*Scenopinus fenestralis* L.
Cecidomyiidae
(36) *Silvestrina tyrophagi* Domb
Dermaptera
Psalididae
(37) *Labidura japonica* de Haan
(38) *Anisolabisannlipes* (Lucas)
(39) *Anisolabis* sp.
Arachnida
Chelonethida
Chernetidae
(40) *Chelifer nodosus* Schrank
(41) *Chelifer cancroids* L.
(42) *Chelifer panzeri* Koch
(43) *Chelifer* sp.
Araneida
Urocteidae
(44)*Uroctea compactilis* Koch

3. Discussion

3.1. Variation in classes, orders, families, genera and species in all investigations

An analysis of the four investigations shows that the number of classes and orders of stored grain insects and their natural enemies increased gradually, as did families, genera and species (Table 2). It is of note that no arachnids were recorded in the earlier three investigations and on this occasion the only arachnids recorded were natural enemies.

Table 2 Variation in classes, orders, families, genera and species in the four national stored grain insect investigations in China.

First investigation	**Second investigation**	**Third investigation**	**Fourth investigation**
Insecta	**Insecta**	**Insecta**	**Insecta**
Coleoptera	Coleoptera	Coleoptera	Coleoptera
Lepidoptera	Lepidoptera	Lepidoptera	Lepidoptera
Corrodentia	Corrodentia	Corrodentia	Corrodentia
Thysanura	Thysanura	Thysanura	Thysanura
Blattaria	Blattaria	Blattaria	Blattaria
Isoptera	Isoptera	Isoptera	Isoptera
Hymenoptera	Hymenoptera	Hymenoptera	Hymenoptera
Diptera	Diptera	Diptera	Diptera
	Hemiptera	Hemiptera	Hemiptera
	Dermaptera	Dermaptera	Dermaptera
			Arachnide
			Chelonethida

First investigation Insecta	Second investigation Insecta	Third investigation Insecta	Fourth investigation Insecta
			Araneida
1 class	1 class	1 class	2 class
8 orders	10 orders	10 orders	12 class
33 families	41 families	52 families	54 families
78 genus	92 genus	106 genus	146 genus
111 species	132 species	171 species	270 species

3.2. Stored grain insects found in all 4 investigations

The four investigations had 56 species in common. These species may be consider to be the main stored grain insects in China and are listed as follows.

Insecta
Coleoptera
Curculionidae
(1) *Sitophilus oryzae* (L.)
(2) *Sitophilus granarius* (L.)
(3) *Rhyncolus chinensis* Voss
Cyladidae
(4) *Araecerus fasciculatus* (Degeer)
Lyctidae
(5) *Lyctus brunneus* (Stephens)
(6) *Lyctus sinensis* Lesne
(7) *Lyctoxylon japonum* Reitter
(8) *Minthea rugicollis* (Walker)
Bostrychidae
(9) *Rhyzopertha dominica* (F.)
(10) *Dinoderus minutus* (F.)
Ostomatidae
(11) *Tenebroides mauritanicus* (L.)
(12) *Lophocateres pusillus* (Klug)
Silvanidae
(13) *Oryzaephilus surinamensis* (L.)
Cucujidae
(14)*Cryptolestes pusillus* (Schönherr)
(15)*Cryptolestes ferrugineus* (Stephens)
(16)*Cryptolestes turcicus* (Grouvelle)
Tenebrionidae
(17) *Tribolium castaneum* (Herbst)
(18) *Tribolium confusum* J. du Val
(19) *Tenebrio obscurus* F.
(20) *Tenebrio molitor* L.
(21) *Palorus ratzeburgi* (Wissmann)
(22) *Gnathocerus cornutus* (F.)
(23) *Latheticus oryzae* Waterhouse
(24) *Coelopalorus foveicollis* (Blair)
(25) *Alphitobius laevigatus* (F.)
(26) *Alphitophagus bifasciatus* Say
(27) *Mesomorphus villiger* Blanchard
Nitidulidae
(28)*Carpophilus dimidiatus* (F.)
Ptinidae
(29) *Ptinus japonicus* (Reitter)
(30)*Niptus holoeucus* (Faldermann)
(31)*Pseudeurostus hilleri* (Reitter)
(32)*Gibbium aequinoctiale* Boieldieu
(33) *Stegobium paniceum* (L.)
(34) *Lasioderma serricorne* (F.)
(35) *Ptilineurus marmoratus* (Reitter)
Lathridiidae
(36) *Dienerellaruficollis* (Marsham)
(37) *Thes bergrothi* (Reitter)
Merophyssidae
(38) *Holoparamecus depressus* Curtis
(39) *Holoparamecus ellipticus* Wollaston
Mycetophagidae
(40) *Typhaea stercorea* (L.)
(41) *Mycetophagus hillerianus* Reitter
Cerylonidae
(42) *Murmidius ovalis* (Beck)
Bruchidae
(43) *Callosobruchus chinensis* (L.)
(44) *Bruchus rufimanus* Boheman
(45) *Bruchus pisorum* (L.)
(46) *Bruchidiusdorsalis* (Fahraeus)
Lepidoptera
Phycitidae
(47) *Plodia interpunctella* (Hübner)
(48) *Ephestia cautella* (Walker)
(49) *Ephestia elutella* (Hübner)
Galleriidae
(50) *Aphomia gularis* (Zeller)
Gelechiidae
(51)*Sitotroga cerealella* (Olivier)
Oecophoridae
(52) *Anchonoma xeraula* Meyrick
Corrodentia
Liposcelidae
(53) *Liposcelis bostrychophillus* Badonnel
Blattaria
Blattidae
(54) *Periplanetaamericana* L.
(55) *Periplaneta australasiae* (F.)
Hemiptera
Anthocoridae
(56) *Xylocoris galactinus* (Fieber)

3.3. New records of stored grain insects in China

The following species were recorded for the first time from grain stores in China - *Myiophanes* sp., *Chlaenius* sp. (not validated), *Chlaenius* sp.(not validated), *Mizodoractoma* sp. (not validated).

3.4. Stored grain insects with potential threats found in the fourth investigation

Stored grain insects found for the first time in the fourth investigation that present a potential threat are as follows:

- *Necrobia violacea* (L.) and *Necrobia rufipes* (Degeer) can damage silkworm cocoons and meat products such as hams. They can also be a problem in oil seeds.
- *Dinoderus japonicus* Lesne may infest bamboo but can also attack hard Chinese medicinal materials (lanceolata, ginseng and gastrodia) and can damage paddy and maize.
- *Nicobium castaneum* (Olivier) feeds on wooden structures, including wooden architecture, antique furniture, woodcarving (Buddha in temples) etc.

3.5. Several major types of stored grain insects

3.5.1. Sitophilus zeamais (Motschulsky)

During the first investigation there was no record of maize weevil (now considered the most important pest in Chinese grain stores). This is because the maize weevil and rice weevil were not recognized as separate species before the 1960s but instead as the large and small strains of the same species, *Sitophilus oryzae*. Species identification requires examination of the male or female genitalia.

3.5.2. Tenebrionidae

In the first and second investigation, 11 insects were recorded, including *Palorus depressus* (F.) *Blaps chinensis* Faldermann, *Cyphogenica funesta* Faldermann, *Leptodes sulcicollis* Reitter, which were absent in the fourth investigation. This may have resulted from inadequate investigation in Northwestern China.

3.5.3. Dermestidae

In all four investigations on stored grain insects, many dermestids were observed, although none was recorded in all four investigations. The reasons may be that all the investigations focused on stored grain insects while dermestids are also pests of oil seeds and of animal products (leather, furs, woolen cloth, animal bones, hams etc.)

3.5.4. Importation of quarantine species

China currently recognizes six quarantine species of storage pests, *Prostephanus truncatus* (Horn), *Trogoderma granarium* Everts, *Zabrotes subfasciatus* Boheman, *Callosobruchus analis* F., *Callosobruchus phaseoli* (Gyllenhal) and *Acanthoscelides* obtectus (Say).

In the fourth investigation, none of the six quarantine pests were found. But in the third investigation *T. granarium*, *Z. subfasciatus*, *C. analis* had been found in the inspection of foreign goods. The main reasons may be that the third investigation was conducted by personnel from the Ministry of Commerce and Ministry of Agriculture and Forestry. This made it easy for the investigators to work with the quarantine departments directly under control of the Ministry of Agriculture and Forestry. With the economic development of China and its entry into WTO, all sorts of pests, including stored grain insects are being imported. In order to prevent the importation of hazardous insects by efficient quarantine control, it is necessary to investigate stored grain insects in foreign imported goods with the collaboration of the Ministry of Agriculture.

3.6. Prospects for the use of natural enemies of stored grain insects.

In the fourth investigation, 44 species of natural enemies of stored grain insects had been identified and recorded. These natural enemies can limit populations of stored grain insects, offering a means of biological control. It is worthwhile for us to explore ways for making good use of these natural resources. There have been some successful cases recorded in the United States and the Czech Republic. The most promising natural enemies to the stored grain insects are parasitoid, predatory bugs and Chelonethida.

In this investigation 4 species of pseudoscorpion were identified, including *Chelifer cancroids* L., *Chelifer panzeri* Koch, *Chelifer nodosus* Schrank and *Chelifer* sp. Psocids (Psocoptera) are a significant problems in grain warehouse in China. It is believed that the reason for this is that their natural enemy Chelonethida had been killed by the application of chemical pesticide including PH_3 fumigation, and then psocids propagate causing a great nuisance in stored grain.

Predatory bugs - this research found and recorded six kinds of predatory bugs, including *Xylocoris flavipes* (Reutes) etc. Those predatory bugs can prey on many kinds of adults and larvae of stored grain insects.

Parasitoids - In this research seven kinds of parasitoid were recorded, including *Lariophagus distinguendus* Förster, *Aplastomorpha calandrae* Howard and *Microbracon hebetor* Say etc. These parasitoids are natural enemies of important stored grain insects, especially, stored grain moths and in the United States have been used to control the larvae of moths in groundnut stores.

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Section: Engineering

Protecting and disinfesting stored products by drying and cooling, and disinfesting stored products during handling by mechanical treatments

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Abstract

Drying and cooling nonperishable products sufficiently to achieve effective protection or disinfestation has been considered difficult in the case of the former and mostly impractical in the case of the latter. Physical methods of disinfestation that could be incorporated into the handling process, such as cleaning and mechanical impact have either not given complete pest control or have caused unacceptable grain damage. Thus chemical options, particularly phosphine fumigation, have remained the main method of pest control. However, minimal use of chemical treatments is becoming increasingly necessary. Apart from market forces and stricter food safety standards, insect resistance to phosphine is growing and chemical alternatives are limited. In light of these circumstances, this paper reviews the effectiveness of grain drying and cooling not only as a means of protecting against insects and mites during storage, but also as a means of disinfesting during the drying and cooling process. Opportunities are identified that might capitalize on a specific pest's response to such conditions. The level of pest control that can be achieved when manipulation of grain temperature and moisture is used in conjunction with mechanical disinfestation is also considered. Recent studies of grain cleaning are reviewed and fresh data from mechanical impact research are presented on the mortality of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum*. These data show real promise that mortality of insect development stages within grain kernels can be obtained at levels above 99% without damage to commodities such as wheat, which would overcome a major limitation to the technology. The results of the mechanical disinfestation research are discussed as part of a pest control strategy that includes the combined effects of grain drying, cooling and handling, to help deliver pesticide-free grain and extend the life of phosphine by restricting the development of resistance.

Keywords: Drying, Cooling, Handling, Aeration, Mechanical impact, Disinfestation

1. Introduction

Storing nonperishable products under dry, cool conditions is essential to maintain the quality of the product and minimize contamination by insects, mites and microbes (Hall, 1970). Drying grain with either ambient or heated air allows the product to be harvested at relatively high moisture content and, then with the active removal of moisture, stored at conditions that protect against fungal growth and infestation of mites and stored product insects. Aeration with ambient or chilled air cools the product to temperatures that maintain the quality required for its subsequent purpose, and reduces or stops the population growth of pest species or the development of fungal contamination. However, achieving the conditions required for effective protection and disinfestation has been considered limited by drying and, apart from limited circumstances, impractical by aeration or chilling.

Mechanical techniques that can be incorporated into handling processes, such as cleaning, can achieve substantial levels of disinfestation. Physical impact has generally been more effective against immature stages of primary pests developing inside grain kernels, but the trade off has been unacceptable levels of grain damage (Banks and Fields, 1995). These technologies have, therefore, been considered either insufficiently effective or having inherent limitations. Another physical method which can kill all stored-product pests without grain damage is heat treatment, but it is costly by comparison (Sutherland et al., 1987). Thus, chemical methods, primarily phosphine fumigation, have remained the mainstay of pest control.

The need for a minimal use of pesticides is becoming increasingly important. This is not only in response to market forces and stricter food safety standards, but also as a means of limiting the development of insect resistance, which is becoming increasingly critical. It is essential in the case with phosphine, where no alternatives are as versatile and effective. In light of these circumstances, it is timely to review not only the limiting effect that grain drying and cooling have on the population growth of stored product insects and mites during storage, but also the disinfesting effect that occurs during the drying and cooling process. This is done with a view to identifying opportunities that might capitalize on a specific pest's response to such conditions.

These opportunities may be enhanced in combination with mechanical disinfestation, either before or after storage. Data from recent research on mechanical impact will be presented that show real promise that mortality of insect development stages within grain kernels can be obtained at levels above 99% without damage to commodities such as wheat. This would overcome a major limitation to the technology and may allow mechanical treatments to play a substantial role in grain disinfestation, especially in a pesticide-free environment where the advantages of drying and cooling can be maximized. This paper will explore these possibilities.

2. Protection and disinfestation from grain drying and cooling

2.1. Drying

Storing a dry product has particular advantages for insect and mite control. The effect of aridity can be demonstrated by the mortality of *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae) during immature development (Beckett and Evans, 1994) (Fig. 1). At 30°C the level of mortality is 14.5% at 70% r.h., but 42% at 30% r.h. (approximately 9% m.c.). However, at 20°C mortality is 46% at 70% r.h., but as much as 99% at 30% r.h. Not only is there a significant level of disinfestation, but development time is also extended by 10 and 12 d respectively, affording additional protection in terms of slower population growth. Drying is particularly important as a means of controlling psocids and mites as both are particularly sensitive to aridity and can not survive at any temperature below about 60% r.h. for psocids (Rees, 2004) and 65% r.h. for mites (Navarro et al., 2002). However, maintenance of such conditions at the grain surface can be a challenge particularly in a maritime climate (Armitage and Cook, 2003).

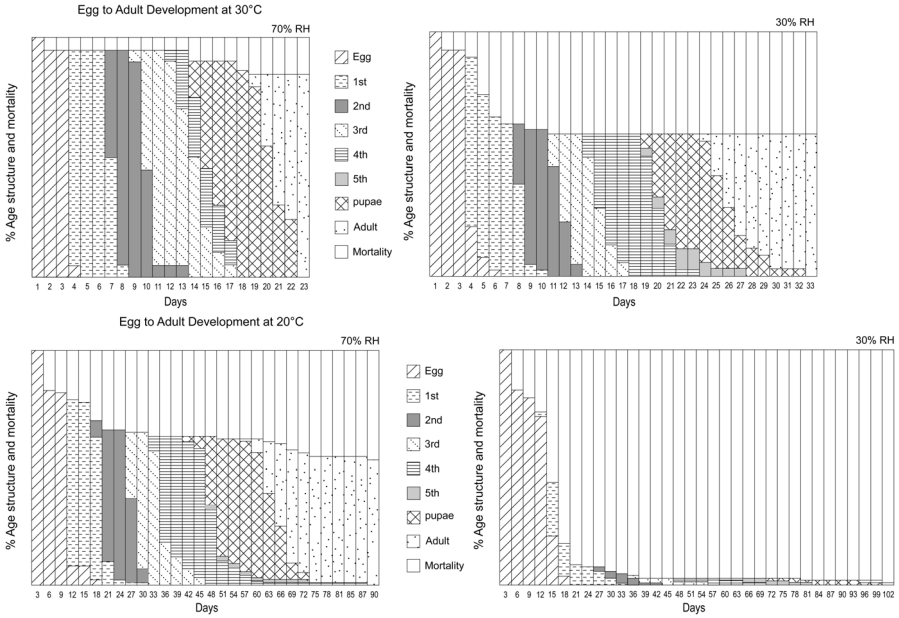

Figure 1 The effect of temperature and relative humidity on immature mortality and development time of *Oryzaephilus surinamensis* (from Beckett and Evans (1994)).

When heat is used, grain drying can be an effective method of thermal disinfestation. Insect mortality can be achieved at grain temperatures as low as 43°C, which is the maximum recommended for malting and seed barley, seed wheat, canola, and grain legumes at high moisture contents (Metz, 2006; Hill, 1999) (Table 1). Heat tolerance at moderately high temperatures is species-dependent so it is valuable to identify a particular infestation. The time required for disinfestation at these conditions can be prolonged, so any opportunity for grain to hold heat before active cooling would increase efficacy.

Table 1 Time (h) required for 99% mortality of the most heat tolerant life stage of a range of major insect pests at different temperatures and recommended grain temperatures during drying for different grain types (from Beckett et al., (2007), Metz (2006) and Hill (1999)).

		LT_{99} (h) for the most heat tolerant insect life stage at different temperatures					
	Moisture conditions	**43°C**	**45°C**	**50°C**	**55°C**	**60°C**	**65°C**
Sitophilus oryzae	12%mc	19	8.6	2.4 (49°C)	-	<0.01	<0.01
Sitophilus granarius	13%mc	-	3.3	0.5	<0.01	<0.01	<0.01
Rhyzopertha dominica	12%mc	-	70.8	8.9	0.3	0.01	<0.01
Tribolium castaneum	22% rh	83.7 (42°C)	13.0 (46°C)	7.2	1.4	0.5	<0.01
Tribolium confusum	22% rh	-	-	1.5	1.0 (54°C)	0.4	<0.01
Psocids	70%rh	40.4	19.9 (46°C)	2.5	-	<0.01	<0.01
Trogoderma variabile	0% rh	-	-	3.6	0.07	0.04	<0.01
Recommended grain temperatures for different grains types	malting/ seed barley	Maximum					
	seed wheat	Maximum					
	canola	Maximum					
	Grain legumes	Maximum					
	milling wheat			Optimum	Maximum		
	feed grains					Optimum	Maximum

There have been a few investigations into the used of grain dryers as a practical means of rapid heat disinfestation, which would allow the capital cost of equipment to be offset (Bruce et al., 2004; Qaisrani and Beckett, 2003a,b). At higher temperatures required for heat tolerant species, potential grain damage due to drying temperature variation was a concern, while disinfestation of other species with lower heat tolerance at moderate temperatures could be achieved successfully. More research is required, but the development of dependable dryer/disinfectors holds some promise.

2.2. Cooling

Cooling grain with ambient or refrigerated aeration has particular advantages for insect and mite control as lower temperatures have considerable effect on population parameters (Aspaly et al., 2007; Beckett et al., 1994). Using the example of immature mortality and development in *O. surinamensis* (Beckett and Evans, 1994), Fig. 1 shows mortality increase from 14.5% at 30°C and 70% r.h. to 46% at 20°C and 70% r.h. and 42% at 30°C and 30% r.h. to 99% at 20°C and 30% r.h., with eggs and first instar larvae most vulnerable. The difference in development time between 30 and 20°C is 23d compared with 90 d and 33 d compared with 102 d for 70 and 30% r.h., respectively. The threshold temperatures for population growth can vary considerably. For mites it can be as low as 7°C (i.e. for *Acarus siro* (L.)(Astigmata: Acaridae)), but for major stored-product beetles it is considerably higher. For example, it is about 14°C for *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) at 70% r.h. (calculated from Nakakita and Ikenaga, 1997), and 15, 17.5, 19 and 20°C for *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.)(Coleoptera: Bostrichidae), *O. surinamensis* and *Tribolium castaneum* (Herbst)(Coleoptera: Tenebrionidae), respectively, at relative humidities ranging from 45 to 65% (Beckett et al., 1994). Populations of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) have also been shown to decline at 20°C and 70% r.h. (Burges, 2008). Major stored-product moths start breeding at 15°C or above, but *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Ephestia elutella* (Hübner) are reported to start breeding at 12 and 10°C, respectively (Rees, 2004).

Predictive models for rates of population growth in relation to temperature and grain moisture have been developed for the major stored-product pests (Aspaly et al., 2007; Driscoll et al., 2000; Kawamoto et al.,

1989). The relatively low rate of population growth at dry, cool conditions near the threshold of population growth may allow storage for some time with minimal risk, if the grain is initially insect-free or the population is very small. A simple demonstration of this is by considering population doubling times (Dt) at such conditions (Table 2) ($Dt = \log_e(2)/\log_e(\lambda)$, where λ is the weekly finite rate of population growth). For example, at 18°C *S. oryzae* can double its numbers in 3.4 weeks at 50% r.h. but can not tolerate 35% r.h. at this or any temperature. *Rhyzopertha dominica* and *O. surinamensis* take about 3 and 6 months to double at 20°C and 35% r.h., and about 1.5 and 2.25 months at 20°C and 50% r.h., respectively. Data for *T. castaneum* are limited, but of the four species it appears to have the slowest rates of population growth at moderately low temperatures. In comparison, at 30°C and 50% r.h., all species will double in number in about a week.

Table 2 Population doubling times (weeks) for four major stored-product insect pests at a range of temperatures at 35 and 50% r.h. (negative numbers refer to population halving times) (Calculated from Beckett et al. (1994)).

	Temperature (°C)							
	18		20		22		30	
	35% r.h.	50% r.h.	35% r.h.	50% r.h.	35% r.h.	50% r.h.	35% r.h.	50% r.h.
Sitophilus oryzae	-34.3	3.4	-6	2.3	-3.5	1.9	-1.5	1.2
Rhyzopertha dominica	-69	∞	11.9	5.7	4	2.8	1.6	1
Oryzaephilus surinamensis	-	-	23.5	9	3.6	3.3	1.2	1
Tribolium castaneum	-	-	-	-	-	9	6.6	1.3

Where lower temperatures can be obtained, disinfestation can be achieved, but the process is slow unless temperatures are subzero. For example, Evans (1987) determined the rate of immature mortality over 12 months of six major stored product beetle species on Australian Standard White wheat at typical refrigerated aeration conditions of 13.5 and 9°C, and 11% m.c. The species tested were *Cryptolestes ferrugineus* (Stephens)(Coleoptera: Laemophloeidae), *O. surinamensis*, *R. dominica*, *Sitophilus granarius* (L.), *S. oryzae* and *T. castaneum*. After 3 months there was 100% mortality of *C. ferrugineus* at both temperatures and *O. surinamensis* at 9°C. After 9 months there was 100% mortality of *R. dominica* at both temperatures and the two *Sitophilus* species at 9°C. Mortality at 13.5°C was 97.5% for *S. granarius* and 99.8% for *S. oryzae* and *T. castaneum*. At temperatures such as these, low grain moisture content continues to have a substantial effect on mortality (Evans, 1983). Rapid cooling counteracts the development of cold tolerance through acclimation, which has been observed in several insect species, particularly *C. ferrugineus* (Fields and White, 1997), where 5% of an acclimated adult population is estimated to survive 75 days at -10°C (Fields, 1990).

Achievable outcomes and whether cooling is with ambient or refrigerated air, are determined by climate, target conditions and cost. Recent studies have shown the potential of aerating Japanese rice to 15°C using ambient autumn temperatures to control *S. zeamais* (Arthur et al., 2003), and demonstrated the ability to achieve that temperature using chilled aeration to control *S. zeamais* in stored maize in the United States (Ileleji et al., 2007). Kaliyan et al. (2007) determined the cost of disinfesting *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) using ambient aeration in regions in the United States where winter temperatures were below -10°C, and Rulon et al. (1999) developed an economic model which demonstrated that chilled aeration in some circumstances could compete with phosphine fumigation as a disinfestation method with a high value crop such as popcorn.

3. Disinfestation during handling

3.1. Grain cleaning

Quantitative disinfestation studies of specific grain cleaning machinery are limited. This author is aware of only two studies in the last 14 years, however, these reports indicate that substantial levels of insect mortality can be achieved. Armitage et al. (1996) evaluated aspirated sieving using a laboratory scale apparatus. The process successfully removed *O. surinamensis*, *S. granarius* and *C. ferrugineus* adults, and 90% of mixed development stages of two species of mites, *A. siro* and *Lepidoglyphus (Glycyphagus) destructor* (Schrank) (Astigmata: Glycyphagidae) with a grain weight loss of 4.5%. However, there was no reduction in the numbers of immature stages of *S. granarius*. Weller et al. (1998) evaluated a commercial laminar air-flow grain cleaner where the process removed 99.5% of mixed aged *T.*

castaneum and 93.5% of mixed-age *R. dominica* with a grain weight loss of less than 1.5%. It was suggested that death by physical shock or impact must play a large part in the reduction of immature *R. dominica* numbers. While cleaners could be modified to disinfest more effectively (Weller et al., 1998) and are generally successful at removing major secondary invaders such as *Tribolium* and *Cryptolestes* spp., *O. surinamensis* and psocids, it is likely that a proportion of internal developers such as *R. dominica* and *Sitophilus* spp. will remain. Subsequent cooling or fumigation is essential with infestations of these species.

3.2. Mechanical disinfestations

The challenge for mechanical disinfestation, ultimately, has been impacting the eggs and larvae of insects such as *Sitophilus* spp. and *R. dominica* that are protected within kernels without causing seed damage. Entoleters, for example, are effective as disinfesters, but they destroy a large proportion of the kernels and so are used prior to milling or for treating flour (Stratil et al., 1987). Bailey (1962; 1969) was able to demonstrate a relationship between grain impact velocity, moisture content and grain breakage on one hand, and impact velocity, impact repetition and insect mortality on the other. Recent trials have further explored the relationship between impact velocity and repetition, and grain damage and insect mortality to determine if a treatment range exists where sufficient mortality can be achieved before grain damage commences.

4. Physical impact trials

4.1. Materials and methods

An apparatus was constructed to discharge a given quantity of grain at a known velocity at a surface so that each grain kernel hit the surface unobstructed (Fig. 2). The grain could then be collected and the process repeated. The apparatus consisted of an aluminium cylinder 82-cm diameter by 60-cm high, in the centre of which was a Perspex grain container/releaser or 20-cm diameter by 1.5 cm impellor attached to a vertically mounted 2.2-kW electric motor. Grain was fed into the container through small holes in the lid. While the container spun on the motor shaft, the lid was forced down onto it by hydraulic pressure. When the container was revolving at an appropriate speed, the lid was lifted and a mono-layer of grain which had formed around the circumference was projected out against the inside wall of the cylinder. The grain was then funnelled down into a collecting bin. The speed of the grain on impact (ms^{-1}) was $2\pi r_{impellor}rpm/60$.

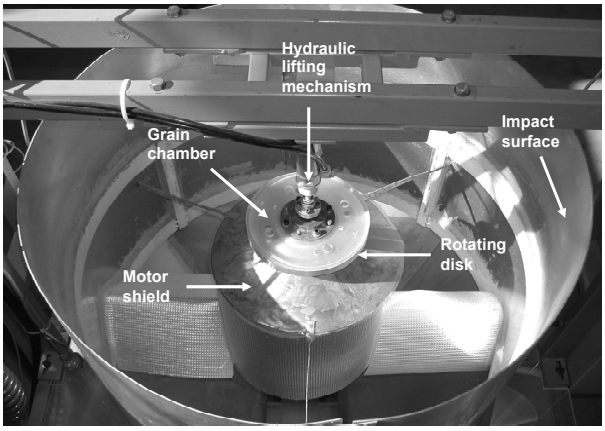

Figure 2 Vertical view of experimental grain impact apparatus.

Mortality data were collected for adults and four immature cohorts of *S. oryzae* and *R. dominica*. The cohorts progressed in age such that cohort 1 was mainly eggs and first instar larvae and cohort 4 was mainly late larvae and pupae. Mortality data were also collected for adults and late larvae of *T. castaneum*. Each replicate tested for *S. oryzae* and *R. dominica* consisted of 10 g of grain infested with 100 adults or an immature cohort, while each replicate for *T. castaneum* consisted of 10 g of grain infested with either 100 adults or larvae.

Experiments were conducted using Australian soft wheat, variety Rosella at grain velocities of 10, 15 and 20 ms^{-1} over a range of repetitions from a single impact to 45 continually repeated impacts. The time between impacts was less than 1 min. Three replicates of each development stage of each species in wheat at 11% m.c. were tested for each impact treatment. Experiments using wheat at 14% m.c. were also conducted on *S. oryzae*. Adult mortality in all three species was assessed against controls after 24 h and 7 d, while immature cohorts were incubated at 30ºC and 55% r.h., and emerging adults assessed on a weekly basis against controls. Grain quality was monitored by subjecting three replicates of 10 g of uninfested wheat at 12 and 14% m.c. to a similar range of treatments. Quality response was determined by observing the levels of germinative energy and germinative capacity (Ghaly and Taylor, 1982; Ghaly and van der Touw, 1982), following the procedure set out by the International Seed Testing Association (Anonymous, 1993). A reduction in germination due to treatment ≥3% was considered to be excessive damage.

4.2. Results

At 20 ms^{-1}, 100% mortality of all development stages of all species in grain at 11% m.c could be achieved by 7 repetitions. However, unacceptable grain damage occurred at 3 repetitions. At 15 ms^{-1}, mortality was at least 99% for all stages of *S. oryzae* and *R. dominica* by 7 repetitions, except for immature cohort 1 of *R. dominica* where mortality was 91%. In this treatment, grain quality was still acceptable, but declined rapidly with subsequent repetitions. At 10 ms^{-1}, 45 repetitions were performed before 100% mortality was achieved in all stages except for immature cohort 2 of *S. oryzae* with 98% mortality, and immature cohort 1 of *R. dominica* with 96% mortality. At this treatment, grain quality still remained acceptable (Tables 3 and 5). In general, early immature stages of *S. oryzae* and *R. dominica* were the most tolerant stages with *R. dominica* slightly more tolerant that *S. oryzae*. Conversely, *S. oryzae* adults were more tolerant than *R. dominica* adults, which were even more susceptible that *T. castaneum* adults. *T. castaneum* adults proved slightly more tolerant than larvae.

The mortality response of *S. oryzae* in grain at 14% m.c. to all impact velocities was less than that in grain at 11% m.c., particularly for immature cohort 1 (91% compared with 100% at 45 repetitions of 10 ms^{-1} and 83% compared with 99% at 7 repetitions of 15 ms^{-1}). However, there was no discernible difference detected in grain quality response (Tables 3 and 4).

Table 3 Percent mortality of *Sitophilus oryzae* in Australian soft wheat at 11% m.c. in response to a range of repeated impacts at three velocities.

		Repetitions									
	Velocity m/s	**1**	**3**	**5**	**7**	**10**	**15**	**20**	**25**	**40**	**45**
Adults	10					42	76	90	95	100	
	15		71	82	99						
	20	70									
Immature cohort 4	10					95	100		100	100	
	15			99	100						
	20	92	100	100							
Immature cohort 3	10			80	91	97	99	100	100	100	
	15	82	95	99	100	100					
	20	95	100								
Immature cohort 2	10				91	95	99	98	97	100	98
	15		95	99	100	100					
	20	88	98	99	100						
Immature cohort 1	10				58	77	85	91	92	96	100
	15		78	90	99	98	100				
	20	55	95	99							

Shaded area: ≥3% germination loss

Table 4 Percent mortality of *Sitophilus oryzae* in Australian soft wheat at 14% m.c. in response to a range of repeated impacts at three velocities.

		Repetitions									
	Velocity m/s	**1**	**3**	**5**	**7**	**10**	**15**	**20**	**25**	**40**	**45**
Adults	10					60	78	88	88	100	100
	15		73	90	99	100					
	20	69	98	100							
Immature cohort 4	10				94	97	98	99	97	100	
	15		96	99	100	100					
	20	92	100								
Immature cohort 3	10					89	96	99	99	100	
	15		95	99	99	100					
	20	90	100								
Immature cohort 2	10				79	85	94	97	97	100	
	15		91	97	99	100					
	20	77	95	99							
Immature cohort 1	10				45	60	68	72	84	87	91
	15		61	81	83						
	20	48	86								

Shaded area: ≥3% germination loss

Table 5 Percent mortality of *Tribolium castaneum* and *Rhyzopertha dominica* in Australian soft wheat at 11% m.c. in response to a range of repeated impacts at three velocities.

		Repetitions									
	Velocity m/s	**1**	**3**	**5**	**7**	**10**	**15**	**20**	**25**	**40**	**45**
Tribolium castaneum											
Adults	10				51	71	93	100	100		
	15		85	97	100	100					
	20	51	100								
Large larvae	10	5	33	45	71	92	99	100	100		
	15	19	97	100	100						
	20	99	100								
Rhyzopertha dominica											
Adults	10				56	83	100	100			
	15		98	100	100						
	20	99									
Immature cohort 4	10			97	100	100	100				
	15	96	100	100							
	20	98	100								
Immature cohort 3	10				99	100	100	100	99		
	15		99	100	99	100					
	20	96									
Immature cohort 2	10				94	98	99	99	100	100	
	15		96	98	99	100					
	20	89	99	99							
Immature cohort 1	10				76	82	91	95	98	96	96
	15		80	90	91						
	20	69									

Shaded area: ≥3% germination loss

Treatments at 3 and 5 impacts at 15 ms^{-1}, which gave moderate levels of mortality, were repeated for both *S. oryzae* and *R. dominica* in grain at 11% m.c. so that a subsequent period of cooling at 15 and 20°C could be included to determine the effects of aeration on mortality after mechanical impact treatment. Results showed 99 to 100% mortality can be achieved within 5 weeks at 15°C and even 97% mortality can be achieved at the higher temperature of 20°C (Table 6). This may in part be due to eggs and first instar larvae being particularly sensitive to cool, dry conditions similar to that exhibited by *O. surinamensis* (Fig. 1).

Table 6 The effects of cooling to 15 and 20°C for 35 days on mortality of *Sitophilus oryzae* and *Rhyzopertha dominica* after 3 and 5 repetitions of impact at 15 ms^{-1}.

Insect		*Treatment*	*Repetitions*	*Insect*
			3	*5*
Sitophilus oryzae	Cohort 1	Impact only	78	90
		+ cooling 20°C	92	99
		+ cooling 15°C	100	100
	adults	Impact only	71	82
		+ cooling 20°C	91	97
		+ cooling 15°C	97	99
Rhyzopertha dominica	Cohort 1	Impact only	80	90
		+ cooling 20°C	90	97
		+ cooling 15°C	99	100
	adults	Impact only	98	-
		+ cooling 20°C	100	-
		+ cooling 15°C	100	-

5. Discussion and conclusions

The degree to which stored grains are kept dry and cool will determine the level and range of infestation problems. If commodities are kept below 65% r.h., mites should not present a problem, and below 60% r.h. psocids should also be controllable. Below 17°C at 45 to 65% r.h., most stored-product beetles will also cause little problem, and if relative humidity is at or below 35%, *Sitophilus* spp. should also be controllable at any temperature. A few moth species will breed at temperatures below 17°C at relative humidities as low as 20% r.h.; this may be a concern. If commodities with little or no infestation can be held in well sealed facilities at appropriate storage conditions, then their status should change little y time of out-loading. However, in the areas of increased moisture (by moisture migration) with possibly increased temperatures, populations of some insects may develop rapidly.

The process of drying using elevated grain temperatures and rapid cooling to relatively low temperatures can cause disinfestion to occur if sufficient time is available. For most stored product beetles 99% mortality can be achieved at 50°C in less than 9 h. Moths are more susceptible with 100% mortality at 50°C usually reached in 2 h (Fields, 1992). Under cool conditions, 9°C for 3 months will kill *C. ferrugineus* and *O. surinamensis*, and after 9 months will kill *R. dominica*, *S. oryzae* and *S. granarius* and *T. castaneum*. However, a practical difficulty with temperature control, as it is with fumigation, is variability, particularly at the periphery of a storage structure. The financial cost of drying and cooling can also be a serious impediment which must be accounted for.

Like drying, grain cleaning also gives the opportunity to in-load grain free of most stored-product pests with the exception of those whose immature stages develop within the kernels. This could be managed if adequate cooling is possible. The removal of dockage also reduces easily accessible food sources for certain pest species (Song et al., 1990).

The use of mechanical impact combined with aeration at moderately cool conditions (20°C) is promising as a way to in-load insect-free grains such as wheat and barley. This technology may not be practical for commodities such as maize which is highly susceptible to breakage during harvesting and handling, especially at lower moisture contents (Hagstrum et al., 1996). Mechanical impact could also be used as a post-storage treatment, particularly if further research shows that complete disinfestation of eggs and first instar larvae can be achieved without the added effect of cooling. A drawback is that mechanical impact treatment requires specific grain-handling equipment designed to provide controlled grain velocities at impact; it may be difficult to achieve uniformity of treatment in commercial grain industries that are provided by drying, cooling and cleaning technologies.

Some stored-product pests show more susceptibility than others to dry, cool conditions, or drying, cooling or handling, either in terms of survival or population growth. With current developments in these technologies, it may become possible in the future to reliably store certain major commodities without chemical treatment. Adding judicious mechanical treatment may deliver them sufficiently pest-free. Moreover, any reduction or break in the use of pesticides is widely regarded as one of the best ways of managing development of pest resistance (Subramanyam and Hagstrum, 1996); this is becoming particularly urgent due to the major reliance on phosphine.

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Economic decision-making model for best ozonation treatment system for stored-product protection

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Abstract

Ozonation treatment systems have been proved as a potential non-chemical, non-residual and environmentally friendly alternative for stored-product protection. Based on scale-up and demonstration trials, static bed ozonation, semi-continuous counter-flow ozonation and continuous ozonation flow systems have been designed and successfully tested for pest control. Each system has different engineering and economic design parameters base on its treatment principle. The purpose of this study was to develop an economic decision-making computer model that will allow full-scale farms and commercial processing facilities to determine the best ozonation treatment option for insect mortality, mold and mycotoxin reduction and off-odor removal for grain based on the different design parameters of each case, equipment and storage availability, projected running cost and treatment time without affecting grain quality. The design set-up prototype of the economic decision-making model resulted into five blocks for user determination of treatment system, input parameters and fixed variables. Based on these blocks, calculations are performed for determination of power consumption per hour, treatment time and total treatment cost per year that lead into a decision of the best treatment system option for each specific case. The economic decision-making tool also determines the best option of either purchasing or renting an ozone generator for treatment during a specific number of usage years.

Keywords: Ozonation, Continuous Treatments, Stored-Product Pests, Molds, Mycotoxins, Economic Models

Comparison of leakage rates of methyl bromide and sulfuryl fluoride during structural fumigations

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Abstract

In structural fumigations, half-loss time (HLT) is the most frequently used indicator for comparing fumigant leakage rates. In practical situations where gas leakage rates during structural fumigations are compared, environmental conditions generally are not analyzed in detail and sealing quality is assumed to be constant or fixed. This gives a false impression that a certain gas fumigant might be contained in a structure better than another fumigant. During commercial structural fumigations at the Hal Ross Flour Mill, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA, leakage characteristics of Methyl bromide (MB) and Sulfuryl fluoride (SF) were compared by taking internal and external environmental conditions into consideration.

Two sets of one 24-h MB and one 24-h SF fumigation experiments were conducted in May and August 2009. Mill sealing and fumigations were conducted by two separate commercial fumigators. After sealing, sealing quality prior to a fumigation was verified by a building pressurization test. The mill was subjected to different pressure levels generated by a specially made fan. At each pressure level, the air flow rate through a calibrated fan was measured. The observed air flow rate plotted as a function of pressure quantified leakage characteristics of the mill. In two MB and SF fumigations, gas concentrations were continuously monitored during the entire fumigation period. A weather station was installed on the roof of the mill to monitor outside barometric pressure, wind speed and direction, temperature, and relative humidity. Inside the mill, a temperature and relative humidity data logger was placed on each of the five floors of the mill.

Results of this study provided a quantitative side-by-side comparison between MB and SF in the same facility. The pressurization test showed that sealing effectiveness can be quantitatively determined ahead of fumigation. It also confirmed the sealing quality for all fumigations was essentially similar. MB and SF showed similar gas distribution and leakage characteristics. Although the observed HLTs of the fumigations were different, those differences could be explained by the differences in environmental conditions, primarily wind speed, and to a certain extent mill temperature, rather than inherent properties of MB and SF gases.

Keywords: Structural fumigation, Half-loss time, Grain-processing facility, Sulfuryl fluoride, Methyl bromide

1. Introduction

In the United States, the primary fumigant used for structural fumigation in food-processing facilities (e.g., flour mills) had been Methyl bromide (MB) until Sulfuryl fluoride (SF) was introduced. SF was registered in the United States for post-harvest use in January 2004 under the trade name ProFume® by Dow AgroSciences LLC, Indianapolis, Indiana, USA. MB was phased out in the United States as of 2005, but it continues to be available to certain end users through the critical use exemption (CUE) process. Two reasons cited for allowing CUE were that the alternatives, in this case SF, should be as cost effective as MB, and the end users needed transition time to embrace SF and other MB alternatives.

Most studies in which fumigation experiments are conducted in commercial food processing facilities primarily focus on efficacy against insects and/or on insect population rebound (Drinkall et al., 2003; Reichmuth et al., 2003; Drinkall et al., 2004; Small, 2007). In a structural fumigation, half-loss time (HLT) is the most frequently used indicator to characterize fumigant leakage rates. Cryer (2008) used

computational fluid dynamics simulations to compare leakage characteristics between MB and SF from a flour mill subjected to various fixed wind speeds, and found that under the same conditions the HLTs for MB and SF were nearly identical. Another computer simulation study by Chayaprasert et al. (2009) supported this view. However, in typical discussions where gas leakage rates during structural fumigations are compared, environmental conditions are not analyzed in detail and sealing quality is not characterized. Therefore, it makes it difficult to interpret the effectiveness of a structural treatment absent such a characterization.

Chayaprasert (2007) reported on data collected from five SF and one MB fumigation experiments in three flour mills which included fumigant concentrations, indoor temperature and relative humidity, and outside weather conditions (i.e., temperature, relative humidity, barometric pressure, solar radiation, and wind speed and direction). Analysis of the data resulted in concluding that sealing quality and environmental factors should be considered when comparing structural fumigants. However, sealing quality effectiveness is generally not quantified whenever a fumigation is done. Therefore, the effectiveness of a fumigant should be based on how the gas behaves and how environmental factors affect how a gas behaves within a structure, and consequently how that affects fumigation efficacy.

The objective of the current study was to experimentally compare leakage characteristics of MB and SF while maintaining all the other influencing parameters as close to identical as possible.

2. Materials and methods

2.1 Fumigation procedure

The Hal Ross Flour Mill has five floors with a total volume of approximately 9,628 m^3 (340,000 ft^3). Figure 1 shows a photo of the mill and its generic floor plan which is the same for every floor. All five mill floors during fumigation were interconnected through stairwell doors and air supply vents, in addition to openings between certain floors to accommodate equipment. Two replications of one 24-h MB and one 24-h SF fumigation experiments were conducted in this mill (Table 1). In each set, the two fumigations were carried out within a three-week time span. All fumigations were done by professional fumigators following label directions. Preparation for all fumigations was similar. Prior to each fumigation, the mill was cleaned and sealed. Sealing quality was verified by building pressurization tests (described in Section 2.2 below). Two 20-inch fans were placed on each floor to facilitate gas distribution. These fans were operating during the entire exposure period. One fumigant introduction point was selected at approximately the same location on every floor (Fig. 1b). The amount of fumigant (i.e., weight) being introduced on each floor and the corresponding introduction time for each fumigation are listed in Table 1. The amount of fumigant used for the August fumigations was less than during May fumigations because of higher inside mill temperatures.

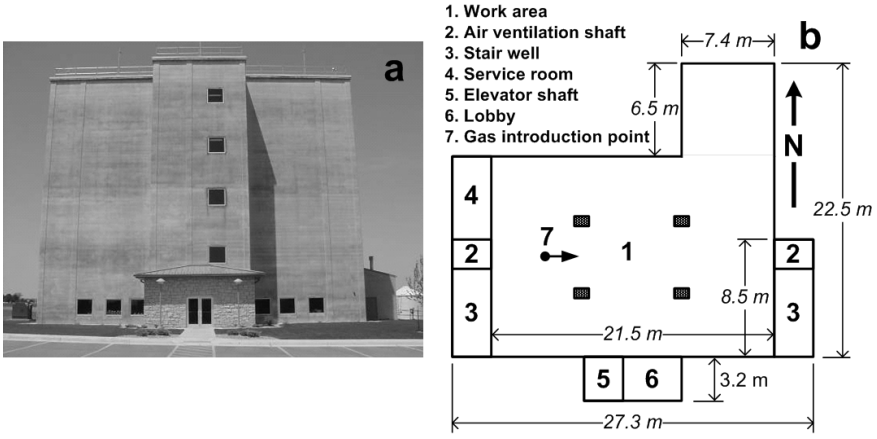

Figure 1 (a) The Hal Ross Flour Mill and (b) its floor plan.

Table 1 The quantity of MB and SF fumigants used and gas introduction times.

	MB1	SF1	MB2	SF2
	Gas introduction time			
	6:40P 6 May 2009	6:00PM 27 May,2009	2:50PM 11 August 2009	2:45PM 19 August 2009
Floor	Introduced amount (kg)			
1st floor	22.7+22.7[a]	113.6	22.7	113.6
2nd floor	22.7	113.6	22.7	56.8
3rd floor	22.7	113.6	22.7	113.6
4th floor	45.4	113.6	45.4	113.6
5th floor	45.4	113.6	45.4	113.6
Total	181.6	568.0	158.9	511.2

[a]Top-up release at 9:50AM 7 May 2009

2.2 Pressurization test

At approximately two hours before the gas introduction of each fumigation, the building sealing quality was quantitatively evaluated by a pressurization test. The pressurization test was conducted using the E3 blower door fan (Infiltec, Waynesboro, Virginia). This fan is capable of delivering a maximum airflow rate of 2.57 m^3/s (5,450 cfm). As the speed of the fan varies, it pressurizes the tested building with different level of airflow rates. The fan was attached to one of the exit doors. During each pressurization test, the building was subjected to different pressure levels between 20 and 130 Pa. At each pressure level, the flow rate through the fan and the static pressure difference across the blower door were measured by the DM4 micro-manometer (Infiltec, Waynesboro, Virginia). The gas-tightness characteristic of the mill was determined by plotting the pressure-flow rate relationship.

2.3 Fumigation monitoring

Six monitoring lines were evenly distributed on each floor of the mill. Fumigant concentrations were continuously monitored automatically by the Spectros Instruments Single Point Monitor (Spectros Instruments, Hopedale, Massachusetts) at 10 locations. The other 20 locations were monitored using either the Spectros Instruments Single Point Monitor or Fumiscope (Key Chemical and Equipment, Clearwater, Florida) manually throughout the 24-h exposure time. In addition to fumigant concentrations, environmental conditions were monitored. A HOBO® U30 weather station (Onset Computer Corporation, Bourne, Massachusetts, USA) was installed on the roof of the mill. The weather station recorded barometric pressure, wind speed and direction, temperature, and relative humidity every minute. Temperature and relative humidity inside the mill were monitored by HOBO® H8 data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) every three minutes (one logger per floor).

3. Results and discussion

A plot of the pressure-airflow rate curves representing the sealing effectiveness of all fumigation experiments is shown in Figure 2. The data points for the MB1, SF1 and MB2 fumigations were on an almost identical curve (i.e., dashed line in Fig. 2), indicating similar gas-tightness levels. The result of the pressurization test for the SF2 fumigation was adversely affected by strong prevailing wind around the mill experienced during the test, resulting in notably more scattered data points. However, the lower bound of the scattered data points, which is the highest building gas-tightness possible for the SF2 fumigation, coincides with the pressure-airflow rate curves for the other three fumigations. Assuming that this lower bound represented the sealing quality for the SF2 fumigation, the plot in Figure 2 in general confirms that the sealing quality of all four fumigations in this study was nearly identical. In other words, if the pressure differences across the flour mill building envelope were the same, the fumigant leakage rates observed from these four fumigations would be the same as well. Thus, any differences in the leakage rates (i.e., HLTs) between the fumigations were caused by the fumigation-to-fumigation variations in environmental conditions.

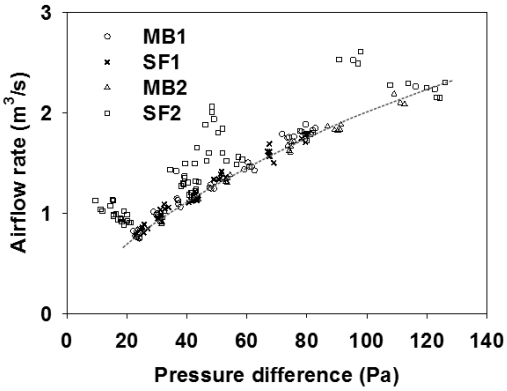

Figure 2 Results of the pressurization tests.

Figures 3a, 3b and 3c show barometric pressures, outside temperatures, and outside relative humidity, respectively, during the four fumigations. Note that the barometric pressure reduction due to the difference in height between the weather station and ground level has been compensated for in the barometric pressure curves in Figure 3a. Substantial variations in these environmental conditions were observed both within each fumigation and between fumigations. Within each fumigation, the differences between the highest and lowest values of barometric pressure, temperature, and relative humidity, were approximately 3-9 mbar, 9-12°C, and 40-50%, respectively. Nevertheless, the inside temperature and relative humidity were relatively stable during the entire exposure periods for all fumigations. Within each floor, the inside temperature and relative humidity varied less than 1°C and 5%, respectively, and the differences in the inside temperature and relative humidity between floors were less than 4°C and 20%, respectively (data not shown). This implied that the heat transfer rate between the inside and outside, and the heat generation and accumulation rates within the mill were balanced. Similar observations can be expected for other buildings with the same gas-tightness level.

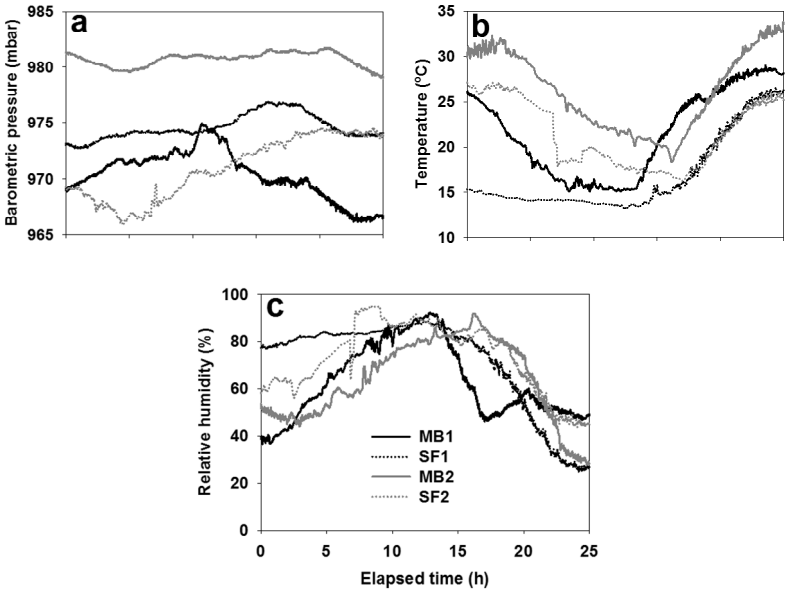

Figure 3 Plots of (a) barometric pressures, (b) outside temperatures, and (c) outside relative humidity during the four fumigations.

The fumigant concentrations at all monitoring locations during the MB1, SF1, MB2 and SF2 fumigations are plotted as curves with markers in Figures 4a, 4b, 4c and 4d, respectively. Both MB and SF showed similar gas characteristics. Initially, the fumigant concentrations increased rapidly. For the first three fumigations, even gas distribution was established throughout the mill within the first four hours. For the SF2 fumigation, however, it took 10 hours. During the first three fumigations, all the stairwell doors were opened, while during the SF2 fumigation only the stairwell doors on the 3rd, 4th and 5th floors were opened. Partitioning of the 1st and 2nd floors caused slower gas movement from these two floors to the other floors. However, in some buildings partitioning very leaky areas as separate fumigated volumes will be beneficial in preventing excessive fumigant loss. The sudden peak in gas concentration at the 15th hour of the MB1 fumigation was due to adding an additional 22.7 kg of MB. Generally, once the fumigant was well mixed, it remained evenly distributed throughout the building and the concentrations gradually decreased over time. The differences in concentrations within the mill were between 2 and 7 g/m^3 most of the time.

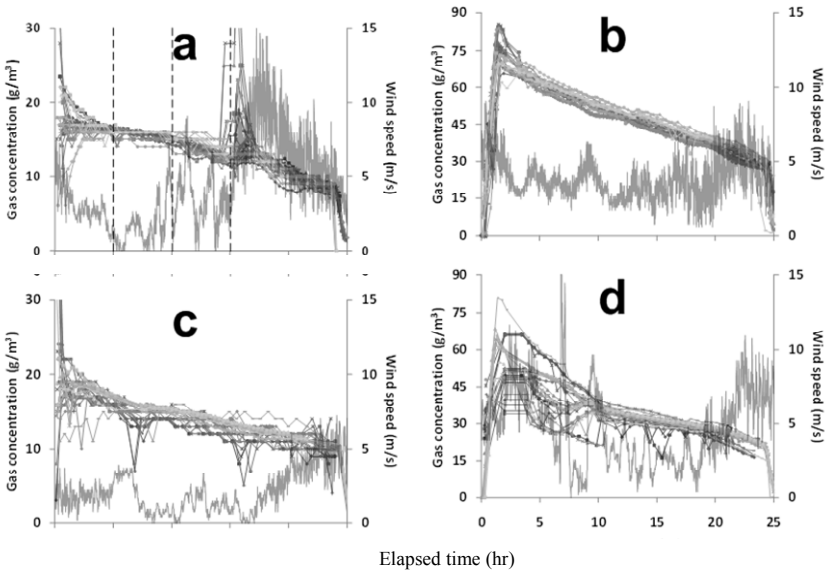

Figure 4 Fumigant concentrations at all monitoring locations and outside wind speeds during the (a) MB1, (b) SF1, (c) MB2 and (d) SF2 fumigations.

After even gas distribution was achieved, the SF1 and MB2 fumigations showed relatively constant HLTs, while the HLTs for the MB1 and SF2 fumigations changed as the fumigations progressed. By observation, the concentration curves for the four fumigations were divided into sections and the average HLT for each section was calculated. The average HLTs and the corresponding elapsed time periods in which the HLTs were calculated are summarized in Table 2. The HLTs for the MB1, SF1, MB2 and SF2 fumigations were 10.2-111, 19.7, 26.0 and 9.9-26.1 hours, respectively. The wind speed data collected during the fumigations are superimposed on the concentration plots in Figure 4 (transparent lines). Although the effect of rapid wind fluctuations could not be seen in the gas concentration curves, a strong correlation between HLT and wind speed could be observed. The average wind speeds corresponding to the HLTs of the same elapsed time periods are listed in Table 2. Regardless of the type of fumigant, when the average wind speed was less than 2 m/s, between 2 and 3 m/s, between 3 and 4 m/s, and greater than 4 m/s, the HLT was much greater than 26 h, approximately 26 h, between 20 and 15 h, and approximately 10 h, respectively. This indicates no noticeable difference in the leakage characteristics of MB and SF. Banks and Annis (1984) analytically showed that the overall ventilation rate (d^{-1}), which is defined as the total volume of the enclosure divided by the volumetric gas loss rate, during fumigation in grain storages is a summation of individual ventilation rate associated with atmospheric pressure, buoyancy and wind forces. However, the effects of other environmental conditions, specifically buoyancy (i.e., inside-outside temperature difference) and barometric pressure pumping forces, were not

observable in the concentration data, and thus no other correlations with the HLTs could be established. It is likely that these forces were overshadowed by wind.

Table 2 Half-loss times (HLT), average wind speeds, and corresponding elapsed time periods in which these two values are calculated.

Fumigation	Elapsed time period (h)	HLT (h)	Avg wind speed (m/s)
MB1	5th-10th	111.0	1.65
	10th-15th	16.4	3.52
	17th-24th	10.2	7.12
SF1	5th-24th	19.7	3.67
MB2	5th-24th	26.0	2.16
SF2	11th-21st	26.1	3.00
	21st-24th	9.9	6.90

4. Conclusions

Results of this study provided a quantitative side-by-side comparison between MB and SF under nearly identical conditions in the same facility. The pressurization test showed that sealing effectiveness can be quantitatively determined ahead of fumigation. It also confirmed the sealing quality of all fumigations to be similar. SF and MB showed similar gas distribution and leakage characteristics. Although the observed HLTs of the fumigations were different, those differences could be explained by the differences in environmental conditions, primarily wind speed, and to a certain extent mill temperature, rather than inherent gas properties of MB and SF.

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A simplified and improved modeling approach for the structural fumigation process using computational fluid dynamics

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Abstract

A 3D Computational fluid dynamics (CFD) model of the fumigation process in the Hal Ross Flour Mill of Kansas State University, Manhattan, Kansas, USA, was formulated for prediction of the gas leakage rate to approximate the gas Half-loss time (HLT) during fumigation with Methyl bromide (MB) and Sulfuryl fluoride (SF). The model consisted of external and internal flow domains. The external domain was used to predict stagnation pressures generated by wind impinging on the mill's walls. The internal domain was used to predict fumigant leakage rates in terms of HLT. Cracks on the mill's walls represented the effective leakage areas on the internal flow domain. This modeling approach had been used by the authors (Chayaprasert and Maier) in a previous study, but it was simplified and improved in the present study. The primary simplification in the modeling approach was exclusion of the flour mill's interior details (e.g., milling equipment), reducing the model formulation and simulation computing times. In the previous study, the gas-tightness of the internal flow domain was identified by varying the flow resistance coefficient of the effective leakage areas until the model yielded a HLT value that was close to the one observed from the experimental fumigant concentration data. In the present study, the domain gas-tightness was verified by building pressurization tests. The model was validated using data from one MB and one SF fumigation experiments. The HLTs provided by simulated fumigations were in good agreement with those determined from the experiments. The result of the present study provides further validation to the modeling approach and emphasizes the importance of building pressurization test for accurate HLT prediction.

Keywords*:* Structural fumigation, Half-loss time, Pilot flour mill, Computational Fluid Dynamics (CFD), Building pressurization test

1. Introduction

In fumigation of large structures such as flour mills or food-processing facilities, fumigant leakage always occurs because it is not practically possible to perfectly seal the structure. The decay of fumigant concentrations can be described by a first-order kinetic approximation (Cryer and Barnekow, 2006):

$$C_t = \frac{C_i}{2^{\frac{t}{HLT}}} \quad (1)$$

where C_t = current concentration (g/m^3) at elapsed exposure time t (h) and C_i = initial concentration (g/m^3). The half-loss time (*HLT*) is the time (h) at which the concentration reduces by half. The concentration × time (Ct) product (g-h/m^3) achieved at any given time can be calculated by integrating Eq. 1:

$$Ct = \frac{C_i HLT\left(1 - 2^{-\frac{t}{HLT}}\right)}{\ln(2)} \quad (2)$$

In other words, the needed amount of fumigant (i.e., the initial concentration times the building volume) for any fumigation is a function of the target Ct product, available exposure time, and half-loss time. Given fixed values for the target Ct product and available exposure time, it can be shown through Eq. 2 that the needed amount of fumigant is minimized when the HLT is known (Chayaprasert, 2007). Thus, being able to predict HLT is an essential part of optimizing the structural fumigation process.

Chayaprasert et al. (2008) used computational fluid dynamics (CFD) software, Fluent® (Fluent Inc., Lebanon, New Hampshire, USA), to develop a structural fumigation model for predictions of HLT and Ct product. The CFD model was validated based on a set of data collected during a fumigation experiment conducted by Chayaprasert et al. (2006). Given the same weather conditions, the coefficient (Eq. 4) which specified the gas-tightness of the building in the model was arbitrarily varied until the model was able to predict essentially the same HLT as observed in the experiment. Although the HLT prediction was accurate, this was rather an indirect approach for validating the model because the building gas-tightness was not directly measured. One possible method for measuring building gas-tightness is the equilibrium pressure-flow pressurization test in which measurements of air volume leakage rates through the building envelope are taken at multiple pressure levels. In the present study, the CFD modeling approach used by Chayaprasert et al. (2008) was implemented to construct a structural fumigation model using the Hal Ross Flour Mill, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA, as the reference structure. The objectives of the study were 1) to simplify the modeling approach so that the model construction and simulation computing times are reduced, and 2) to improve the modeling approach by incorporating the building pressurization test data.

2. Materials and methods

2.1 Model construction

The flows of wind surrounding the mill and fumigant distribution inside the mill were separately simulated. The external flow domain was used to predict stagnation pressure profiles on the flour mill's walls created by prevailing wind. Sixty steady-state flow simulations, each of which was specified with a different fixed wind speed and direction, were performed. Five wind speeds (i.e., 2, 4, 6, 8 and 10 m/s) and 12 wind directions (i.e., 0, 30, 60, 300 and 330 degrees with respect to the north) were selected. Note that Chayaprasert et al. (2008) performed external flow simulations with the six wind speeds (i.e., 1, 2, 4, 6, 8, and 10 m/s) and 24 wind directions (i.e., 0, 15, 30, 330 and 345 degrees with respect to the north). Primary features included in the external flow domain were the flour mill building, surrounding structures, and perimeter boundaries. Figure 1 shows the external flow domain when the wind direction was between 270 and 0 degrees with respect to the north. Note that the north direction used in this paper and as shown in Figure 1 was a fictitious north which was approximately 135 degrees rotated from the true north in the clockwise direction. Given the mill's height H (i.e., 22 m), the distances from the upwind and downwind perimeter boundaries to the mill building were at least 4H and 10H, respectively, to ensure that recirculation flows in the downwind region of the domain did not influence the simulation result. For different wind directions, the upwind and downwind perimeter boundaries were relocated such that this criterion was met. A uniform gauge static pressure of 0 Pa was specified at the downwind boundaries. There was no velocity gradient (i.e., symmetry) through the top boundary.

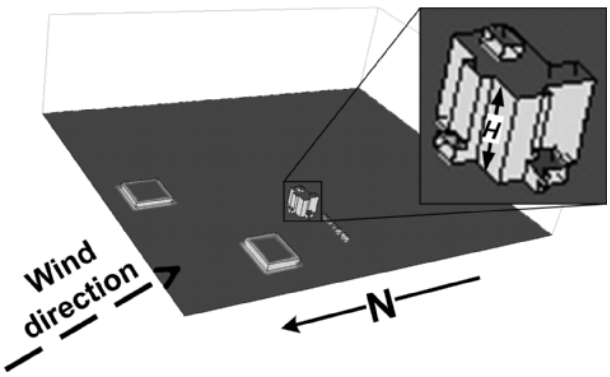

Figure 1 External flow domain when wind direction was 120 degrees with respect to the north. Note that the upwind and top perimeter boundaries are not shown.

To represent the atmospheric boundary layer, the velocity profile at the upwind (i.e., flow inlet) boundaries was calculated as (ASHRAE, 2001):

$$U_h = U_{ref}\left(\frac{h}{h_{ref}}\right)^a \quad (3)$$

where U_h is the local wind speed (m/s) at height h (m) and U_{ref} is the reference wind speed (2, 4, 6, 8 or 10 m/s) specified at a reference height, h_{ref} (m), of 10 m. The exponent, a, was 0.14, representing an atmospheric wind boundary layer in an open terrain. The internal flow domain was a rough representation of the Hal Ross Mill building (Figure 2) which has five floors and a total volume of 9,200 m^3. Unlike the model of Chayaprasert et al. (2008), the flour mill's interior details (e.g., milling equipment) were not included in the internal flow domain, reducing the model construction and simulation computing times. However, major structural features such as elevator shaft, stair wells, and ventilation shafts were incorporated. All the floors were interconnected through various openings which represent opened doors and air vents located on these structural features. A square 0.025 m^3 fluid zone was placed around the middle of each floor. The velocity vector in each fluid zone was fixed parallel to the floor and pointed to the west, simulating a fan flow rate of 0.94 m^3/s. Note that the number and location of this simulated fan were different from those in the experiments in which two 20-inch floor fans with unknown flow rates were placed in each floor. All physical cracks and crevices on the actual building envelope were represented by equivalent leakage zones (ELZs). Two 0.09 m^2 ELZs were placed on each of the north, south, west, east and top sides of the building. The locations of these ELZs were chosen arbitrarily. For the north, south, west and east sides (i.e., vertical walls), the ELZs were located on the first and fifth floors. As an example, the ELZ on the south side of the fifth floor is magnified in Figure 2.

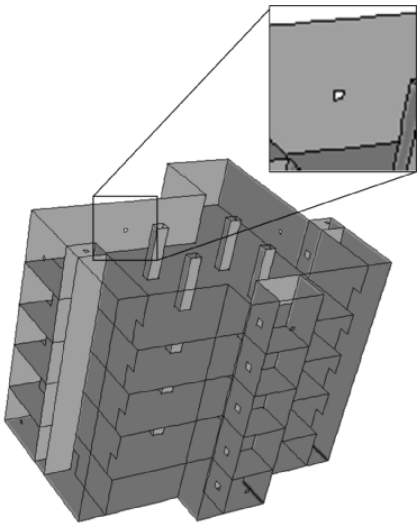

Figure 2 Internal flow domain divided into five floors.

For the top side (i.e., roof), an ELZ was placed at the top of each of the air ventilation shafts. All ELZs were assigned pressure boundary conditions with a flow resistance. The pressure difference, Δp, across each ELZ was calculated as:

$$\Delta p = k_L \frac{1}{2}\rho v^2 \quad (4)$$

where ρ and v are the density and velocity of the gas flowing through the ELZ, respectively. The gas-tightness of the mill building could be changed by adjusting the dimensionless loss coefficient, k_L, which was the same for all ELZs.

2.2 Model validation

The Hal Ross Mill model was validated using data from one 24-h methyl bromide (MB) and one 24-h sulfuryl fluoride (SF) fumigation experiments which are discussed in detail in another paper presented in this conference entitled, "Comparison of Leakage Rates of Methyl Bromide and Sulfuryl Fluoride during Structural Fumigations" by the same authors as this paper. Before each fumigation experiment, a building pressurization test was conducted. During each test, the building was pressurized by a specially calibrated fan to different pressure levels. At each pressure level, the flow rate through the fan and the static pressure difference across the building envelope were measured. The gas-tightness of the mill was characterized by the relationship between the pressure difference (ΔP, Pa) and airflow rate (Q, m^3/s) according to the following equation (ASHRAE, 2001): where b and n are the flow coefficient and pressure exponent, respectively. Instead of performing fumigation simulations with various values of the coefficient, k_L, until the model yields the correct HLT, the loss coefficient of the internal flow domain of the Hal Ross Flour Mill model was determined, before performing fumigation simulations, by simulating the pressurization test. The loss coefficient was satisfactorily determined when the simulated pressurization test provided the flow coefficient and pressure exponent (Eq. 5) that were almost identical to the ones obtained from the actual test.

$$Q = b\Delta P^n \quad (5)$$

One MB and one SF fumigation were simulated. The simulations were unsteady state flows with a time step of one minute. At each simulation time step, the pressure value assigned to each ELZ was a summation of the average stagnation pressure, which was predicted by the external flow model and was different for different walls, and the stack effect pressure (p_s), which was calculated by:

$$p_s = (\rho_o - \rho_i) g (H_{NPL} - H) \quad (6)$$

where ρ_o is the outside air density (kg/m^3), ρ_i is the fumigant–air mixture density in the building (kg/m^3), g is the gravitational constant (9.81 m/s^2), and H is the height of the ELZ (m). H_{NPL} is the height of the neutral pressure level (NPL) which was assumed at the middle height of the mill. Provided that the inner surface temperature of the walls of the internal flow domain was specified equal to the average ambient temperature measured inside the mill, the fumigant-air mixture was obtained from the Fluent® solution. The outside air density was explicitly calculated using the ideal gas law:

$$P = \frac{\rho_o R T_o}{M} \quad (7)$$

where M is the air molecular weight of 28.966 g/mol, and R is the universal gas constant of 8.3145 (m^3-Pa/K-mol). The ambient atmospheric pressure, P (Pa), and outside air temperature, T_o (K), were obtained from the fumigation experiments. Chayaprasert et al. (2008) conducted a simulation of an entire fumigation including the fumigant introduction phase, but in the present study only sections of the exposure period, where the fumigant concentrations were decreasing, were simulated. During the MB and SF experiments which lasted 24 h, three and one HLTs were observed in different elapsed exposure times, respectively. The HLT values and corresponding elapsed exposure times are listed in Table 1. Each elapsed time was simulated separately. For each simulation, the initial concentration was set to the value observed at the beginning of the respective period. At each time step, the average concentration in the internal flow domain was recorded. To determine the HLT, the resulting average concentration data were fitted to Eq. 1.

Table 1 Comparison between the average HLTs calculated from the actual concentration curves and those from the simulated curves. The last column lists the average wind speed measured at the flour mill during each elapsed exposure time.

Fumigation	Elapsed exposure time (h)	HLT (h)		Avg. wind speed (m/s)
		Experiment	Simulation	
MB	5th-10th	111.0	29.6	1.65
MB	10th-15th	16.4	17.0	3.52
MB	17th-24th	10.2	10.5	7.12
SF	5th-24th	19.7	20.0	3.67

3. Results and discussion

The pressurization test results for the MB and SF fumigations are plotted in Figures 3a and 3b, respectively. For both tests, the flour mill was pressurized between 20 and 80 Pa. The mill could not be tested for pressure levels lower than 20 Pa because prevailing wind was interfering with the test results, yielding unstable flow rate and pressure readings. On the other hand, simulations permitted pressurization tests for this lower pressure range. It can be seen that the results of the simulated pressurization tests were in good correlation with those of the actual test. This provided prior confirmation that the internal flow domain had essentially the same gas-tightness as the actual mill building. Comparing the two fumigations, the flow coefficients (0.10 versus 0.11) and pressure exponents (0.65 versus 0.63) were similar, indicating comparable sealing effectiveness.

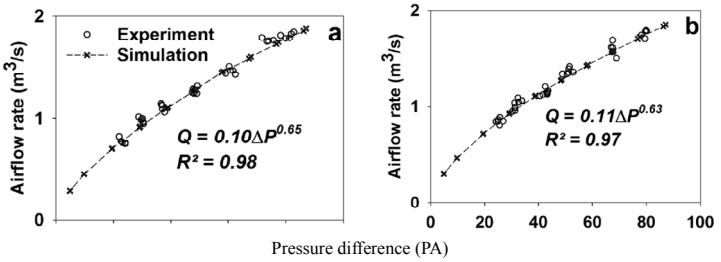

Figure 3 Experimental and simulation results of the pressurization tests for (a) MB fumigation and (b) SF fumigation.

The actual measurements of fumigant concentrations at all monitoring locations during the MB and SF fumigations are compared with the average concentrations obtained from the simulations in Figures 4a and 4b, respectively. By observation, it can be seen that the SF fumigation showed a relatively constant HLT, while the HLT for the MB fumigation changed as the fumigation progressed. Therefore, the entire exposure time of each fumigation was divided into sections according to the observed HLT values and each elapsed exposure time was simulated separately. For both fumigations, the differences in the observed concentrations within the mill were within 5 – 6 g/m^3 for most of the time, implying even fumigant distribution. At any point in time of each elapsed time, the simulated concentration curve stayed within this 5 – 6 g/m^3 band and followed the decreasing trend of the corresponding actual concentration curves relatively well. Table 1 compares the average HLTs calculated from the actual concentration curves with those from the simulated curves. While the simulation predicted a relatively long HLT (i.e., 29.6 h) for the first elapsed time of the MB fumigation, it was substantially different from the HLT calculated from the experimental concentration curves (i.e., 111 h). This substantial difference can be explained by the fact that at low fumigant leakage rates changes in the HLT are highly sensitive to changes in the gas. Given the initial concentration = 16 g/m^3 and elapsed time = 5 h, it can be shown using Eq. 1, that the final concentration will be 15.51 and 14.23 g/m^3 when the HLT is 111 and 29.6 h, respectively. While the difference in the final concentrations is approximately 8%, the difference in the HLTs is 73%. Except for the first elapsed time of the MB fumigation, the HLTs were accurately predicted with error of less than 1 h.

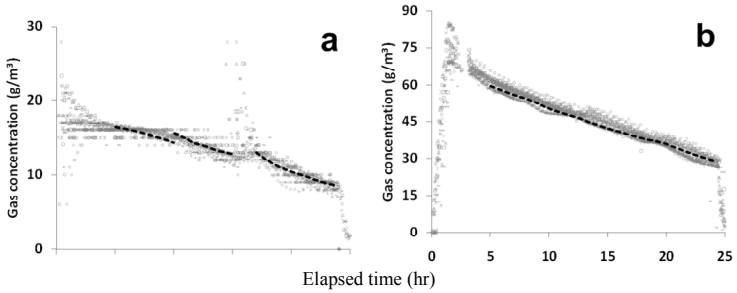

Figure 4 Comparison between the actual and average simulated gas concentrations for the (a) MB and (b) SF fumigations.

Variations in the environmental conditions, especially the wind speed, had a noticeable effect on the HLTs. The average measured wind speed for each elapsed exposure time is also given in Table 1. It is clear that the HLTs were well correlated with the wind speed measured at the flour mill during the fumigations. An argument could be made against the validity of the modeling approach of Chayaprasert et al. (2008) because these authors selectively varied the loss coefficient, k_L, until their model provided a satisfactory HLT prediction and the gas-tightness of the reference building was not directly quantified. A slightly different modeling approach was implemented in the present study. The environmental effect on fumigant leakage rates was effectively captured by the CFD model as indicated by the accurate HLT predictions after the leakage characteristic of the simulated building had been quantitatively specified to match that of the actual building. This shows that the CFD modeling techniques, mainly the implementation of ELZs and separation of the internal and external flows, used by both the previous and present studies are acceptable. In addition, exclusion of the milling equipment in the internal flow domain appeared to have negligible effect on the HLT prediction accuracy.

4. Conclusions

A CFD model of the structural fumigation process in the Hal Ross Flour Mill was formulated using the modeling methodology established by Chayaprasert et al. (2008). The model was validated using data sets from two fumigation experiments conducted in the flour mill. The actual building gas-tightness before the fumigation experiments was quantified by building pressurization tests. This quantified gas-tightness was incorporated into the model. While the model was simplified by reducing the number of external flow simulations and excluding the milling equipment in the internal flow domain, it was able to accurately predict the HLT values observed in the experiments. The result of the present study provides further validation to the modeling approach and emphasizes the importance of building pressurization test for accurate HLT prediction.

Acknowledgements

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The specific heat of wheat

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Abstract

Specific heat of Nanduan 1 variety of wheat was determined by Differential Scanning Calorimetry (DSC) in the temperature range of -10 to 110°C and initial moisture content from 19.90% wet basis reduced to 6.23% dry basis by 130°C oven drying. The specific heat ranged from 1.0792 to 5.5336 kJ/kg ºC. A multiple regression model relating specific heat to temperature and moisture content was developed in the temperature range of 0-70°C. The calculated curve fit the observed curve well, with an R-square of 0.99. An equation describing the relationship between density and moisture content was developed. The peak value of the density of wheat was observed in the moisture content range 8% to 10% wet basis.

Keyword: Specific heat, Wheat, Differential Scanning Calorimetry, Physical Properties, Model

1. Introduction

Specific heat is an important property of wheat for analysis of thermal processes because it indicates how much heat is required to change the temperature of a material. It is necessary for the design of drying, storage, aeration and refrigeration systems of grain storage.

Various methods of measuring the specific heat were mentioned by Jayas et al. (1996), and the mixture method was the most common method used for specific heat determination. In addition, differential scanning calorimetry (DSC) was regarded as the most reliable method to measure specific heat of biomaterials, and it is well suited for determining the effect of temperature on specific heat.

Most determinations of specific heat of wheat were carried out by mixture methods. Muir and Viravanichai (1972) used mixture method to study the specific heat of hard red spring wheat. Results showed a linear function of both temperature range -33.5 to 21.8°C and of moisture content in the range 1% to 19% wet basis; however, moisture contents of wheat were obtained by drying at 100ºC or adding weighed amounts of distilled water. ASABE D243.4 (2007) mentioned the specific heats of five kinds of wheat, at a limited range of moisture contents and temperatures, the major factors that affect the specific heat of wheat.

Satoshi (1987) measured the specific heat of 8 species of cereal grains by DSC in the range of about 10 to 70°C, and about 0% to 35% wet basis moisture content. Results suggested a linear relationship between the specific heat and moisture contents, and a quadratic relationship between the specific heat and temperature. However, the moisture contents were adjusted artificially and few other properties of cereal were mentioned.

The major objectives of this study were to determine the specific heat of Nanduan 1 variety of wheat with the moisture content reducing naturally in the temperature range of -10 to 110°C by DSC, to indicate the relationship between the specific heat and the interaction of temperature and moisture content and the relationship between density and moisture content.

2. Materials and methods

2.1. Sample Preparation

Wheat samples (Cultivar: Nanduan 1) used in this test were obtained from a farmer's field in Shandong province in east China during the 2008 harvest season and thoroughly cleaned by hand. Initial moisture content of the wheat sample was 19.90% wet basis. With the moisture content reducing naturally, the moisture content percent wet basis, density and specific heat capacity were determined at the same time. Dry basis wheat samples were obtained by drying samples in a forced draft oven for 19 h at 130°C; all weight loss was considered to be moisture.

2.2. Moisture content

The moisture content of the wheat sample was determined by the oven drying method, oven drying 19 h at 130°C,and all weight loss was considered to be moisture, according to the standardized procedure for moisture content determination by ASABE Standard S352.2 (2007). Three replicated measurements were carried out at each moisture content.

2.3. Density

The density of the wheat bulk (test weight) was determined by filling a one-litre graduated cylinder and weighing the amount of grain according to state standard of China (GB/T: 5498-85). The density of grain kernels (specific gravity) was determined by using a density balance (XS/XP-Ana Density Kit and Mettler xp205) scale graduated to 0.01 mg.

2.4. DSC Procedure

DSC-200PC (NETZSCH Germany) was used to determine the specific heat of Nanduan 1 variety of wheat. After calibrating the equipment for temperature and power, two empty aluminum pans of the same weight were placed in the sample and reference holders respectively. The calorimeter was adjusted to the initial, base-line temperature and allowed to isothermally equilibrate at -20°C, then scanned dynamically at 10ºC per min over the temperature range from -20°C to 120°C for the baseline. A sapphire run then followed: a 0.12 mg sapphire was placed in the sample pan and in the sample holder with an empty pan in the reference holder, and the same procedure was carried out for the standard sample line. The primary samples of wheat were divided into test samples of about 0.12 mg, which was near the weight of the sapphire, and the procedure was repeated for each of the replications of wheat at different moisture contents. The specific heats were calculated by comparison methods in the Netzsch thermal analysis system.

2.5. Statistical analysis

Average and standard error were calculated by PROC MEAN of Statistic Analysis System (SAS), figures were made by Microsoft Excel, the PROC REG procedure of SAS was performed to develop a multiple regression model of the specific heat as a function of temperature and moisture content, and the significance of the regression coefficients was determined at 0.05 levels.

3. Results

3.1. Density

Table 1 shows the moisture content, percent wet basis, and the density of samples of Nanduan 1 variety of wheat used in our studies.

Table 1 Density of samples used for determining specific heat

Moisture content[1] (%), wet basis	Density of kernels[2] (g/cm^3)	Density of grain bulk[1] (g/L)
19.90±0.03	1.3320±0.0027	748.09±0.02
19.41±0.07	1.3291±0.0024	739.00±0.60
18.17±0.04	1.3345±0.0017	759.34±1.08
17.16±0.04	1.3388±0.0016	770.43±0.27
16.77±0.03	1.3404±0.0015	776.87±0.49
15.88±0.01	1.3454±0.0010	783.03±0.20
15.32±0.01	1.3331±0.0071	783.93±0.18
14.60±0.12	1.3410±0.0030	789.80±0.25
14.13±0.09	1.3516±0.0018	794.10±0.50
13.53±0.01	1.3475±0.0047	794.80±0.05
13.45±0.01	1.3553±0.0008	796.95±0.25
12.58±0.11	1.3546±0.0015	796.76±0.20
12.08±0.08	1.3590±0.0009	797.50±0.10
9.95±0.01	/	802.05±0.65
8.17±0.05	1.3822±0.0011	805.00±0.70
6.23±0.01	/	805.03±0.42
0[3]	1.3663±0.0009	/

[1]Average of triplicates; [2]Average of 300 kernels; [3] Dried at 130ºC for 19 hrs; / indicates missing values; Means±SE.

3.1.1. Relationship between moisture content and density of wheat bulk

The relationship between moisture content and density of the wheat bulk is not linear, as shown in Figure 1, so a binomial equation was used to describe the relationship between moisture content and density of the wheat bulk:

$$\rho_b=765.61+9.1664M-0.5189M^2 \quad (1)$$

Where: ρ_b and M are density of bulk and moisture content on a percent wet basis, respectively; R-square is 0.9709, C.V. % is 0.46.

According to equation 1, the peak value of density of the wheat bulk is 806.09 g/L at 8.82% wet basis moisture content. So, the density of the wheat bulk is reduced as moisture content increases at high moisture contents, and it is reversed at low moisture contents.

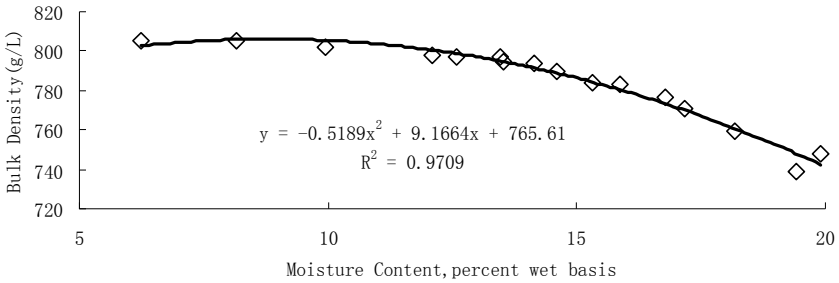

Figure1 Relationship between moisture content and the density of the wheat bulk.

3.1.2. Relationship between moisture content and density of wheat kernels

Figure 2 shows the linear relationship between moisture content and density of wheat kernels:

$$\rho_k=1410.5-4.1695M \quad (2)$$

Where: ρ_k and M are density of kernels and moisture content on a percent wet basis, respectively; R-square is 0.9521, C.V. % is 0.25.

According to equation 2, the dry basis density of kernels should be 1410.5 g/L; however, when the sample was oven dried for 19 hours at 130ºC, the density of kernels was only 1366.3 g/L. These results showed the same trend as the relationship between moisture content and the density of wheat bulk.

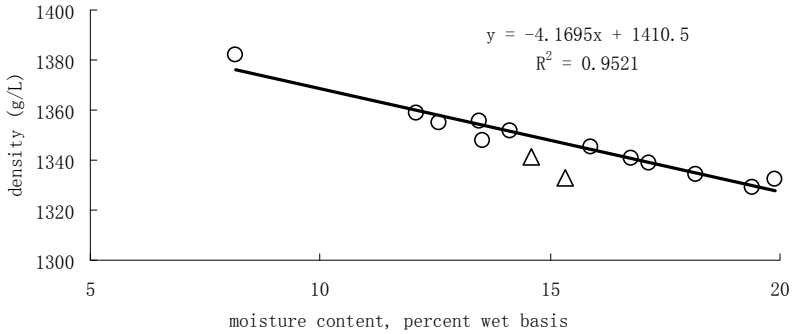

Figure 2 Relationship between moisture content and the density of wheat kernels

3.2. Specific heat

Specific heats of Nanduan 1 variety of wheat at 0.00%, 6.23%, 8.17%, 12.08%, 14.60%, 18.17 and 19.90% wet basis moisture contents were determined using DSC over a temperature range from -10 to 110ºC. The tests were carried out in a randomized order with nine replicates at each moisture content.

3.2.1. Relationship between the specific heat and temperature

The specific heat increased with temperature, but not always linearly, as was observed by other researchers. At low moisture content, the specific heats linearly increased with temperature, however, the specific heats increased at higher temperature more rapidly than at low temperature, and peak values occurred at about 85-90ºC and then were reduced, as shown in Figure 3. We checked the sample weight before and after DSC tests, and the results indicated that the reason for reduction in specific heat was the loss of water in the samples. The average weight loss was 1.10 mg (1.10±0.064 mg, sample weight 12 mg) at 19.90 percent wet basis and 0.33 mg (0.33±0.044 mg) at 8.17 percent wet basis; however, at 0.00 percent wet basis, the weight loss was almost nil. The specific heat could be related to temperature quadratically at the temperature range of 0-70ºC, and these equations are shown in Table 2.

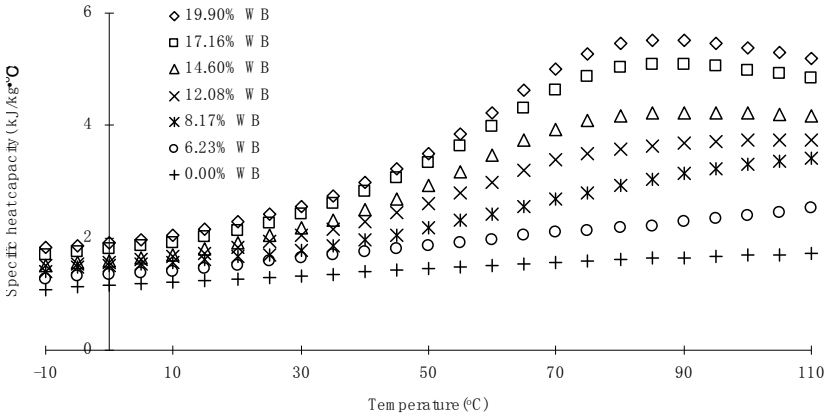

Figure 3 Relationship between the specific heat and temperature

Table 2 Relationship between specific heat and temperature at range of 0-70ºC

Moisture content		Quadratic equation*	R-square
Percent wet basis	Dry basis in decimal		
19.90	0.2484	c=1.9636+0.0021T+0.0006T^2	0.9992
17.16	0.2071	c=1.8073+0.0054T+0.0005T^2	0.9998
14.60	0.1710	c=1.5964+0.0076T+0.0004T^2	0.9995
12.08	0.1374	c=1.5709+0.0072T+0.0003T^2	0.9998
8.17	0.0890	c=1.5073+0.0033T+0.0002T^2	0.9996
6.23	0.064	c=1.3174+0.01T+0.00002T^2	0.9982
0	0	c=1.1399+0.0058T+0.000006T^2	0.9982

* T=temperature (ºC)

3.2.2. Relationship between the specific heat and moisture content

The specific heat data were linearly dependent on the moisture content, and these results were the same as many empirical investigations; therefore, linear regressions were applied as shown in Figure 4. For example:

$$c_{t10} = 1.2031 + 3.3292M \quad (3)$$

$$c_{t30} = 1.3145 + 5.1320M \quad (4)$$

Where c_{t10}, c_{t30} are the specific heat (kJ/kgºC) at 10 and 30ºC, respectively, and M is moisture content in decimal fraction, dry basis.

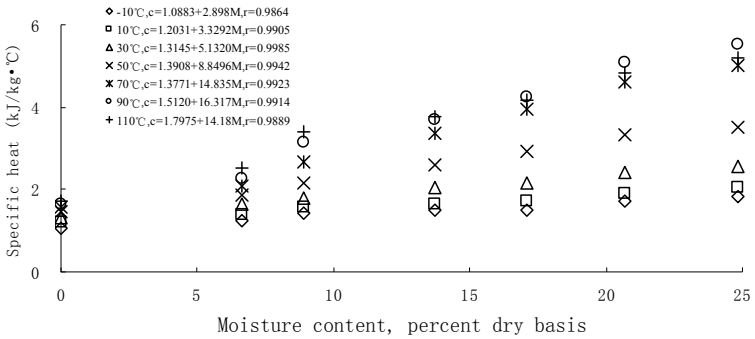

Figure 4 Relationship between the specific heat and moisture content

However, the coefficient of M in the linear equation of Nanduan 1 variety of wheat is affected significantly by the temperature. The relationship could not be simply estimated as the sum of the specific heat of its dry mass and the specific heat of water held in the product at the temperature range of 0-70°C and 4186J/kgºC of water, as described by Jayas et al. (1996) or Young and Whitaker (1973) that plots of specific heat versus moisture content at a constant temperature should have a slope of difference between the specific heat of water and dry mass and pass through the specific heat of water at a wet basis moisture content of 1.0.

3.2.3. Effect of Temperature and Moisture Content on specific heat

Table 3 shows the experimental values of the specific heat of Nanduan 1 variety of wheat every 5 degree at a temperature range of -10 -110ºC and at 0.00%, 6.23%, 8.17%, 12.08%, 14.60%, 18.17, and 19.90% wet basis moisture contents, and the specific heat capacity of wheat-nanduan 1 ranged from 1.0792 to 5.5336kJ/kgºC. In general, the specific heats of wheat increased with moisture contents and temperatures in the prescribed moisture content regions and temperature range from 0 to 70ºC.

Table 3 Experimental values of specific heat of wheat kJ/kgºC

Temp.	M (%WB)						
(ºC)	**19.90**	**17.16**	**14.60**	**12.08**	**8.17**	**6.23**	**0.00**
0	1.9168	1.7903	1.5961	1.5733	1.5145	1.3450	1.1518
5	1.9679	1.8455	1.6410	1.6160	1.5282	1.3746	1.1729
10	2.0475	1.9073	1.7045	1.6626	1.5611	1.4013	1.1981
15	2.1549	2.0172	1.8039	1.7336	1.6024	1.4541	1.2243
20	2.2773	2.1381	1.9169	1.8286	1.6560	1.5113	1.2538
25	2.4128	2.2650	2.0354	1.9278	1.7061	1.5838	1.2815
30	2.5663	2.4184	2.1663	2.0351	1.7787	1.6385	1.3163
35	2.7535	2.5982	2.3207	2.1578	1.8587	1.6926	1.3515
40	2.9761	2.8201	2.4995	2.2911	1.9511	1.7453	1.3872
45	3.2238	3.0667	2.6992	2.4404	2.0581	1.8025	1.4206
50	3.5052	3.3427	2.9296	2.6085	2.1746	1.8573	1.4533
55	3.8401	3.6435	3.1860	2.7950	2.3020	1.9176	1.4866
60	4.2108	3.9708	3.4574	2.9975	2.4289	1.9767	1.5151
65	4.6250	4.3141	3.7314	3.2073	2.5587	2.0341	1.5430
70	4.9967	4.6276	3.9391	3.3773	2.6839	2.0867	1.5673

Average of nine repeats

Based on the analysis of the relationship among the specific heat, temperature and moisture content, respectively, above, the specific heat could be approximately related to temperature quadratically and to moisture content linearly. So a multiple regression model with interaction terms correlating the specific heat to temperature quadratically and moisture content linearly was developed:

$$c=1.11479+3.37138M+0.00913T-0.00028778MT-0.00009249T^2+0.00003354MT^2 \quad (5)$$

Where, C=Specific heat, kJ/kgºC; T=Temperature range from 0 to 70ºC; M=Moisture content, dry basis in decimal, ranging from 0.00% to 19.90% wet basis.

In an analysis using PROC REG of SAS, R-square is 0.9903, root MSE is 0.0819, and C.V.% is 3.6756. Equation 5 can be changed to

$$c=1.11479+0.00913T-0.00009249T^2+(3.37138-0.00028778T+0.00003354T^2)M \quad (6)$$

$$c=1.11479+3.37138M+(0.00913-0.00028778M)T-(0.00009249-0.00003354M)T^2 \quad (7)$$

Equation 6 and 7 indicate that the specific heat linearly increases with moisture content at certain temperatures and quadratically increases with temperature at certain moisture contents, respectively.

4. Discussion

Few reports mentioned, as shown in our studies, that the density of Nanduan 1 variety of wheat decreases with the increase of moisture content at high moisture contents, and that this is reversed at low moisture contents, or that the peak value of density was at the moisture range 8%-10% wet basis. The hardness index, weight and diameter of the sample kernels were tested. The results shown in Table 4 indicated that the average weight loss of kernels was linearly reduced with the decrease of moisture content; however, the average decrease of diameter of kernels was reduced with reduced moisture content (shown in Figure 5). These results confirmed our finding of relationship between densities of wheat and moisture contents.

Table 4 The hardness, weight and diameter of Nanduan 1 variety of wheat

Moisture content Percent wet basis	Hardness index		Weight(mg)		Diameter(mm)	
	Avg.*	S.Dev.	Avg.	S.Dev.	Avg.	S.Dev.
19.90	52.44	16.17	35.32	8.26	3.20	0.55
8.17	69.65	18.12	31.38	7.45	2.98	0.46
0.00	74.82	23.23	28.24	6.62	2.90	0.51

*Average of 300 kernels.

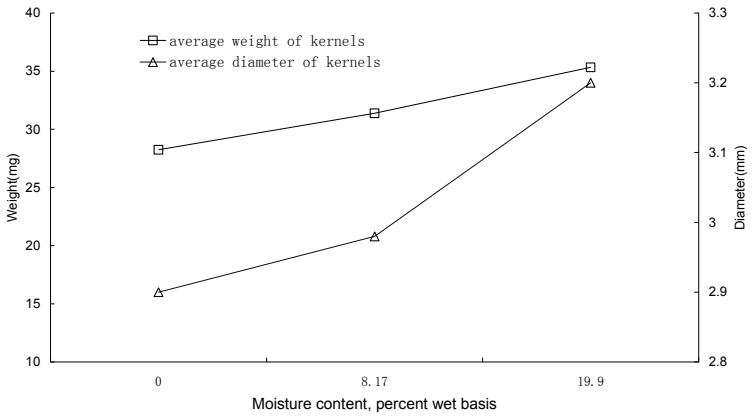

Figure 5 Relationship among moisture content and weight, diameter of kernels

Determination of the specific heats of Nanduan 1 variety of wheat over the temperature range -10-110°C and over the range of moisture content wet basis from zero to 19.90% by the DSC method showed that: 1) The specific heat was linearly dependent on the moisture content at the range of 0-19.9% wet basis; 2) The specific heat could be approximately related to temperature quadratically at the temperature range of 0-70°C moisture loss significantly affected the specific heat tests at high temperatures by DSC; and 3) A multiple regression model with interaction terms correlating the specific heat to temperature quadratically and moisture content linearly was developed at the moisture and temperature range mentioned above with an R-square of 0.99.

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Dust explosions: A report on recent major explosions in Argentina and Brazil

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Abstract

In South America, grain elevators have not been exempt from dust explosions. Between 2001 & 2002 there were three major dust explosions, TOEPFER, Puerto San Martin (Argentina), San Lorenzo Terminal (Argentina) and COINBRA, Paranaguá (Brazil), with both great loss of lives and material. This presentation has three specific targets:

1. Explanation of how each of these explosions occurred.

2. How local industries reacted to prevent future explosions and reduce their severity.

3. Reinforce safety issues offering new perspectives on the explosion threat.

In Argentina and Brazil, due to this recent explosion frequency, there is a bigger industry alert on the hazard of grain dust explosions. We will see specifications and regulations to guide the building new facilites.

Facilities must be kept thoroughly clean; dust control systems must be installed and up-graded, together with the installation of adequate electronic hazard monitors which add higher levels of security. I hope this article will help prevent future explosions by alerting and reminding the industry of the need to reinforce safety issues and standards.

1. Introduction

Since these three major explosions happened, as part of my professional and institutional activities, I have been giving presentations on this issue, and I have been astonished to learn of the number of other smaller incidents involving dust explosions, where people have been killed, not only in Argentina, but also in the hot and humid heartlands of central Brazil.

In October 2001, a severe explosion left three dead and seven injured in the Terminal of A.C. TOEPFER in Puerto San Martín, Santa Fe province, Argentina. A month later a similar disaster destroyed the port terminal of COINBRA, Louis Dreyfus´ Brazilian grain subsidiary, in Paranaguá, Paraná State, Brazil. Fortunately, on this occasion, it was without casualties, but it did inflict complete material damage.

In April 2002, the San Lorenzo Terminal exploded in the Santa Fe province, Argentina. The result was tragic: three people were killed, nineteen injured and there was total destruction of the main infrastructure, resulting in millions of dollars worth of lost structures, equipment and material.

Other recent explosions, although less damaging, include the terminal of PRODUCTOS SUDAMERICANOS, in Punta Alvear, near Rosario, Argentina, on the Paraná river, in August 2000; the LOUIS DREYFUS Terminal in General Lagos, north of Rosario (where the world's largest oil-seed crushing plant is located, with a production capacity of 12 000 t per day) - sadly one person was killed in this explosion – and an explosion in a flour silo at MOLINO ARGENTINO (a wheat flour mill) in 1995 in the Buenos Aries metropolitan area that killed three, but incredibly occurred without any material losses. It is not surprising that these explosions occurred, but the frequency with which they have done so in recent years is remarkable. In the previous 15 years, South America had not suffered any serious explosions.

Nevertheless, Argentina has had its share of accidents. One of the largest was in 1990, when we were surprised by the explosion of GENARO GARCIA Terminal in the port of Rosario, resulting in ten deaths. But the worst explosion was the tremendous tragedy that occurred in 1985 at the silos of JUNTA NACIONAL DE GRANOS, in Bahía Blanca, an ocean terminal, killing twenty-two and injuring more than ten people.

2. TOEPFER Puerto San Martín explosion, Argentina

The explosion happened in a tunnel underneath five steel bins during lunch on a sunny and dry spring day, one hour after loading a ship. Parallel and beside these bins there was a horizontal flat warehouse, that was empty, where five workers were doing civil maintenance works. Between the steel bins and the horizontal warehouse there was a connecting tunnel, that held a belt conveyor that collected both from these silos, the warehouse and from others, conveying to the shipping bucket elevators (Fig. 1).

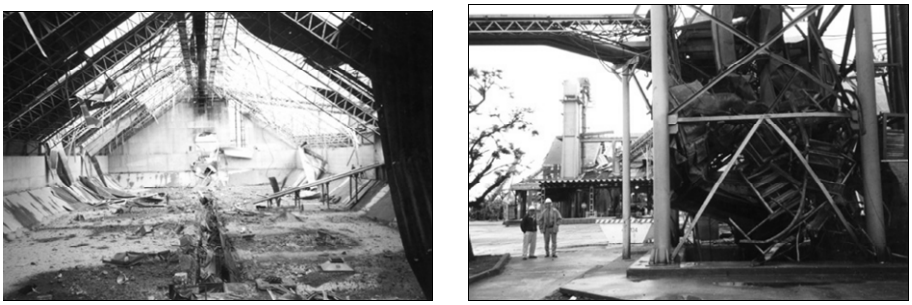

Figure 1 TOEPFER Puerto San Martín explosion (Santa Fe, Argentina) October, 2001

The first or primary explosion started underneath the steel bins, in the tunnel. Standing dust on the floors and edges was stirred up by the shock wave caused by this primary explosion, and provided the fuel for a secondary explosion, which was much more violent than the first, expanding quickly through the connecting tunnel to the tunnel underneath the warehouse, where the workers were. The warehouse was empty but two workers inside the tunnel were killed instantly and a third, working on the floor of the warehouse, was killed as the concrete tunnel roof blow up. A chain reaction of ever-increasing intensity had been set in motion that culminated with a third explosion that completely destroyed the reception area (about 300 m away from the starting point), and other concrete and metallic structures.

Curiously, the conveyor belt had stopped operating inside the tunnel where the explosion had started one hour before the explosion. Therefore, everything was still and quiet, and there was no dust-air mixture in suspension. An ignition source of sufficient energy, temperature and duration to initiate the explosion had to be present. Without an electrical spark or an overheated bearing in a confined area with fuel, and with no dust in suspension and nothing moving in the tunnel, an explosion seems impossible!

Experts think that the TOEPFER explosion was due to hexane gas rather than grain dust. Since rebuilding of the facility, hexane monitors have been installed in the tunnels which will detect gas leaking into the tunnels under the storage from the crushing plant next door. Steps were taken on both sides to correct this dangerous situation.

3. COINBRA Paranaguá explosion, Brazil

Again the explosion occurred at midday, whilst loading maize onto a ship, so luckily the main personnel were out for lunch, and no one was killed. Nevertheless, six people were injured, and there was massive damage to the facility. The information I have on the causes is acquired from the testimonies of people operating the neighboring terminal, who witnessed the explosion and whose facilities also suffered serious damage from huge pieces of airborne concrete that hit their facility during the explosion (Fig. 2).

The explosion started in the shipping bucket elevators were in operation, and was most probably due to belt misalignment. This primary explosion expanded quickly throughout the whole facility. The first explosion caused dust within the facility to be blown into suspension in the air, thereby contributing to a series of subsequent much more powerful secondary explosions. The secondary explosion was so strong that all resistant structures collapsed, even rail cars were turned over like toys, big pieces of concrete that weighed over 5 t were blown 300 m away, the steel shipping tower collapsed to earth. The destruction was followed by fire, which ignited the grain and continued burning for nearly three weeks.

Figure 2 The COINBRA Paranaguá Terminal explosion (Paraná, Brazil) November, 2001

4. Terminal San Lorenzo explosion, Argentina

This facility had a comprehensive housekeeping program that ensured dust accumulations were promptly and regularly cleaned. It included a brand new and highly efficient dust collection system, fitted with modern filters (bag houses) with low pressure automatic cleaning. A thorough maintenance program was in place as well as a training program for employees and contractors on the hazards of handling and collecting dust.

The explosion occurred while loading soya onto a ship on a dry and sunny autumn day. Again, luckily, the main group of personnel was out for lunch. Even so, the explosion left three dead, nineteen injured, and caused massive destruction of the terminal (Fig. 3). They had been loading a ship that was receiving simultaneously from three spots: from the horizontal silo, directly from trucks through the receiving pits and directly from railcars

Figure 3 The San Lorenzo Terminal explosion (Santa Fe, Argentina) April, 2002

All three spots were connected through a tunnel that collected from the horizontal silo, passed through the truck reception pits, through the railcar pits and continued to the shipping header house tower that supported the shipping-bucket elevators. This header house was built on a concrete structure, but with no walls, that could have helped to stop or deflect and dissipate the power of the destructive explosion energy wave.

An unknown ignition source ignited dust within the facility and resulted in a series of explosions that severely destroyed the heart of the port facility. The actual ignition source may never be known due to the damage that occurred in the tunnel beneath the horizontal silo, where the first or primary explosion started, and because the employee working in this area at the time of the explosion was killed.

This underneath tunnel in the flat storage, connected to another underneath collector tunnel, an underground avenue that led to the shipping tower. The collector tunnel collected grain from the truck reception area, from other tunnels under other horizontal warehouses and from the railcar reception area arriving to the bucket elevator pits in the shipping tower.

This underground infrastructure was a long network of confined spaces that distributed and accelerated the propagation and intensity of the explosion. A second worker was killed while operating the railcar gates and a third was found dead 3 days later in the shipping bucket elevator pit, 15 m below ground level, were the shipping tower stands.

Figure 4 The San Lorenzo Terminal explosion (Santa Fe, Argentina) April, 2002

4.1. San Lorenzo terminal reconstruction

The former idea of extended interconnecting conveying tunnel lay-outs with no bucket elevators has changed. It is now believed that the installation of bucket elevators will prevent the transmission of primary explosions to the rest of the facility.

It is interesting to observe the thinking behind the reconstruction. The design is supported by three main axes designed to minimize or eliminate confined spaces.

4.1.1. Minimizing explosion risks

- Eliminating tunnels where possible and instead using open galleries and catwalks that operate above ground, loaded by new bucket elevators at the end of each horizontal warehouse.
- Confining the risks to certain sectors by installing a bucket elevator at the end of every tunnel, eliminating connections between tunnels, avoiding the propagation of the explosion.
- All the mechanical handling is now fitted with hazard monitors, controlling speed, belt misalignment, belt slip, plugging and maximum belt extension, with emergency stop.
- The elevator towers are open and made of steel. Elevator pits are also open.

4.1.2. Reduction of environmental pollution

- Replacing cyclones with low pressure filters (bag houses).
- Adding thirty three aspiration systems with filters (bag houses), that collect the dust emitted during operations, in different sections of the facility.
- Installation of a white mineral oil application system for dust emission control.
- Installation of dust suppression systems in two of the four ship loading tubes, with telescopic hoses, minimizing the dust emission during loading of a vessel.

4.1.3. Increasing operational efficiency

By reducing belt speeds and increasing capacities, with wider belts to reduce dust generation. For example: Previously the shipping belt ran at a capacity of 1000 t/h at 400 m/s. By comparison, in the new facility the same conveyors run at a capacity of 1200 t/h at 300 m/s.

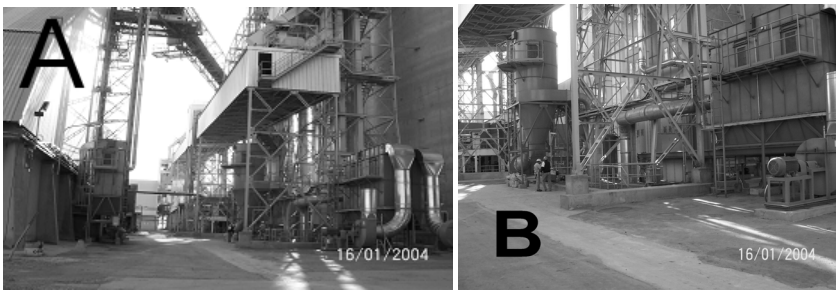

Figure 5 San Lorenzo terminal reconstruction: A. Open steel galleries above ground. B. Some of the 33 large aspiration systems with filters (bag houses) and open elevator pits

5. How the grain industry responded

In South America, there is still no specific legislation to prevent dust explosion hazard. However, in Argentina and Brazil, due to the frequency of recent explosions, there is now greater awareness within the industry on the hazard of grain dust explosions. Our vegetable oil industry is both very strong and conscientious so the companies are applying North American specifications and regulations to orientate new facility designs. Facilities are kept thoroughly clean, mineral oil applications are frequent, and dust control systems are installed and up-graded. The installation of adequate electronic hazard monitors is adding higher levels of security.

In order to assure that an explosion is not produced, the equation "dust + oxygen + confinement + ignition" must be altered. The elimination of any one of these requirements will prevent the reaction. This can be done by neutralizing ignition sources or eliminating or reducing the emissions of dust. Dust is eliminated by controlling its generation or installing vacuum systems that collect the dust in filter sleeves at each point of emission.

Sources of ignition can be eliminated principally by preventing electrical failures, by use of adequate non-explosive materials (cables, lighting, electrical components and motors) in equipment, and plants that are well designed and maintained. Mechanical failures, like the temperature of bearings and slipping and misalignment of the belts, can be controlled by the installation of adequate electronic monitors. These automatic monitors add a higher level of security as they are connected to an alarm system and are strategically located so they can shut down a conveyor and/or the entire facility if necessary.

Modern plants are constructed with extended lay-outs, to avoid elevators and confined spaces where possible, but some will always exist out of operational necessity: bucket elevators, tunnels under silos, linking tunnels, etc. It is very difficult to prevent an explosion. However, it is always possible to minimize the damage by designing light open structures and explosions reliefs that can dissipate the pressure created by a ‘primary’ explosion. Using openings that act like vents to prematurely dissipate the initial pressure wave and avoid its destructive and lethal propagation.

Figure 6 Open towers and explosion reliefs for tunnels.

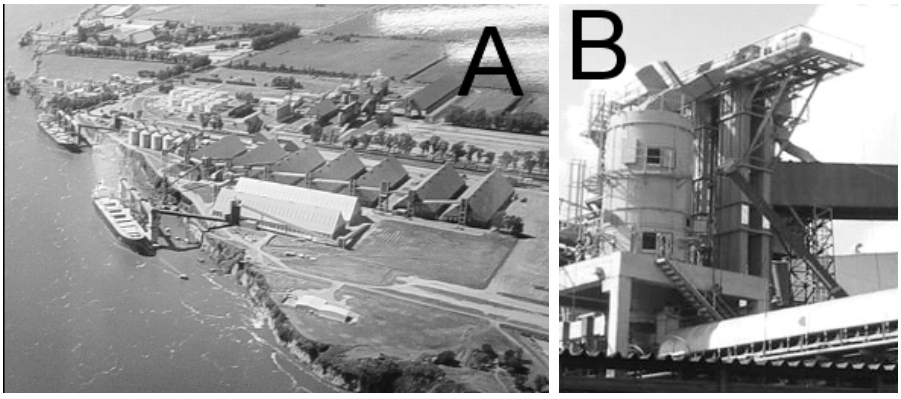

Figure 7 A. Facility with extended tunnel conveyor lay-outs with no elevators, B. Filter bag houses.

6. Conclusions

Grain dust is explosive, and by far the biggest risk in the grain storage and processing industries is a dust explosion. Wherever grains are handled, there is a potential to generate dust and consequently, the risk of explosion. The most important preventive practices to limit the dangers of dust explosion are to avoid the formation of explosive air-dust concentrations in confined spaces and limit any source of ignition that could ignite a primary explosion, thereby minimizing the risk of expansion that would generate secondary explosions.

Training and education must be intensive and essential. Facilities not properly designed and if poorly maintained, can be the source of expensive consequences. I hope this article will help prevent future explosions by both alerting and reminding the industry of the need to reinforce safety issues.

Identification of stored-grain insects using microwave/RF electric fields

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Abstract

Identification of the species and quantities of seven stored-grain insects were investigated with a new measuring method using a sensor and an electric field device in the frequency range of 0.3MHz to 1200MHz.. Three different constant voltages, 0, 20, and 40V and three different frequencies, 0.01Hz, 0.5MHz, and 5MHz alternating electric fields were tested to address the possibility of improving the identification of insects. Frequency ranges were optimized to maximize the identification and detection recognition rate using neural network techniques. Strong recognition rates of identification of species and quantities of insects were achieved at the band-pair (660.1 and 768.1 MHz) under 0, 20, and 40V constant electric fields and at the band-pair (174.3 and 432.2 MHz) under 0.01Hz, 0.5MHz, and 5MHz alternating electric fields. (Jones et al., 2009; Ding et al., 2009)

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The sorption isosteric heats of rice grains in China

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Abstract

Equilibrium moisture content (EMC) data for the grains of thirteen rice cultivars were obtained by gravimetric method at 11% to 96% equilibrium relative humidity (ERH) and 10, 20, 25, 30, and 35°C above nine saturated salt solutions. Six commonly used mathematic models, namely, BET, Modified-Guggenheim–Anderson-deBoer (MGAB), Modified-Chung-Pfost (MCPE), Modified-Henderson (MHE), Modified-Oswin (MOE), and Strohman-Yoerger (STYE), were fitted to the data with evaluating the coefficient of determination, residue sum-of-squares, standard error of estimate, mean relative percent error, and residual plots. The best fitted equations to the EMC/ERH data were MCPE and STYE, but MCPE is three-parameter, readily transformed equation and adopted in this study. The isosteric heats for both rice desorption and adsorption, and for both the sorption of Japonica and Indica rice, decreased rapidly with an increase in seed moisture content until the moisture content of 20% d.b. (dry basis) was reached, and thereafter they decreased smoothly with increasing moisture content. The isosteric heats of rice desorption were higher than those of adsorption below 22.5% d.b. EMC, but thereafter there was no difference found between desorption and adsorption. The sorption isosteric heats of Indica rice were slightly higher than those of Japonica rice under all moisture contents at a constant temperature.

Keywords: Equilibrium moisture content, Rice, Adsorption, Desorption, Mathematic model, Ventilation, Drying

1. Introduction

Rice is the major grain in China, with its annual production being around 180 million metric tons in recent years. Due to the large population, a portion of the rice grain is stored for a longer period of time (2 years) in China than in developed countries, with deterioration controlled largely through moisture content and temperature. In order to describe the process of drying, the ambient cooling of grain, and improving physical control in storage, a sound knowledge of the relationship between equilibrium moisture content (EMC) and equilibrium relative humidity (ERH) is essential. The most useful way to define this relationship is fitting the data to a suitable equation (Jayas and Mazza, 1991; Sun, 1999; Li, 2009). Many investigators have developed equations, theoretically, semi-theoretically or empirically to describe the sorption isotherms of rice. Chen and Morey (1989) compared four EMC/ERH equations such as Modified-Chung-Pfost (MCPE), Modified-Halsey (MHAE), Modified-Henderson (MHE), and Modified-Oswin (MOE) for rough and brown rice from three sources, and found except the MHAE, the other three equations could fit some or all of the rice data. In the 1996, version of the ASABE Standard D245.5 “Moisture Relationships of Plant-Based Agricultural Products”, the MCPE, MHAE, MHE, and MOE were recommended. Sun (1999) analyzed 17 source sets of rice EMC/ERH data with four commonly cited models, i.e. MCPE, MHE, MOE, and Strohman-Yoerger (STYE), and considered the STYE as the preferred equation. However, such suitable equation for Chinese rice varieties is rather deficient.

Nomenclature	
C_1, C_2, C_3, C_4	equation coefficients
d.b.	dry basis
EMC	equilibrium moisture content
ERH	equilibrium relative humidity
m.c.	moisture content
M_m	monolayer moisture content (% d.b.)
m_{mi}	the average of experimental value
m_{pi}	the predicated value

Nomenclature	
MRD	mean relative percentage error (%)
n	the number of observations
h_s	the isosteric heat of sorption (KJ/Kg)
h_v	the latent heat of vaporization of free water (KJ/Kg)
h_w	the differential heat of wetting (KJ/Kg)
R^2	coefficient of determination
P_s	saturate vapor pressure (Pa)
r.h.	relative humidity
RSS	residue sum of squares
SE	standard error
T	absolute temperature (^oK)
t	temperature (^oC)
w.b.	wet basis

Knowledge of the heat of sorption is important in understanding the mechanism of grain sorption. It is a valuable tool in designing the drying process (Iglesias et al., 1976). Design of more efficient drying systems could be achieved by stating the correct mathematical models to estimate the heat and mass transfer mechanisms (Thorpe, 2001; Li, submitted). The purpose of the present work is to determine the most suitable ERH/EMC model corresponding to the sorption isotherms of thirteen rice varieties in China, and calculate the isosteric heat of water sorption from the experimental data.

2. Materials and methods

2.1. Materials and experimental procedures

Thirteen rice varieties in China were used in the sorption isotherm experiments. These rice varieties, including three species of Japonica rice and ten species of Indica rice, were respectively collected from eight provinces of the major rice grain production regions in China during 2007 to 2009. The rough rice grains, paddy, used for this study were intact, clean and plump. For adsorption experiment, the rice grains were dried to the moisture content (m.c.) of 7-8% w.b. at 39.0°C in an oven, and then dehydrated by P_2O_5 solid in a dessicator to below 5% w.b. as being samples. For the samples of desorption experiment, the rice varieties were re-moisturized from below 5% w.b. to the m.c. of 22% w.b., and equilibrated at 4°C for two weeks.

The gravimetric static method, with standard saturated salt solutions in Table 1 to maintain constant vapor pressure (Jayas and Mazza, 1991), was used to obtain nine equilibrium moisture contents at each of five constant temperatures (10, 20, 25, 30, and 35ºC). Twenty-seven glass bottles with a volume of 250 ml each contained 65 ml salt solution, and were kept in one temperature controlled cabinet to maintain nine groups of different relative humidity levels ranging from 11.3 to 96% ERH. Every relative humidity at one temperature was triplicated and a total of 135 bottles was used in the experiment for five sorption isotherms of a rice variety. The temperature of cabinets was often proofed with a standard thermometer ranging from 0 to 50^{o}C, and controlled to an accuracy of ±0.5^{o}C. The sample of rice seeds (about 4-5 g) was put into a small bucket (3 cm diameter × 4 cm length) made from copper wire gauze, and hung into the 250 ml bottle on a copper wire pothook under a rubber plug, just 2-3 cm above saturated salt solutions. The rubber plug was tightly plugged into the bottle mouth. From three weeks after exposing the samples in the saturated vapour at 35^{o}C, the copper wire buckets with samples were weighed every other day until the change in mass between two successive readings was less than 2 mg. When the sample was exposed to a lower temperature, the sample was left several days to equilibrate. However, the rice grains exposed over the saturated potassium nitrate solution for 5 to 7 days at higher temperatures were susceptible to grow molds, and immediately taken out, once one seed had mould. The moisture content of the sample at this constant stage was defined to be the equilibrium moisture content and was determined by the vacuum oven method (AOAC, 1980). The sample was dried to constant weight under 103.0±0.5°C for 22-28 hours.

Table 1 The equilibrium relative humidity (%) produced by nine saturated salt solutions[a].

	Equilibrium Relative Humidity (%)					
	Temperature (°C)					
Saturated salt solution	**10**	**15**	**20**	**25**	**30**	**35**
Lithium chloride	11.29	11.30	11.31	11.30	11.28	11.25
Potassium acetate	23.38	23.40	23.11	22.51	21.61	21.50
Magnesium chloride	33.47	33.3	33.07	32.78	32.44	32.05
Potassium carbonate	43.14	43.15	43.16	43.16	43.17	43.16
Magnesium nitrate	57.36	55.87	54.38	52.89	51.40	49.91
Cupric chloride	68.40	68.40	68.30	67.00	66.50	66.00
Sodium chloride	75.67	75.61	75.47	75.29	75.09	74.87
Potassium chloride	86.77	85.92	85.11	84.34	83.62	82.95
Potassium nitrate	95.96	95.41	94.62	93.58	92.31	90.79

[a]Source: Jayas and Mazza (1991)

2.2. Analysis of the adsorption and desorption data

The adsorption and desorption equilibrium moisture content data of rough rice were fitted to the six moisture sorption isotherm equations given in Table 2, using the non-linear regression procedure in SPSS 13.0 for Windows, which minimizes the sum of squares of deviations between experimental and predicted data in a series of iterative steps. The goodness-of-fit of each equation was evaluated using determination coefficient (R^2), residue sum of squares (RSS), the standard error (SE), mean relative percentage error (MRE). The determination coefficient (R^2) was one of the primary criteria for selecting the best equation to fit the experimental data. In addition to R^2, the other statistical parameters, MRE as a percentage, RSS and SE were used to determine the quality of the fit. The equations (1) - (4) were used for calculating R^2, RSS, SE, and MRE, respectively.

$$R^2 = \sqrt{1 - \frac{\sum_{i=1}^{n}(m_i - m_{pi})^2}{\sum_{i=1}^{n}(m_i - m_{mi})^2}} \quad (1)$$

$$\mathrm{RSS} = \sum_{i=1}^{n}(m_i - m_{pi})^2 \quad (2)$$

$$\mathrm{SE} = \sqrt{\frac{\sum_{i=1}^{n}(m_i - m_{pi})^2}{(n-1)}} \quad (3)$$

$$\mathrm{MRE} = \frac{100\sum_{i=1}^{n}\left|\frac{m_i - m_{pi}}{m_i}\right|}{n} \quad (4)$$

where m_i is the experimental value m_{pi} the predicated value m_{mi} the average of experimental values, and n the number of observations. The fit of an equation is good enough for practical purposes when MRE is less than 10% (Aguerre et al., 1989).

Table 2 The cited EMC/ERH equations in the study.

Equations	**Formula**[a]
Modified-BET	$M = \frac{(C_1 + C_2 \times t) \times C_3 \times r.h.}{(1 - r.h.) \times (1 - r.h. + C_3 \times r.h.)}$ $(r.h. < 50\%)$
Modified-Chung-Pfost (MCPE)	$r.h. = \exp[-\frac{C_1}{t + C_2}\exp(-C_3 \times M)]$ or $M = -\frac{1}{C_3} \times \ln[-\frac{(t + C_2) \times \ln(r.h.)}{C_1}]$
Modified GAB (MGAB)	$r.h. = \frac{2 + \frac{C_3}{t} \times (\frac{C_1}{M} - 1) - \{[2 + \frac{C_3}{t} \times (\frac{C_1}{M} - 1)]^2 - 4 \times (1 - \frac{C_3}{t})\}^{\frac{1}{2}}}{2 \times C_2 \times (1 - \frac{C_3}{t})}$ or $M = \frac{C_1 \times C_2 \times (\frac{C_3}{t}) \times r.h.}{(1 - C_2 \times r.h.) \times (1 - C_2 \times r.h. + \frac{C_3}{t} \times C_2 \times r.h.)}$

Equations	Formula[a]
Modified-Henderson (MHE)	$r.h. = 1 - \exp[-C_1 \times (t + C_2) \times M^{C_3}]$ or $M = [-\frac{\ln(1 - r.h.)}{C_1 \times (C_2 + t)}]^{\frac{1}{C_3}}$
Modified-Oswin (MOE)	$r.h. = \frac{1}{1 + (\frac{C_1 + C_2 \times t}{M})^{C_3}}$ or $M = \frac{C_1 + C_2 \times t}{(\frac{1}{r.h.} - 1)^{\frac{1}{C_3}}}$
STYE	$r.h. = \exp[C_1 \times \exp(-C_2 \times M) \times \ln(P_s) - C_3 \times \exp(-C_4 \times M)]$

[a] *r.h.* represents moisture content, *M* is equilibrium moisture content (% dry basis), *t* is temperature (°C), and P_s is saturated vapor pressure. C_1, C_2, C_3, and C_4 are the coefficients of equations.

2.3. Determination of the isosteric heat of sorption

The total energy required to remove a unit mass of water from grain kernels, the differential heat of sorption, h_s, is conveniently partitioned into two components, namely the latent heat of vaporization of free water (h_v) and the differential heat of wetting (h_w). The h_v of adsorption and desorption of rice were respectively calculated by the following six equations according to Thorpe (2001).

$$\frac{h_s}{h_v} = 1 + \frac{p_s}{r.h.} \times \frac{dT}{dP_s} \times \left.\frac{\partial r.h.}{\partial T}\right|_{m.c.} \quad (5)$$

$$h_v = 2501.33 - 2.363 \times t \quad (6)$$

$$P_s = \frac{6 \times 10^{25}}{(273.15 + t)^5} \times \exp(-\frac{6800}{t + 273.15}) \quad (7)$$

$$\frac{dP_s}{dT} = \frac{P_s}{(t + 273.15)} \times (\frac{6800}{t + 273.15} - 5) \quad (8)$$

$$\left.\frac{\partial r.h.}{\partial T}\right|_{m.c.} = \frac{C_1 \times r.h.}{(t + C_2)^2} \times \exp(-C_3 \times m.c.) \quad (9)$$

The equation (5) enables one to calculate

h_s/h_v, provided dP_s/dT and $\left.\partial r.h./\partial T\right|_{m.c.}$

can be evaluated by equations (8) and (9), respectively. The h_v of free water in equation (6) is dependent on temperature. The saturated vapor pressure, P_s, can be calculated by equation (7). The derivative of *r.h.* with respect to t,

$$\left.\partial r.h./\partial T\right|_{m.c.}$$

Depends on the sorption isotherm equation used, and the Modified-Chung-Pfost（MCPE）in equation (9) used in this study.

3. Results

3.1. Fitting of sorption equations to experimental sorption data

The results of nonlinear regression analyses of fitting the sorption equations to the experimental data of desorption and adsorption isotherms were respectively evaluated with the indices such as correlation coefficient (R^2), residue sum of squares (RSS), the standard error (SE), and mean relative percentage error (MRE). Of the six commonly used equations, namely BET, MCPE, MGAB, MHE, MOE, and STYE (Table 2), five equations such as STYE, MCPE, MHE, MOE and MGAB gave the better fit to the experimental data of adsorption and desorption isotherms in a wide range of 11.3 to 96.0% ERH, but the BET equation gave the better fit in the range of 11.3 to 49.9% ERH (data not shown). The further comparisons of the sorption equations with a form of

$$r.h. = f(M, t) \text{ or } M = f(r.h., t)$$

for twenty-six sets of isotherm data are given in Table 3. The average values of R^2 and error parameters (RSS, SE, and MRE) for the twenty-six sets of isotherm data were calculated. For the form of

$$r.h. = f(M, t),$$

the equations were ranked for accuracy in an order: STYE, MCPE, MHE, MOE, MHE and MGAB, but for that of

$$M = f(r.h., t),$$

the order was: BET, MCPE, MHE, MOE, and MGAB. However, STYE is four-coefficient, temperature independent equation and can not be explicitly inverted to give EMC as a function of ERH. MCPE, MHE, MOE and MGAB equations all are three-coefficient, temperature dependent and easily invertible equations (Table 2). Thus, the MCPE with a form of

$$r.h. = f(M, t),$$

or with a form of

$$M = f(r.h., t)$$

was considered to best describe the equilibrium moisture data of thirteen rice varieties in a wide range of 11.3 to 96.0% ERH, and the best fitted coefficients for both adsorption and desorption isotherms of rough rice data were summarized in Table 4. For MCPE model, the three coefficients C_1, C_2 and C_3 of adsorptive isotherm equation were different from those of desorptive isotherm equation, and there were some difference in three coefficients C_1, C_2 and C_3 of between Japonica rice and Indica rice.

Table 3 Summary of the results of fitting equations to the data sets of thirteen pairs of rice desorption and adsorption.

Model function	Equation	Statistical parameters[a]			
		R^2	RSS	SE	MRD %
$r.h. = f(M, t)$	MCPE	0.99570	0.01440	0.00020	3.67480
	MGAB	0.99045	0.03092	0.00074	5.90547
	MHE	0.99192	0.02624	0.00042	5.04545
	MOE	0.99208	0.02421	0.00085	6.30338
	STYE	0.99594	0.01414	0.00044	3.65875
$M = f(r.h., t)$	BET	0.98096	1.91840	0.10670	2.98630
	MGAB	0.97031	47.73069	1.13642	8.46685
	MCPE	0.99225	12.24169	0.29150	3.02528
	MHE	0.98675	21.32694	0.50775	4.52040
	MOE	0.98031	31.34288	0.74628	6.02336

[a]The statistical parameter is average of the data sets of thirteen pairs of rice desorption and adsorption

Table 4 The best fitted coefficients of MCPE for sorption isotherms of rice.

Model	Data sets[a]	Model coefficients			Statistical parameters			
		C_1	C_2	C_3	R^2	RSS	SE	MRD %
$r.h. = f(M, t)$ (MCPE)	Desorption	412.543	35.300	0.181	0.9986	0.0046	1.094E-04	1.7654
	Adsorption	677.146	110.639	0.184	0.9981	0.0060	1.430E-04	2.1502
	Average	483.486	57.569	0.182	0.9985	0.0048	1.131E-04	1.6690
	Japonica rice	455.064	63.182	0.175	0.9978	0.0071	1.682E-04	2.5580
	Indica rice	504.668	58.354	0.184	0.9985	0.0048	1.136E-04	1.6934

[a]Data sets derived from the average sorption data of thirty rice varieties. Desorption, desorption isotherm; adsorption, adsorption isotherms; Average, the average values obtained from adsorption and desorption isotherms.

3.2. Isosteric heat of sorption

The isosteric heat of sorption (h_s) was calculated from the equations (6) to (9). The coefficients C_1, C_2, and C_3 of MCPE equation in Table 4 were used as the coefficients in equation (9). Figure 1 shows the influence of moisture content on rough rice adsorption and desorption isosteric heats, and on the sorption isosteric heats of Japonica and Indica rice determined from adsorption and desorption isotherms. The isosteric heats for both rice desorption and adsorption (Fig. 1A), and for both the sorption of japonica and India rice (Fig. 1B) decreased rapidly with increase in seed moisture content until the moisture content of 20% d.b. was reached, but above 20% d.b. they decreased smoothly with increasing moisture content. At lower moisture contents below 22.5% d.b., under lower temperatures the isosteric heats of both desorption and adsorption of rough rice, and of both sorption of Japonica and Indica rice were higher than those under higher temperatures. The isosteric heats of rough rice desorption were higher than those of adsorption below 22.5% d.b., but thereafter there was no difference found between desorption and adsorption (Fig. 1A). The sorption isosteric heats of Indica rice were slightly higher than those of Japonica rice at all moisture contents under a constant temperature (Fig. 1B).

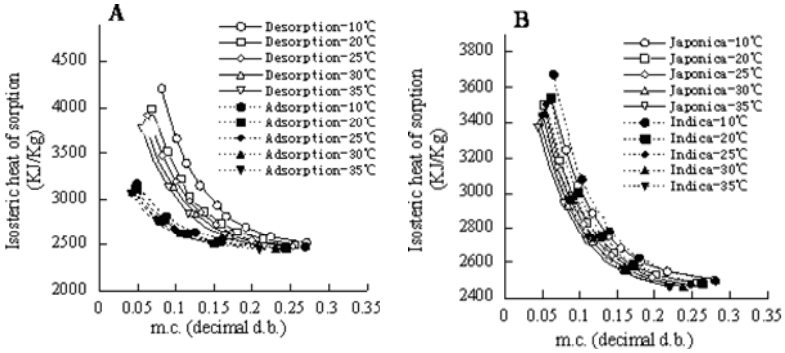

Figure 1 Comparison of adsorption and desorption isosteric heats of rice (A), and of the sorption isosteric heats of Japonica and Indica rice (B) at different temperatures (°C) predicted by the Modified-Chung-Pfost equation.

4. Discussion

Sun (1999) analyzed 17 source sets of rice EMC/ERH data with four commonly cited models such as MCPE, MHE, MOE, and STYE, and considered the STYE as the preferred equation. In this study, for the form of

$$r.h. = f(M, t),$$

the equations were ranked for accuracy in an order: STYE, MCPE, MHE, MOE, MHE and MGAB, but for that of

$$M = f(r.h., t),$$

the order was: BET, MCPE, MHE, MOE, and MGAB. We regarded MCPE as the but fitted equation due to it being three coefficients, invertible equations. Thus, MCPE was adopted in this study to calculate the rice isosteric heat of sorption.

The isosteric heats for both rice desorption and adsorption, and for both the sorption of Japonica and Indica rice, decreased rapidly with an increase in seed moisture content until the moisture content of 20% d.b. was reached, and thereafter they decreased smoothly with increasing moisture content (Fig. 1). The isosteric heats of rice desorption were higher than those of adsorption below 22.5% d.b. EMC, but thereafter there was no difference found between desorption and adsorption (Fig. 1A). These results show a little difference from the results reported by Öztekin and Soysal (2000) that the isosteric heats of rice desorption were higher than those of adsorption below 11% d.b. EMC, but above 11% d.b. EMC the isosteric heats of desorption were lower than those of adsorption. The sorption isosteric heats of Indica rice were insignificantly higher than those of Japonica rice under all moisture contents at a constant

temperature (Fig. 1B). The rapid increase in the heat of sorption at low moisture content might be due to the existence of highly active polar sites on the surface of rice grains, which were covered with water molecules forming a mono-molecular layer (Tsami, 1991). The decrease in the isosteric heats with higher amounts of sorbed water can be quantitatively explained by considering that sorption initially occurs on the most active available sites giving rise to high interaction energy. As these sites become occupied, sorption occurs on the less active ones, resulting in lower heats of sorption (Wang and Brennan, 1991). In low moisture contents, the values of the isosteric heats were higher than the latent heat of vaporization of water, indicating that the energy of binding between the water molecules and the sorption sites was higher than the energy which holds the molecules of pure water together in the liquid phase (Al-Muhtaseb, et al., 2004). At high moisture contents, there was no significant difference between the sorption isosteric heat and the latent heat of vaporization of water over the broad range of moisture contents. In the present study, the heat of sorption of rice grains might approach that of pure water at the moisture content of about 22.5%d.b.

It has been noted that h_s/h_v was calculated to be dependent on temperature, but the dependence was small (Thorpe, 2001). This temperature dependency of h_s/h_v was rather clear in Figure 1, which might arise from experimental errors in measuring the sorption isotherm, or from the sample properties such as variety, harvest time, pre-treatment, and so on.

In summary, MCPE was the best fitted equation to describe the EMC/ERH data of thirteen Chinese rice varieties, and was adopted to calculate the isosteric heat of sorption. The isosteric heats of rice desorption were higher than those of adsorption below 22.5% d.b. EMC, but thereafter there was no difference found between desorption and adsorption. The sorption isosteric heats of Indica rice were slightly higher than those of Japonica rice under all moisture contents at a constant temperature.

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Effects of preconditioning on quality of dried blueberries

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Abstract

Blueberries are a rich source of phenolic compounds, especially anthocyanins, which contributes to their high level of antioxidant activity. However, these compounds of significant health benefit will be degraded after the blueberries undergo various air drying processes. Drying of blueberries can be difficult due to the wax layer surrounding the fruit. The health properties may be reduced to a large extent due to long exposure to high temperature. In this study, a mechanical wax abrasive pretreatment is used to carefully remove the wax layer and reduce drying time. The abrasive drum was lined with medium grain sand paper and attached to a constant speed rotator. The amount of blueberries and the rotating time were optimized to reduce the damage to the blueberries. Rabbiteye blueberries cv. Climax were used as the test fruit. A laboratory scale cabinet dryer was operated at temperatures between 50 and 90ºC with single stage and multistage drying strategies. The drying rates, colour and level of phenolic compounds and antioxidant activity were evaluated.

Keywords: Blueberries, Air drying, Wax abrasive pretreatment

1. Introduction

It has been reported that consumption of blueberries contributes to a decrease in risk of cardiovascular diseases, inhibition of the growth of cancer cells and prevention of neurodegenerative diseases such as Alzheimer's disease (Heinonen et al., 1998; Seeram, 2008; Singh et al., 2008). The protective effects against diseases have been attributed to various antioxidants contained in blueberries. In addition to well-known vitamin C, phenolic compounds, especially anthocyanins, make a significant contribution to the total antioxidant activity due to their high concentration in blueberries (Prior et al., 1998). However, the antioxidants can be affected by processing conditions after harvest including heat, pH, oxygen and various storage conditions which result in alteration of the biological activity (Kalt et al., 2000).

The issue of seasonal availability may limit the consumption of fresh blueberries. Drying is, therefore, one of the methods employed to extend the availability of blueberries out of season. The drying process involves reduction of water content to the level at which the spoilage by micro-organisms or other reactions that cause deterioration are minimized or eliminated. Drying of blueberries can be difficult because blueberries contain a wax outer skin layer which acts as a barrier to moisture movement across the membrane. Therefore, various drying methods and pretreatments have been investigated and introduced. The food industry generally uses chemical pretreatment or osmotic dehydration to improve the drying rate and other related quality parameters. However, the cost of chemicals can be high. In addition, consumers increasingly prefer fruit that are naturally dried without chemicals. Dipping blueberries in an osmotic solution prior to drying is another alternative. It involves the use of inexpensive sugar to remove large amount of water from the fruits. Nevertheless, even though osmotic solutions can aid moisture loss and solid gain before drying, nutritive components from inside such as acids and phenolic compounds can be leached out during soaking (Raoult-Wack et al., 1992).

Due to these problems, mechanical means of wax removal have gained interest. The idea is derived from the abrasive mechanical skin/peel removal for crops such as potatoes. The raw materials are fed onto an abrasive roller or into a rotating bowl, the surface of which is made of carborundum or protrusions in a stainless steel sheet. The rough surface helps in the removal of the waxy skin coating that is then washed away by water. The benefits of the mechanical pretreatment include low energy cost, low capital cost and no heat damage. The factors which need to be considered include time, load, and drum speed (Singh and Shukla, 1995).

In this study, we aim at investigating the efficiency of wax abrasive pretreatment that will be used with single-stage or mult-stage hot air drying. It is expected that the investigated drying method could have

benefit in lowering the cost of production as well as preserving the quality and nutritional value of the dried blueberries.

2. Materials and methods

2.1. Blueberries

Rabbiteye blueberry (*Vaccinium ashei* Homebell) cv. Climax was obtained from Berry Exchange Farm, Corindi, New South Wales, Australia. The fruits were harvested based on colour at mature stage and stored in a box at ambient temperature for approximately 6 h before reaching the laboratory. The sample was further stored in the freezer at -18ºC until analysis.

Table 1 Level of damage after skin abrasive pre-treatment.

Level	Appearance
1	The berries remained in the same condition as the raw material (unbruised and undamaged). Cuticle was partially removed.
2	The berry's texture was softer than that of the raw material. There was no obvious damage observed on the skin of blueberries (see Figure 2).
3	1-3 damaged berries in a 50 g batch due to excessive skin abrasion. This damage could cause the loss of the pigment during the process (see Figure 3).
4	More than 3 damaged berries. Large amount of pigments and juice from the berries were lost. The berries were very soft.

2.2. Mechanical skin abrasive pre-treatment

A rotating drum was constructed for removing the cuticle of blueberries. The main part of the machine was an abrasive drum made of tin, lined on the inside with medium-grain sand paper. The tin sheet was 13.9 cm in length and 6.5 cm in diameter. The drum was fixed on a horizontal shaft which was attached to a constant-speed rotator. A blueberry sample of approximately 50 g was rotated in the drum for 7 min prior to drying. The drum speed was set at 100 rpm. Under these optimum conditions, the level of damage that was observed was minimised, based on the appearance of blueberries after abrasion..

2.3. Drying procedure

Drying of blueberries was carried out using our laboratory scale cabinet dryer. The dryer was turned on to achieve a steady-state temperature before loading the sample. The selected drying temperature was 70ºC for single-stage drying and for the multistage drying, the temperature profile was 90ºC for 90 min, then 70ºC for 120 min and finally 50ºC until a moisture content of 10-12% was reached which was equivalent to water activity (A_w) of 0.60 - 0.65. At this A_w products are believed to be stable and less likely to be subject to microbial spoilage. Relative humidity was maintained at 10±2% throughout the experiment and the air velocity was 2 m s^{-1}.

2.4 Extraction of anthocyanins and other phenolics

A 10-g sample of dried blueberries was ground in a mortar and then mixed with 75 mL of extracting solvent consisting of methanol:water:acetic acid at a ratio of 25:24:1. The extract was centrifuged at 15ºC, 13,000 rpm for 20 min. After removing the supernatant, the residue was mixed again with 75 mL of the extracting solvent followed by centrifugation. The combined supernatants were evaporated using a rotary evaporator at 35ºC. The residue obtained after evaporation was mixed with 5 mL of 3% (w/v) formic acid in water. Then it was loaded on a C18 Sep-Pak cartridge by using 3% (w/v) formic acid in methanol to elute the anthocyanins and other phenolic compounds. The eluted solution from Sep-Pak was evaporated by the rotary evaporator at 35ºC until a dry residue was obtained. The residue was then dissolved with 3 mL of 3% (w/v) formic acid in 15% methanol and filtered through 0.45 µm Millipore filter for further analysis.

2.5. Determination of total anthocyanins

Total anthocyanins determination was performed using spectroscopic pH-differential methods of Giusti and Wrolstad (2001). Samples were diluted in two buffers which were potassium chloride (pH 1.0) and sodium acetate (pH 4.5), then measured at 520 and 700 nm using UV-1601 Shimadzu UV-visible spectrophotometer (Shimadzu Scientific Instruments, Oceanic Pty. Ltd., NSW, Australia). Total

anthocyanin content was based on cyanidin-3-glucoside with a molar extinction coefficient of 26900 and molecular weight of 449.2. The results were expressed as mg of cyanidin-3-glucoside equivalent per g dried weight (mg C3G eq g^{-1} DW).

2.6. Determination of total phenolics

Total phenolics were determined by colorimetric method using Folin-Ciocalteu reagent (Slinkard and Singleton, 1977). A 150 µL volume of the diluted extract was mixed with 2.4 mL of distilled water and 150 µL of 1:10 diluted Folin-Ciocalteu reagent (2N). The mixture was allowed to react for 2 min before adding 300 µL of Na_2CO_3 solution (7.5% w/v) and mixed well. After 2 h incubation in darkness at room temperature, the absorbance of the solutions was measured at 765 nm. Gallic acid (0-0.1 mg ml^{-1}) was used to establish a standard curve. The results were expressed as mg gallic acid equivalents per g dried weight (mg GAE g^{-1} DW).

2.7. Determination of antioxidant activity

Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a source of free radical. 100 µL of diluted extract was allowed to react with 2,900 µL of 60 µM DPPH solution (in methanol) for 24 h in darkness at ambient temperature. The absorbance was then taken at 515 nm. Trolox (50-500 µM) was used as a standard and the results were expressed as µM Trolox equivalent per g dried weight (µM TE g^{-1} DW).

2.8. Identification of anthocyanins

A water alliance 2690 HPLC was used in anthocyanins identification. The column 2.1 mm x 150 mm Xterra™ MS C_{18} column (Waters Corp., Milford, MA, USA) was used to carry out the separation.

2.9 Measurement of colour

The surface colour of sample was measured by the colorimeter which offers three values of *L* (lightness), *a* (redness or greenness) and *b* (yellowness or blueness). The sample was packed in a transparent Petri dish. The mean of 10 readings was taken.

3. Results

3.1, Effects of pretreatment and drying temperature on drying time

To compare the effect of wax abrasive pretreatment and temperature on the drying rate, single-stage drying was used to establish the drying curve as shown in Figure 1. The drying curve at 90ºC was steeper than at 70ºC which indicated faster drying rate. Besides, the drying curve of wax removal pretreatment sample was steeper than the sample that had not been pretreated for both temperatures. As a result, the drying time of the pretreated sample required to reach a certain moisture level was shorter. The drying time was reduced from 20 h to 6.5 h at 70ºC and from 15 h to 4 hat 90ºC. In multistage drying, the drying of untreated sample was completed after 27 h while the pretreated sample was 7 h.

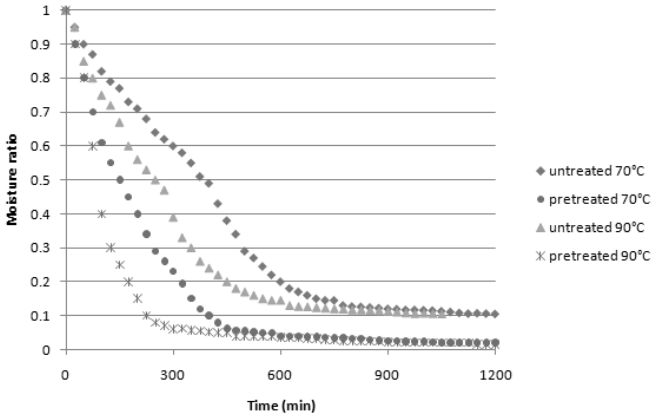

Figure 1 Drying curve of blueberries.

3.2 Determination of total anthocyanins, total phenolics, and antioxidant activity

The results for the effect of each treatment are summarized in Table 2. Fresh blueberries had the highest anthocyanin content, total phenolics, and antioxidant activity which were 14.5, 31.9 and 185.5, respectively. Generally, the wax abrasively pretreated sample showed higher values than the untreated ones. Total anthocyanins in untreated and pretreated samples were highest in drying at 90ºC followed by 70ºC and multistage drying, respectively. The same trend was also observed for total phenolics except between the pretreated sample dried at 70ºC and multistage dried where the values were not significantly different. The antioxidant activity in pretreated samples did not vary significantly between berries dried at 70ºC in the single-stage or multi-stage processes

Table 2 Effects of different treatments on total anthocyanins, total phenolics and antioxidants activity.

Treatments	Total anthocyanins (mg C3G eq/g DW)	Total phenolics (mg GAE/g DW)	Antioxidant activity (μM TE/g DW)
Fresh	14.5 ± 0.5^{a}	31.9 ± 2.1^{a}	185.5 ± 4.7^{a}
Untreated 70 °C	4.9 ± 0.9^{b}	17.5 ± 2.6^{b}	154.6 ± 5.7^{b}
Untreated 90 °C	6.2 ± 1.2^{c}	20.6 ± 0.7^{c}	159.3 ± 3.0^{c}
Untreated multistage	3.9 ± 0.5^{d}	12.3 ± 1.0^{d}	148.5 ± 4.7^{d}
pretreated 70 °C	10.6 ± 0.7^{e}	24.8 ± 1.7^{e}	164.9 ± 5.1^{d}
pretreated 90 °C	12.9 ± 0.8^{f}	$28.1. \pm 3.1^{f}$	174.4 ± 6.5^{e}
pretreated multistage	7.5 ± 0.1^{g}	24.5 ± 0.9^{e}	163.6 ± 10.6^{d}

Mean values followed by the same superscripts within the same column are not significantly different ($P<0.05$)

3.3. Colour characteristics

Mean colour values are shown in Table 3. The *L* and *a* values decreased for the dried blueberries while ***b*** value was more negative compared with the fresh sample. There was no difference in colour between dried samples**.**

Table 3 Effect of drying on the surface colour of blueberries.

Treatments	*L*	*a*	*b*
Fresh	38.41 ± 0.91^{a}	2.57 ± 0.60^{a}	-3.36 ± 0.51^{a}
Untreated 70 °C	34.61 ± 1.32^{b}	1.47 ± 0.32^{b}	-5.72 ± 0.57^{b}
Untreated 90 °C	35.83 ± 1.78^{b}	1.03 ± 0.85^{b}	-5.67 ± 0.79^{b}
Untreated multistage	34.58 ± 2.75^{b}	1.08 ± 0.93^{b}	-5.71 ± 1.06^{b}
pretreated 70 °C	36.20 ± 1.82^{b}	0.98 ± 1.09^{b}	-5.85 ± 0.42^{b}
pretreated 90 °C	35.52 ± 0.43^{b}	1.35 ± 0.74^{b}	-5.77 ± 1.53^{b}
pretreated multistage	35.81 ± 1.33^{b}	1.46 ± 0.50^{b}	-5.79 ± 1.19^{b}

Mean values followed by the same superscripts within the same column are not significantly different ($P<0.05$)

4. Discussion

4.1. Effect of pretreatment and drying temperature on drying time

Drying temperature significantly affected the moisture removal rate during drying of blueberries. Higher temperatures resulted in shorter times required to dry to specified moisture contents. Overall drying rates increased with increase in temperature for both fresh and pretreated blueberries.

Abrasive pretreatment was relatively effective in terms of drying time reduction. Total drying time decreased nearly 4 times for all drying temperatures. The results agreed with the work carried out on plums where abrasive pretreatment can markedly reduce the drying time while product quality were comparable to those that had undergone chemical pretreatment (Cinquanta et al., 2002; Di Matteo et al., 2002). However, additional research is recommended to determine optimum conditions for the process time, load and speed of the rotator. Different blueberry cultivars may require different conditions because the characteristic of their pigment-containing epidermal cell are varied (Allan-Wojtas et al., 2001). In this experiment, damage level was based on their appearance and divided into four levels as described in

Table 1. The sample that was subsequently used in the drying experiment was in level 1 or 2 in order to prevent the loss of anthocyanin pigments.

Figure 2 Blueberry appearance at level 2 damage after skin abrasive pre-treatment

Figure 3 Blueberry appearance at level 3 damage after skin abrasive pretreatment

4.2. Determination of total anthocyanins, total phenolics and antioxidant activity

During the drying process the blueberries were exposed to high temperature and air for a prolonged period of time which contributed to the loss of their antioxidants since anthocyanins are unstable in the presence of heat and oxygen (Kalt et al., 2000). Table 2 shows how different drying treatments cause loss of anthocyanins and also of phenolic content. The multi-stage drying strategy was used due to the fact that the rate of moisture removal is high at the beginning and become slower after a certain period. Thus, instead of using only one drying temperature, samples were dried at 90ºC for 90 min then at 70ºC for 120 min with a final temperature of 50ºC being used at the end of the drying treatment. The aim of using such temperature profile was to prevent blueberries from extended exposure to high temperature which can result in reduction of their antioxidant activity. However, the result revealed highest loss of antioxidants in this drying strategy especially for blueberries that had not been subjected to the abrasive pretreatment. This may explain the long drying time, 27 h, compared with 20 h and 15 h in single-stage drying tests, namely at 70ºC and 90ºC, respectively. With the aid of abrasive pretreatment, the time required to complete the drying process was similar to that used to dry at 70ºC and multistage drying (6.5 and 7 h). There was also no difference between the total phenolics and their antioxidant activity values. The results reflected that the loss of anthocyanins and phenolic compounds may be attributed mainly to the duration of drying. Thus, high temperature drying with shorter time could lessen the reduction of antioxidant activity versus using low temperature for a longer time.

4.3 Colour

Dried products had a darker colour compared to fresh blueberries indicated by their lower *L* values. The ***a*** values were lower than the fresh sample which can be explained by the degradation of the anthocyanin pigments during the drying process. It is suggested that anthocyanins can be converted to a colourless carbinol base upon heating (Nsonzi and Ramaswamy, 1998). This may also account for the more negative *b* value. Thus, dried blueberries had more bluish-brown appearance. Similar results were also found in blueberries dried with various air-drying methods studied by Nsonzi and Ramaswamy (1998) and Yang and Atallah (1985). Within the dried sample, the colour of either pretreated or untreated blueberries differed in a relatively narrow range. There was no significant trend observed visually or by using colorimeter between each drying treatments.

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Effect of nut-in-shell storage conditions on volatile profile in macadamia nuts

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Abstract

In order to study the effect of storage conditions on the volatile profile of macadamia nuts, both temperature and the presence of oxygen were controlled. Nuts-in-shell of variety 246 were stored at -18, 10 and 15°C. At each temperature, samples were stored in net bags and vacuum packed in EVOH (ethylene vinyl alcohol) for a period of 2 months. Prior to testing, samples were cracked and roasted. Analytical tests included peroxide value, p-anisidine and TBA (thiobarbituric acid number). Volatile compounds were isolated using Head-Space-Solid-Phase-Micro Extraction (DVB/PDMS/CAR). Volatiles were analysed by chromatography/mass spectrometry (GC/MS). The results show that at –18°C peroxide, p-Anisidine and TBA values were lower compared to the other 2 storage temperatures. This indicates that lipid degradation was lowest at this temperature. The main compounds found in volatile components of both fresh and roasted macadamia nut were hydrocarbons, aldehydes, and ketones. Several compounds deriving from lipid degradation and Maillard reaction were detected such as hexanal, thiazole. This indicates that changes in volatiles of macadamia nuts result from compounds present in macadamia nuts which were the precursors of volatile compounds produced during roasting. Storage materials which were net bag and EVOH showed no significant effect on volatile produced during 2 months storage trial. Volatiles generated depend mainly on processing steps such as roasting. The lipid degradation was minimised at low temperature. Therefore storage conditions for nut-in-shell influence the final macadamia kernel quality, especially shelf-life, as peroxide value and other lipid degradation product were used as criteria of shelf-life changes while volatile produced by Maillard reaction indicated roasting quality.

Keywords: Macadamia, Volatiles, Storage conditions, Head-Space-Micro Extraction, Lipid changes

1. Introduction

Macadamia nuts are among the most nutritious and highest in monounsaturated oil content among edible nuts (AMS, 2009). They are widely grown in Australia, USA, especially in Hawaii, South Africa and Guatemala (USDA, 2008). According to World Horticultural Trade & U.S. Export Opportunities (2002), Australia is the biggest macadamia producer and exporter, accounting for 46% of total macadamia world production. As the industry expands, the importance of maintaining kernel quality is increasing. High quality will increase competitive advantages and increase profits. Usually during harvest season, which is March to October in Australia, macadamia processors receive 10 tonnes or more per week. It is difficult to process all the nuts in the limited time. Therefore, in practice, macadamia nuts are de-husked and dried to 8-10% wet basis moisture content and stored as nut-in-shell until the processor is able to handle the nuts, which might take up to 1-2 months. During that time, kernel deterioration might occur. Due to the large amount of unsaturated fat that they contain, macadamia nuts are susceptible to rancidity. Rancidity is influenced by post-harvest treatments which could contribute to inferior nut quality. As macadamia nut quality is based on oil content and oil is its major component, it is important to investigate the relationship between post-harvest treatment and fatty acid content in macadamia nuts. Other biochemical changes such as changes in enzyme activity may occur. Changes in lipids influence the volatile compounds produced during roasting affect macadamia's unique aroma and flavour. Such changes may also affect consumer preferences. Hence this study focused on how storage conditions influence volatile profile in macadamia nuts. The aim of this study was to:

a. to determine the volatile compounds produced under defined storage conditions,
b. to evaluate the impacts and consequences of nut-in-shell storage conditions on macadamia nut volatile profile.

2. Materials and methods

2.1. Storage conditions

Macadamia nuts-in-shell (NIS) of variety 246 received from Northern New South Wales, (Australia) were stored for 2 months at -18, 10 and 15°C, respectively. Initial moisture content of macadamia was approximately 8-10% wet basis. For each temperature, samples were stored in both net bags and vacuum packed in EVOH. Prior to testing, samples were cracked and roasted at 125°C for 2 hours in an incubator until the kernels became slightly brown.

2.2. Lipid degradation tests

Analytical chemistry tests were performed such as peroxide value, p-anisidine and TBA (thiobarbituric acid test). Samples were analysed by an indirect colorimetric method (Yaacoub et al., 2009*)*.

2.3. Analysis of volatile compounds

Treated macadamias were placed in glass jars (height 10 cm, radius 3.15 cm total volume 117 cm^3). Volatile compounds were isolated by Head-Space-Solid-Phase Micro-Extraction. Sample and fiber (DVB/PDMS/CAR) were left to reach equilibrium for 1.5 hours at 30°C. The absorbed sample was manually injected into a gas chromatograph (Agilent 6890 N and 5975 inert mass selective detector, Agilent Technologies, USA) coupled with a mass spectrometry (GC/MS). The column used was a fused silica capillary with 5% phenylpolysiloxane as the non polar stationary phase (60 m long × 220 µm i.d. × 0.25 µm film thickness, SGE, USA).The injection port was operated in spiltless mode. Colum pressure was 165.5 kPa with average carrier gas velocity of 26 cm/s .The temperature program started at 30°C and temperature was increased 2°C/min until it reached 180°C. At the final stage, temperature was increased at 50°C/min until the oven temperature was 250°C. Data were collected with HP CHEMSTATION software and peak mass spectra were searched in the Wiley registry of mass spectral data to facilitate identification of individual volatile components.

3 Results and discussion

3.1. Lipid degradation

Measurement of lipid degradation by-products was performed by a colorimetric method (Yaacoub et al., 2009*)*. The change of colour varied in proportion to the amount of compounds in the sample. Figure 1 shows that overall, lipid oxidation was minimised if the sample was stored at 18°C especially with the absence of oxygen (Fig.1a) and (b). However, lipid oxidation rate as indicated by the amounts of lipid oxidation compounds including peroxide, TBA and p-anisidine value were not significantly different between samples with presence (net bags) and absence of oxygen (EVOH vacuum packing). Therefore macadamia processors may not need to invest in oxygen control system to maintain nut quality. However, this study was conducted for only 2 months, and different outcomes may result for longer storage time, as oxygen plays an important role in lipid degradation processes. Peroxide value of after-roasted samples was higher compared to before-roasting (Figure 1). This was because the high roasting temperature accelerated lipid oxidation and decomposition of hydroperoxides was lower than production of hydroperoxide compounds. In contrast to other samples, peroxide value of vacuum pack stored at -18°C was reduced after roasting, which can be seen from Figures 2b and 2c. This indicated there might be a correlation between the peroxide value and TBA at -18°C. TBA, which measures the amount of malondialdehyde, was the only value that increased as storage temperature (net bag) became lower. This explanation could be that peroxide compound underwent secondary oxidative reaction, resulting in the increasing of TBA values at the lower temperatures.

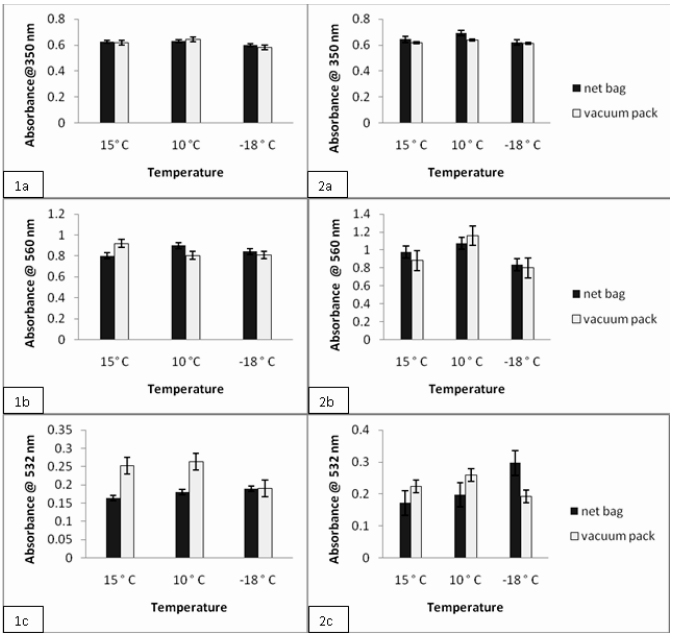

Figure 1 Accumulation of lipid degradation by products of macadamia nut expressed as absorbance against storage temperatures; (a) p-anisidine value before (1a) and after roasting (2a), (b) Peroxide value before (1b) and after roasting (2b), (c) TBA value before (1c) and after roasting (2c).

3.2. Volatile profile

Regardless of packaging material and storage temperature, the concentrations of volatile compounds found in the stored unroasted nuts were not significantly different. From Table 1, hexanal was found in all vacuum packed unroasted nut samples in storage, irrespective of storage temperature. Other volatiles detected were octanal, heptanal and hydrocarbons while in roasted nuts, compounds detected were pyrazine, furan, thiazole, pyranose and pyran. Compounds present in macadamia nuts before and after roasting were qualitatively and quantitatively different. Volatile compounds generated during roasting can occur through Maillard reaction and lipid oxidation pathways. More compounds arose from Maillard reaction than from lipid degradation.

Macadamia is mainly composed of lipids, proteins and sugars. These chemical elements can participate in chemical reactions to form new compounds. Macadamia has high contents of oleic and linoleic acids which oxidize and decompose into hexanal or octanal (Shahidi, 2001) which is supported by the results shown in Table 1.

Table 1 Classification of compound presence in before and after roasting (oven at 125°C) of macadamia nut-in-shell stored for 2 months.

Compounds	
Before roasting	After roasting
Hexanal	Pyridinamine
Octanal	Pentanamine
Butenal	Pyrazine
Ester	Thiazole
Indole	Oxazine
Nonyl aldehyde	Hexanol
Heptanal	Cyclobutanone
Butanone	Pyrrolidine
Propenal	Propenal

Compounds	
Octyldodecan-1-ol	Butanone
Propenal	Pyran
	Furan
	Pyranose
	Pyrrolepyran
	Aziridine
	Amine

Predominant volatile compounds produced during storage of macadamia vary between different nuts. Hexanal was the major volatile compound formed in almond, Brazil nut, hazelnut, pecan, pine nut and pistachio by lipid oxidation while propanal was highest in walnut (Miraliakbari and Shahidi, 2008). Crain and Tang (1975) reported that methyl sulphide, methylpropanal, 2-methylbutanal, 3-methylbutanal were present in high concentrations in roasted macadamia nut. The major volatiles such as pyrazine, furan, thiazole, pyranose, and pyran are formed through Maillard reactions as the nut is subjected to thermal processing steps. In addition to Maillard reaction compounds, Figure 2 shows that there were several other compounds in roasted macadamia. These included pyran, propenal, pyrazine, furan, pyranose, amine, thiazole, pyrrolidine, pyridinamine, pentanamine and oxazine. These findings agreed with the results of others including Pino et al. (2009) and Alasalvar et al. (2003). Studies on other nuts showed similar trends. Morini and Maga (1995) studied volatile compounds in roasted and boiled Chinese chestnut and also concluded that heating processes induce Maillard reactions based on amino acids and sugars as substrates. This process is accompanied by colour development. These results show that volatile compounds produced after roasting were not dependent on storage conditions but rather on how the roasting process was controlled.

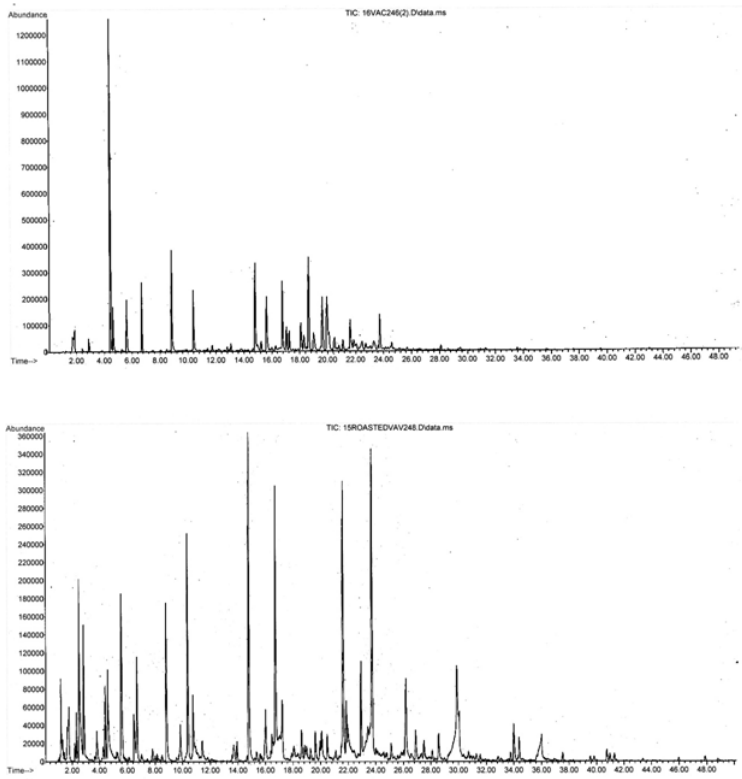

Figure 2 Comparison of volatiles produced in macadamia nut stored at 15°C in vacuum pack before (above) and after roasted (below) at 125°C.

4. Conclusions

This study investigated the effect of storage conditions and temperature on the volatile compounds in macadamia nuts. The results indicated that the changes to lipids were reduced under low temperature storage conditions regardless of packaging material. Therefore storage conditions for nut-in-shell can influence final macadamia kernel quality. Close control of storage and roasting treatments could minimize degradation of fatty acids in macadamia kernels. Volatile compounds produced before and after roasting were mainly due to reactions of precursors in macadamia nut including lipids, sugars and amino acids, which showed no difference between packaging in net bags or vacuum packed in EVOH. However, lipids and others compounds undergo different reactions to produce different end-products, especially volatile compounds which could affect consumer preferences. Volatile compounds generated from Maillard reactions are a good indicator of the effectiveness of roasting, as they play an important role in determining overall flavor while by-products of lipid degradation are good indicators for shelf-life determination of macadamia nut.

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Modelling of seed drying in fluidised and spouted bed dryers

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Abstract

Drying experiments were conducted in the fluidised bed dryer (FBD) and spouted bed dryer (SBD) at temperature 40-80°C using maize, rice and wheat seed samples. The experimental data were fitted into four thin-layer drying models by least square method. As a result, Page's model and two-compartment model were the best-fitted models. Due to the limitation of these models, Page's model and the two-compartment model were modified by adding the drying temperature term. Subsequently, these models could efficiently predict the drying curves under a wider range of temperatures with root mean square (RMS) of the $MR_{predicted}$ - $MR_{measured}$ not over 0.035.

Keywords: Drying model, Seed drying, Fluidised bed dryer, Spouted bed dryer, Drying air temperature

1. Introduction

To date, a number of drying models have been developed to estimate drying times. Several studies proposed simplified models for predicting the drying time of various products. Quite often, a semi-empirical relationship, so-called "thin layer" equation that is analogous to Newton's law of cooling, was introduced to obtain the drying rate of food grains (Soponronnarit, 1997).

Initially, a thin-layer drying model was proposed by Lewis (1921). Regularly, Lewis's equation is referred to as the exponential model and is used for modelling the drying rate of biological materials, especially food grains (Ghaly and Sutherland, 1984; Byler et al., 1987; Shei and Chen, 1998). After Lewis's work, several thin-layer drying models have been developed for food materials (See summary in Table 1). Some researchers (Byler et al., 1987; Shivhare et al., 2000; Tan et al., 2001) claimed that Page's model could predict the drying behaviour of biological materials more accurately than the exponential model, and the relationship between the drying rate constant and air temperature could be represented by the Arrhenius law.

Another thin-layer drying model that has been widely used for modelling the drying of food grain is the 'two-compartment model'. It was found appropriate for application to paddy drying (Sharma et al., 1982; Noomhorm and Verma, 1986). Due to the uniformity of moisture and temperature of samples in FBD and SBD resembling the samples in thin-layer dryer, in this work, a number of thin-layer drying models were developed for maize, rice and wheat seeds dried in FBD and SBD. The aim of this research was to develop thin-layer drying models for predicting the drying curves of maize, rice and wheat seeds in FBD and SBD. These models were expected to be useful for the drying industry.

2. Materials and methods

2.1. Conditioning of seed samples

Australian paddy variety "Amaroo", wheat variety "Westonia", and waxy maize variety "33A63" were used in the experiments. These raw seed samples had moisture contents lower than the designed levels for drying experiments, therefore, seeds were rewetted by adding a calculated amount of distilled water. Seeds were then mixed daily and kept at a temperature of 2-5°C for about 7 d to allow for mosuture equilibration within the seed and to avoid spoilage. The moisture content of seed samples was determined in accordance to ASAE standards (ASAE, 1988).

2.2. Drying experiments

Drying experiments were conducted in two types of dryers consisting of FBD and SBD as outlined in Table 1. During drying experiments, seed samples were collected in each time step for moisture content determination. The data of moisture content change of rice, maize and wheat seeds under each drying condition were used for the purpose of model development.

Table 1 Summary of conditions used in drying experiments

Drying method	Materials	Studied conditions	Remarks
Fluidised bed drying	Maize, rice and wheat seeds	Three levels of initial moisture content (20, 23, 25%wb) and three levels of temperature (40, 60, 80 °C).	Bed depth 0.1 m. Air velocity ≈ 2.8-3.0 m/s. The FBD was designed and constructed at the UNSW, Dept. Food Science & Technology.
Spouted bed drying	Rice and wheat seeds	Three levels of initial moisture content (20, 23, 25%wb) and three levels of temperature (40, 60, 80 °C).	Use sample amount equal to that used in FBD experiment. Air velocity ≈ 8-10 m/s. SBD and its accessories were designed and constructed at the UNSW, Dept. Food Science & Technology.

2.3. Drying model development

Data from drying experiments were fitted into four thin-layer drying models, consisting of Page's model, two-compartment model, Wang and Singh's (1978) model and exponential model (see Table 2) by least square method. The software Statistica 5.5 StatSoft™ (StatSoft Inc., Tulsa, OK, USA) was used for model fitting. In developing thin-layer drying models, the required data consisted of initial moisture content, seed moisture content during drying process, seed equilibrium moisture content, temperature and relative humidity of drying air, and drying time. Equilibrium moisture contents were calculated by using the equations in Table 3 while the other data were obtained from the experiments.

Table 2 List of thin-layer drying models used in this study

Models	References
Lewis's model (Exponential model) $MR = (M-M_e)/(M_i-M_e) = \exp(-kt)$ k = drying constant	Lewis, 1921; Ghaly and Sutherland, 1984; Shei and Chen, 1998
Two-compartment model $MR = (M-M_e)/(M_i-M_e) = Ae^{-k_1 t} + Be^{-k_2 t}$ A, B, k_1, k_2 = drying constants	Henderson, 1974; Sharaf-Elden et al., 1980
Thompson's model $t = A(\ln(MR)) + B(\ln(MR))^2$ A, B = drying constants	Thompson et al., 1968
Page's model $MR = (M-M_e)/(M_i-M_e) = \exp(-kt^N)$ k = f(air temp, air velocity) N = f(initial MC, RH)	ASAE, 1995; Sogi et al., 2003
Wang and Singh's model $MR = (M-M_e)/(M_i-M_e) = A\exp(-kt)$ A,k = drying constants	Wang and Singh, 1978

Table 3 Equations used for estimating the equilibrium moisture content of seeds

Product	Equations	References	Remarks
Maize	$M_e = \frac{1}{100}\left(\frac{\ln(1-RH)}{-0.000030744(T+273.15)}\right)^{\frac{1}{1.8185}}$	Pudpong et al., 1990	Where M_e = Equilibrium moisture content (db, decimal) RH = Relative humidity of air, decimal T =Inlet air temperature, °C
Rice	M_e= --	Steffe and Singh, 1980	
Wheat	M_e= --	Bakker-Arkema et al., 1978	

3. Results

There were two major groups of drying models developed in this work namely models for FBD and those for SBD.

3.1. Drying models for FBD

Thin-layer drying models were developed for seeds of each of the three crops and each drying temperature. The coefficients of determination (R^2) and mean residual square (MRS) were calculated for each drying treatment and crop. The highest values of R^2 and the lowest of MRS were obtained for the Page's and two-compartment models. The results are shown in Tables 4 and 5. These models were not particularly useful because each could be used for one specified drying temperature.

Table 4 Page's model for FBD

Product	Drying air temperature (°C)	Page's model	R^2	MRS
Maize	40	--	96.20	5.2E-04
	60	--	98.10	4.7E-04
	80	--	97.72	1.1E-03
Rice	40	--	98.33	7.4E-04
	60	--	99.40	3.9E-04
	80	--	98.60	9.2E-04
Wheat	40	--	98.85	5.7E-04
	60	--	98.37	1.1E-03
	80	--	98.31	1.2E-03

Table 5 Two-compartment model for FBD

Product	Drying air temperature (°C)	Two compartment model	R^2	MRS
Maize	40	--	95.14	6.6E-04
	60	--	97.75	5.6E-04
	80	--	96.91	1.4E-03
Rice	40	--	98.44	7.0E-04
	60	--	98.96	6.8E-04
	80	--	99.09	6.0E-04
Wheat	40	--	98.68	6.5E-04
	60	--	98.66	8.6E-04
	80	--	99.29	5.2E-04

Thus, two additional models were developed by modifying Page's and two-compartment models; a term for drying air temperature was added (Table 6). In fitting each modified model, the entire range of experimental data between 40 and 80°C drying air temperatures were used (Tables 7 and 8).

Table 6 Modifications to Page's and modified two-compartment model

Description	Equations	Remarks
Modified Pages model	$MR = (M-M_e)/(M_i-M_e)$ $= \exp(-kt^N \exp(-B/T_K))$	Where MR = Moisture ratio M = Moisture content of sample at time t (db), decimal M_e = Equilibrium moisture content (db), decimal M_i = Initial moisture content of sample (db), decimal T_K = Drying air temperature (K) t = Drying time (minutes) $k, k_1, k_2, A_1, A_2, B, N$ = Drying constants
Modified two-compartment model	$MR = (M-M_e)/(M_i-M_e)$ $= A_1 \exp(-k_1 t \exp(-B/T_K))$ $+A_2 \exp(-k_2 t \exp(-B/T_K))$	

Table 7 Modified Page's model for FBD

Product	Drying air temperature (°C)	Modified Page's model	R^2	MRS
Maize	40-80	--	98.07	6.9E-04
Rice	40-80	--	98.57	9.4E-04
Wheat	40-80	--	98.26	1.2E-03

Table 8 Modified two-compartment model for FBD

Product	Drying air temperature (°C)	Modified two-compartment model	R^2	MRS
Maize	40-80	--	97.11	1.0E-03
Rice	40-80	--	98.38	1.1E-03
Wheat	40-80	--	98.75	8.8E-04

The comparison between the experimental data and the models for FBD (Page's, two-compartment, and modified Page's and modified two-compartment) is shown in Figure 1. There is a good agreement between the experimental and the model-predicted values for all models. Additionally, there is a slight improvement in the agreement between experimental and model predicted values for the modified models.

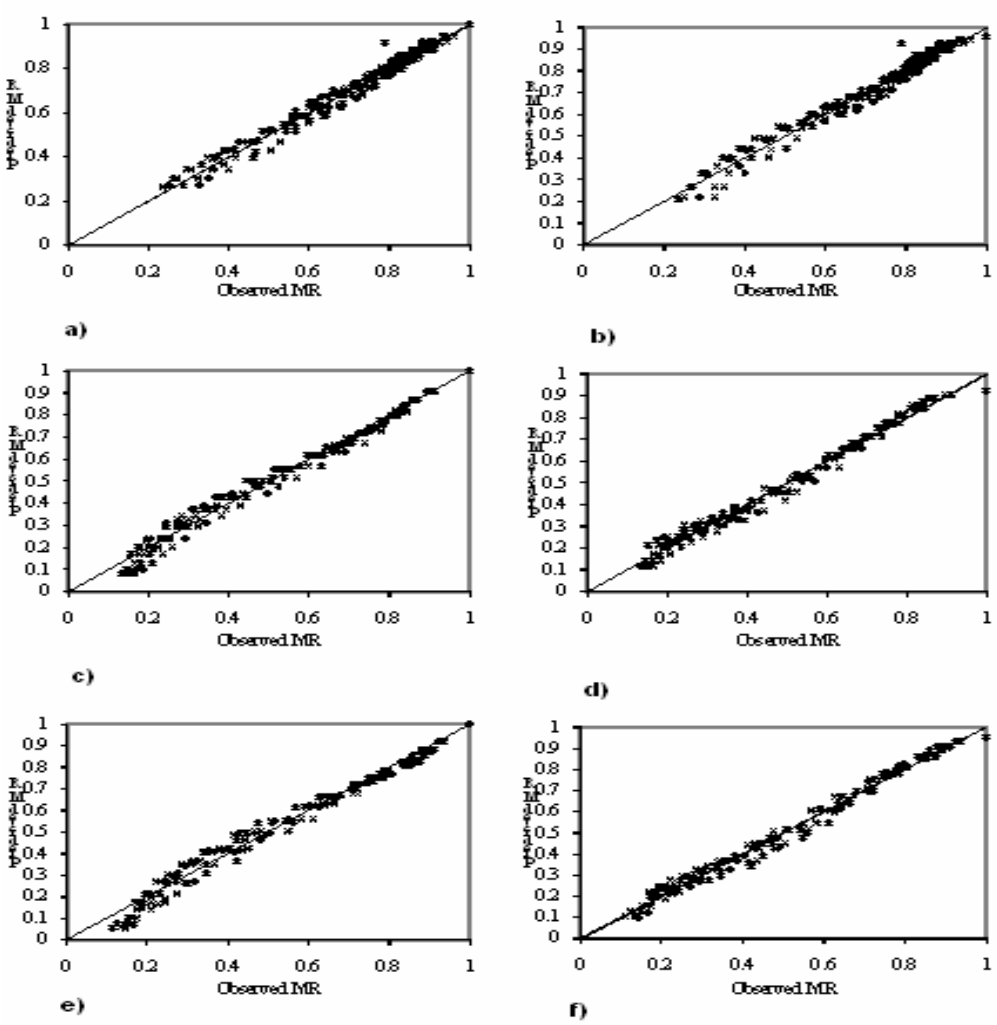

Figure 1 Comparison between $MR_{observed}$ and $MR_{predicted}$ in FBD: a) Maize, modified Pages model b) Maize, modified two compartment model, c) Rice, modified Pages model d) Rice, modified two compartment model e) Wheat, modified Pages model f) Wheat, modified two compartment model

3.2. Drying models for SBD

Best drying results of model fitting for both FBD and SBD were obtained using Page's and two-compartment model (Tables 9 and 10). As explained previously, these two models were modified to allow use over a wide range of drying air temperatures (between 40 and 80°C). The modified models are shown in Tables 11 and 12.

Table 9 Page's models for SBD

Product	Drying air temperature (°C)	Page's model	R^2	MRS
Rice	40	-	98.97	3.4E-04
	60	-	99.27	4.8E-04
	80	-	98.80	9.5E-04
Wheat	40	-	98.88	4.4E-04
	60	-	98.63	9.3E-04
	80	-	98.81	1.1E-03

Table 10 Two compartment models for SBD

Product	Drying air temperature (°C)	Two compartment model	R^2	MRS
Rice	40	-	99.07	3.1E-04
	60	-	99.42	3.8E-04
	80	-	99.68	2.5E-04
Wheat	40	-	98.92	4.3E-04
	60	-	98.84	7.9E-04
	80	-	99.62	3.5E-04

Table 11 Modified Page's model for SBD

Product	Drying air temperature (°C)	Modified Page's model	R^2	MRS
Rice	40-80	-	98.78	8.5E-04
Wheat	40-80	-	98.81	8.5E-04

Table 12 Modified two-compartment model for SBD

Product	Drying air temperature (°C)	Modified two-compartment model	R^2	MRS
Rice	40-80	-	99.44	3.9E-04
Wheat	40-80	-	99.17	5.9E-04

The comparison between the experimental data and the models for SBD (Page's, two-compartment, and modified Page's and modified two-compartment) is shown in Figure 2. There is good agreement between the experimental and model-predicted values for all models with an overall better agreement for the modified models.

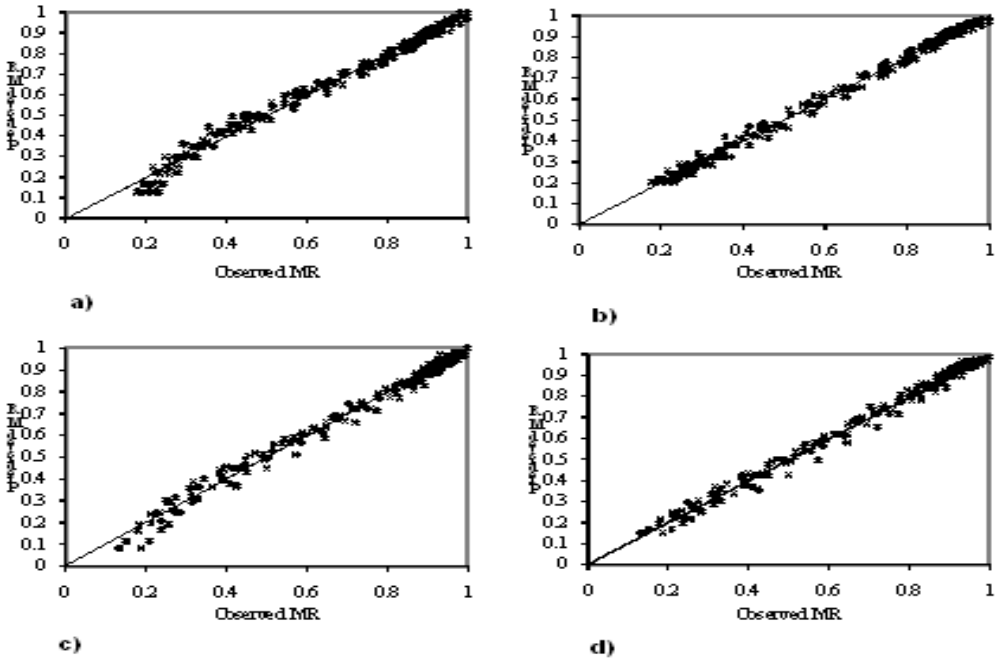

Figure 2 Comparison between $MR_{observed}$ and $MR_{predicted}$ in SBD: a) Rice, modified Page's model ; b) Rice, modified two compartment model; c) Wheat, modified Page's model; d) Wheat, modified two compartment model

4. Discussion

Based on the results of this study, the modified drying models for both types of dryers could accurately predict drying curves for maize, rice and wheat seed under a range of drying temperature from 40-80°C with the MRS values of deviations between predicted moisture ratio and the measured values ($MR_{predicted}$ - $MR_{measured}$) being ≤ 0.0012 (or RMS ≤ 0.035). Moreover, because these models were not complicated, users can use them on scientific calculators or personal computers.

Acknowledgments

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Omni-science: transformative approaches to postharvest technology

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Abstract

Postharvest technology is predicted to experience a transformation over the next couple of decades. Some of the changes may be wrought by as yet unforeseen developments in science, but others will result from the rapid evolution of computer software and hardware. Commercial software is presently available that integrates many strands of engineering science such as structural mechanics, the flow of particulate solids, the distribution of gases within buildings and thermal analysis. The software also enables interactions between these various processes, and the approach is referred to as multi-physics. Commercially available software can be tailored to account for biological phenomena such as the effects of the microenvironments in grain stores on the viability of seeds, the rate of decay of pesticides, the propensity of insect populations to increase and so on. The time is ripe to integrate these chemico-biological aspects of grain storage with multi-physics to form what might be dubbed an omni-scientific approach to postharvest technology. The development of such an approach will help unify the disparate sciences involved in grain handling, and it will provide an explicit overarching intellectual framework into which individuals' work will fit. Information and communications technology will not only enable technical problems to be addressed, but it will enable a range of specialists to contribute simultaneously to solving particular problems. Such a scenario will have a profound effect on the postharvest profession, and it will require a radically new approach to the education and formation of stored grains technologists. These specialists must continue to have deep knowledge of specialised areas of science such as genetics, analytical chemistry, fluid dynamics and so on, but they must also be familiar with the integrating software tools and a broad range of science. Postharvest professionals will need to be familiar with several scientific disciplines, i.e. they will need to be omni-scientists, whilst recognising that omniscience is unattainable.

Keywords: Omni-science, Multiphysics, Postharvest, Grains, Storage.

1. Introduction

Postharvest technology is intensely multidisciplinary. Its principal aim is to maintain desirable properties of agricultural produce between its being harvested and its end-use, which could be processing or direct consumption. In the case of stored grains technology, food grains must be stored free from insect pests and moulds, and properties such as baking quality and germination must be preserved. These goals are generally met by manipulating the physical and chemical environments within grain stores, such as grain moisture content and temperature, the intergranular atmosphere and concentrations of pesticides. If we are to manipulate the stored grains ecosystem we clearly require a deep understanding of the physics of hygroscopic porous media and fluid mechanics, the biology of insects and moulds, the biochemistry of food grains and chemical kinetics. Properties such as grain moisture content are often manipulated ex-store using grain driers, and grain may be disinfested of insects by means of equipment such as fluidized beds, aspiration cleaners and by percussive processes associated with the acceleration and impact of grain kernels.

The physics of heat and mass transfer in ventilated bulks of stored grains has been studied for several decades. Most early research was limited to studies of one-dimensional systems, and some could deal only with grain bulks that have initially uniform properties and time-invariant boundary conditions such as the temperature and humidity of the air entering the system (Barre et al., 1971, Sutherland et al, 1971). Numerical solutions, such as that developed by Boyce (1966), allow the latter restrictions to be lifted. Analyses of heat and mass transfer in three-dimensional systems were ultimately developed, firstly for grain stores that had simple geometries (Singh and Thorpe, 1993) and then for those with more realistic shapes (Thorpe, 1997). One of the main problems with early mathematical models of heat and mass

transfer in stored grains is that they require a considerable degree of mathematical and computational expertise to formulate and to obtain results. The computer codes were usually developed in particular laboratories and their documentation was rarely designed to be user-friendly: the result was very little interoperability of the software between laboratories.

One impediment to progress in postharvest technology may be that some of its practitioners view the activity as a specialism. Evidence for this can be found in the postharvest literature on the development of mathematical relationships between the pressure gradient and the velocity of air flowing through stored grains. Some of the earliest attempts by Shedd (1953) and Hukill and Ives (1955) were purely empirical and have no justification on physical grounds, and they are also inconvenient to manipulate mathematically. Despite these difficulties postharvest technologists continue to use them (Kashaninejad and Tabil, 2009). An alternative approach is to regard the problem of the flow of air through bulk grains and other commodities as the flow of a fluid through a porous medium. Early attempts at tackling this more general problem were made in the 1930s by researchers such as Forschheimer (1930) and Carman (1937). Their work was later refined by Ergun (1952) whose expression for the relationship between pressure gradient and fluid flow rate accounts for physical properties such as the diameter of the particles composing a bed of porous media and the porosity of the bed. Furthermore, Ergun's (1952) equation accounts for the effects of the viscosity and density of the fluid flowing through the porous medium. An additional feature of Ergun's (1952) equation is that it has an extremely simple mathematical form. The possible isolation of postharvest technology from mainstream engineering science is also evidenced by the work of Lukaszuk et al. (2009) that purports to present data on the resistance to air flow through grains in the horizontal and vertical directions. The authors' data indicate that there is a finite pressure gradient within the grain, even when the prevailing air velocity is zero. Had the work been presented within a mainstream engineering framework, the authors may have been alerted to this paradox. More recent approaches to flow through porous media involve studying interstitial flow fields from which the behaviour of the macroscopic system can be estimated (Breugem and Rees, 2006).

Pragmatism and commercial pressures often conspire to produce *ad hoc* approaches to other areas of postharvest technology. For example, whilst experiments on the efficacy of microwave radiation as a means of disinfesting grains are valuable, they tend to be scoping studies rather than detailed definitive studies. A possible reason for this is that microwave engineering is highly specialised, and in depth studies restricted to disinfesting grains would require expertise beyond that usually available in stored grains laboratories.

Information is lost when data are averaged. However, a feature of modern science is that complex systems are being studied in unprecedented detail. Had Vayias and Stephou (2009) been working in this paradigm, they may have chosen to have presented their results on the insecticidal efficacy of an enhanced diatomaceous earth formulation in somewhat more detail. Unfortunately they chose to aggregate their insect mortality data obtained under different temperatures and present them as a function of the relative humidity of the air in which their grain samples were in contact. It is argued in this paper that if postharvest research were carried out within a framework that makes its usefulness explicit authors would present their work in a more coherent form.

Stored grains insect pests have proved remarkably sensitive to rapid acceleration as occurs when grain kernels containing developmental stages of insect pests impact with surfaces and with each other. Experiments have been carried out on practical applications of these percussive effects when grain is being turned or pneumatically conveyed (Paliwal et al., 1999), but if we are to optimise devices, we need to be able to predict the number and nature of the collisions experiences by the individual grain kernels. Insights into the behaviour of individual particles remain in the province of specialists in a domain of engineering computation known as the discrete element method.

2. Multiphysics – a contemporary approach to engineering analysis

The above discussion has highlighted two problems facing stored grains technologists. Firstly, specialised knowledge in areas such as heat, mass and momentum transfer in grain bulks, microwave heating, tracking the paths of individual grain kernels often does not exist in postharvest laboratories. Secondly, it is very difficult to transfer any expertise that may exist in one laboratory to another laboratory with the objective of making it generally accessible. However, the engineering software industry is making considerable progress in overcoming these difficulties by developing suites of

software that incorporate multiphysics. The software integrates several branches of physics such as strength of materials, computational fluid dynamics, heat transfer and electromagnetic waves such as microwaves. Furthermore multiphysics software also incorporates *interactions* of several simultaneously occurring physical phenomena such as the dynamics of the motion of individual grain kernels and heat and mass transfer that may occur in fluidised bed driers. Modern software is also relatively easy to operate and much of the data entry and operation is carried out using drop-down menus. The geometry of the system being studied, a grain silo for example, is relatively straightforward to generate, and the specialised process of generating the finite difference mesh on which the governing differential equations are discretised is automated. Post processing of the results is extremely sophisticated and it allows solutions, such as the rate of cooling grain in an aerated grain silo to be animated.

Modelling the stored grains ecosystem relies on the solution of very large sets of coupled non-linear differential equations. Proprietary software incorporates efficient solvers and the codes can be parallelised so that they can be run several orders of magnitude more quickly than on a single processor. Importantly, the solution procedures are largely automated and the solution algorithms remain invisible to the user; this situation is quite different from the one prevailing when postharvest technologists wrote their own, often idiosyncratic codes.

Some of the problems in the domain of postharvest technology that multiphysics software is able to analyse include:

a) The design and operation of grain aeration systems.
b) The design and operation of grain fumigation systems.
c) An exploration of the behaviour of grain kernels in pneumatic conveying systems.
d) Tracking the locations and temperatures of individual grain kernels in fluidised bed grain drying and disinfestation devices.
e) Microwave heating of grains and insects.

By its nature, multiphysics software is extremely general and it must be tailored to suit specific postharvest applications. In some cases, such as investigating the performance of pneumatic conveyors, this may entail little more that entering physical properties of grains such as their thermal conductivity, density, coefficient of restitution and so on. In other cases, such as studying aeration systems, it is necessary to supply source terms that account for the heat of sorption when grains adsorb or desorb moisture. Contemporary multiphysics software can be accessed by many users, each of whom can contribute to solving the problems at hand. An important feature of multiphysics software is that the various branches of physics are driven by standardised interfaces, and the interactions of the branches are very simple to program.

3. Omni-science – a syncresis of the sciences

If postharvest technologists are to exploit the benefits of the rapidly developing approach to engineering science through multiphysics existing software must be supplemented by the addition of postharvest-specific information. For example, there is a need for models of the sorption and chemical kinetics of fumigants on grains, the response of insect pests of insecticides and fumigants needs to be further refined, and we need a better understanding of how insects distribute themselves throughout bulk of stored grains. The syncretisation, or seamless melding together of these chemical and biological phenomena with multiphysics gives rise to what might be dubbed omni-science. The idea of omni-science enhancing multiphysics software has several advantages for postharvest technology.

4. The postharvest research project in an omni-scientific framework

4.1. The stored grain ecosystem

An abiding interest of grains postharvest technologists is the manipulation of the stored grains ecosystem to control the spoilage of grains. One widely used method of preserving stored grains is to ventilate them with low enthalpy ambient or refrigerated air. It is important to calculate the distribution of air flow within a grain store to ensure that all regions are cooled. Thorpe (2008) has shown that these calculations are easily accomplished by multiphysics software that incorporates built-in expressions for local pressure gradients that are based on Ergun's (1952) equation. Thorpe (2008) has also shown in detail how to

account for simultaneous heat and moisture transfer in beds of aerated grain. This allows the temperature and moisture content of the grains to be calculated throughout the grain store and it becomes possible to extend the physical model to include expressions for the increase in populations of insect pests. This latter extension transforms the multiphysics model into an omni-science model. Using this technique the ratio of a population to its initial population of *Rhyzopertha dominica* (F.) has been estimated in a bulk of wheat with an initial temperature of 35°C and a moisture content of 12% (wet basis) and ventilated with air that has a temperature of 10°C and a humidity of 0.007 kg water per kg dry air. Figure 1 shows the distribution ratio of the potential population to the initial population after 150 hours of aeration. This example is given to demonstrate the potential of omni-science, and to perhaps suggest areas for further research, rather than to provide a definitive representation of the phenomena involved.

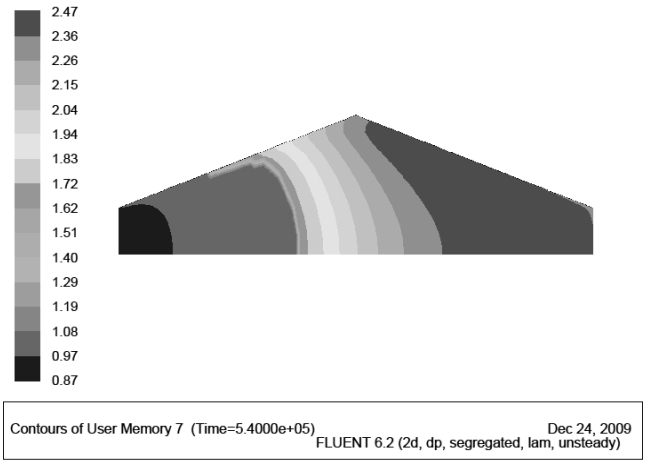

Figure 1 The ratio of potential population increase of *Rhyzopertha dominica* in wheat that has an initial moisture content of 12% (wet basis) and temperature of 35°C, after having been aerated for 150 hours with air that has a temperature of 10°C and a humidity of 0.007 kg water/kg dry air. The specific flow rate of the air is 2 litres per second per tonne of grain.

Fumigant gases such as phosphine are widely used to combat insect pests in stored grains. The low cost and simplicity of application make fumigation with phosphine a powerful weapon in the postharvest manager's armoury, but there are reports that insects are developing resistance to the fumigant over widespread geographical locations. It is therefore essential that grains be fumigated so that the concentration-time product of the fumigant in every location of a grain store ensures that all development stages of insects succumb. Multiphysics software can help postharvest technologists explore the design and operation of fumigation systems. It is straightforward to generate the geometry of a grain store of arbitrary shape – a circular silo with a conical hopper, say, and the interior of the silo is portioned into two regions that comprise the grain and the headspace. The software allows users to define an opening in the silo wall through which the fumigant is introduced and the software automatically calculates its distribution within the grain store. This requires no more than standard operation of the software, but the sorption characteristics of the fumigant on a given type of grain must be specially programmed. Figure 2 shows the estimated distribution of phosphine in a silo partially filled with cottonseed after an elapsed time of 100 hours using the sorption and chemical reaction model presented by Darby (2008). This particular example illustrates how the design of a fumigation system might be improved, and it provides an impetus to investigate the importance of sorption and chemical reaction on the uniformity of the distribution of the fumigant. An omni-scientific approach enables one to incorporate biological data into the model and calculate the concentration-time produce at each point in the grain store, and therefore infer the likely mortality of insect pests throughout the grain store.

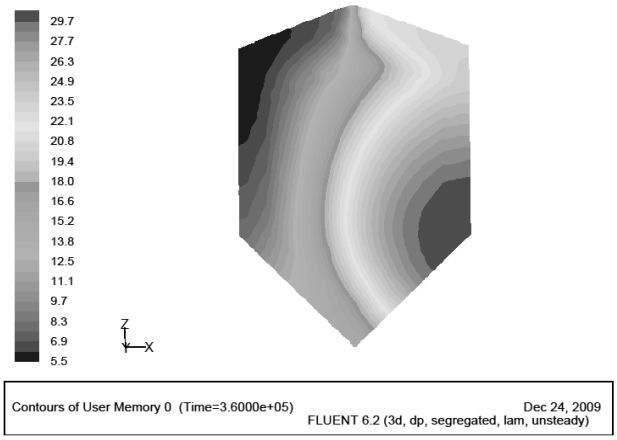

Figure 2 The distribution of phosphine concentration (mg/L) on a diametrical plane in a circular silo containing cotton seed after 100 hours of operation. The phosphine is introduced into the silo through a small hole on the lower right of the plane whence it disperses non-uniformly through the grain, the effect of which is exacerbated by the sorption on and reaction by of the fumigant on the grain.

The two applications of an omni-scientific approach to ecosystem modelling briefly outlined above demonstrate the power of the approach, and they also highlight areas in which the models can be refined. For example, further quantitative research is required in areas such as a) the movement of insects in response to temperature and humidity gradients within grain stores, and the migratory behaviour of insects that enter and leave grain stores, b) the need to generate mathematical models of the sorption and chemical reaction of fumigants in a wider variety of grains, and importantly under a wide range of temperatures and intergranular humidities, and c) more detailed models of the rates of the stages of insect development need to be produced so it is no longer necessary to rely on expressions that relate to populations with stable age distributions.

It is worth pointing out that research on stored grains should be carried out not only under those conditions expected in practice, but also at extrema of temperatures, moisture contents, gas concentrations and so on because it is the latter that are of interest if insects are to be controlled. This is a very general issue, but the requirement to consider extrema continues to recur as evidenced by the work of Vayias and Stephou (2009) who restricted their study if the insecticidal properties of pesticide-enhanced diatomaceous earth to three temperatures, namely 20°C, 25°C and 30°C, and relative humidities of 55% and 70%. These temperatures and relative humidities are typical of those found in grain stores and it may be argued that they are therefore relevant, but they are of less concern from the point of view of controlling pests. Extrema are also of great importance for evaluating mathematical models that must apply over a wide range of conditions.

4.3. Tracking individual grain kernels

There are certain operations in which it is highly desirable to be able to track the location of individual grain kernels. One operation may be the design of pneumatic conveying systems that also incorporate devices that promote insect mortality by percussion. It is also important to model the behaviour of individual grain kernels to ensure that grain silos are designed to empty thoroughly, and possibly in designing devices that sort grain kernels not only by grade, but possibly that can discriminate between infested and uninfested kernels. Fluidised bed driers are used to dry grain, and their effectiveness as thermal disinfestors is well established. One of the advantages of fluidised beds is that they promote extremely good mixing of the grains, so that grains spend only very short times in regions where the air temperature is high. This prevents grain kernels being damaged and it promotes rapid heat transfer between the air and the grain kernels. However, a disadvantage of the rapid mixing of grains in fluidised beds is that it gives rise to longitudinal dispersion that results in some grain kernels having a very short

residence time, whilst others linger in the fluidised bed. A net result of this is that the efficiency of fluidised bed driers is reduced because some kernels are over dried.

In this work we shall outline one potential multiphysics application that highlights the potential power of discrete element modelling, namely the development of a novel device for emptying grain silos. Many grain silos used on farms in Australia empty be the grain falling through an opening near the base of conical hoppers, and because of the shallowness of the hoppers or their design some silos fail to empty completely. The silos therefore provide refuges for insects that may infest the following grain harvest. McDiarmid et al. (2009) have invented a device that aims to promote more complete emptying of silos. The idea is to locate the resonant frequency of the hopper containing the grain and vibrate the hopper at this frequency using a proprietary low frequency audio transducer powered by a consumer audio amplifier. This results in the hopper vibrating with a large amplitude thus promoting the emptying of the silo. The resonant frequency is established by measuring the output from an accelerator attached to the vibrating audio transducer. As the silo empties the resonant frequency changes and this is continuously monitored and the input frequency of the low frequency audio transducer is adjusted appropriately. This is an archetypal example of multiphysics in which a structure (the silo hopper) interacts with particles (the grain kernels) and from which the resonant frequency and the locations of the grain kernels may the tracked. If the silo does not empty completely biological data can be used to assess the risk of insect pests being harboured until the following harvest. This represents an extension of multiphysics into the realm of omni-science.

5. The impact of omni-science on postharvest technology

A principal motivation for proposing the idea of applying an omni-scientific approach to postharvest technology is that it provides the discipline with a coherent intellectual framework. This is because postharvest technology is a distinctly applied subject, and the ultimate aim of most laboratory and field experiments is to produce useful data. By incorporating the results into high level computer software one is likely to construct a metanarrative through which individual researchers in postharvest technology will interpret their work.

Omni-science is likely to guide research, and gaps in knowledge will become apparent as researchers strive to develop useful analytical tools. The approach will highlight the importance of studying extrema as these are not only useful from the point of good agricultural practice, but they also provide severe tests of mathematical models.

The development of multiphysics and omni-science software draws on many scientific, computational and mathematical disciplines. This will have several impacts on postharvest technology. It will help to ensure that scientific advances become available to postharvest technologists in a timely manner, and the emphasis on the ease of use and extremely thorough documentation of the software will facilitate its adoption. It will be possible to call on the advice and expertise of practitioners from a diversity of scientific and engineering disciplines not normally associated with postharvest technology. This free flow of knowledge across disciplines will result in postharvest technology being less of a specialism, and it will give rise to far more mobility of personnel into and out of the field. This will result in postharvest technology being constantly renewed with contemporary methodologies and insights. A feature of modern information technology is that it also allows information to be shared rather than simply being transmitted. This will also result in the cross-fertilisation of ideas. If end-users share the technology an omni-scientific approach could result in new developments being adopted more quickly that at present.

Postharvest technologists of the future – possibly within a couple of decades – will reflect the prevailing scientific and technological Zeitgeist. It is impossible to predict what form this will take, but it is likely that boundaries between scientific disciplines will be more porous and transparent. Institutions dealing with postharvest technology would be advised to be adventurous in their recruitment, and to be more reticent in recruiting scientists and engineers specifically with a stored products background. Educational institutions should be prepared to explore the possibilities of multiphysics or omni-science through high level graduate student projects. Exchanges of personnel between institutions with complementary missions should also be encouraged.

Although omni-science may become widespread, omniscience will remain beyond the reach of human beings but it may be approached by computer networks as presaged by Lyotard (1984). The generation and sharing of knowledge is more likely to be a result of computer mediated team work.

6. Conclusions

Stored grain technology is a distinctly applied area of activity. It is also very diverse and it encompasses disciplines such as engineering, physical chemistry, biochemistry, biology, genetics and so on. It is argued that if these disciplines are to be integrated with the aim of obtaining useful practical outcomes it is desirable to devise an overarching and unifying intellectual framework. Multiphysics is a rapidly evolving concept in the physical sciences. It is driven largely by the increasing sophistication of computer software that enables many branches of physics – mechanics, heat, sound, radiation and so on – to be integrated into relatively easy-to-use software packages. The software not only enables problems in many branches of physics to be solved, it enables *interactions* of different physical phenomena to be studied. It is further argued that multiphysics software should be supplemented by incorporating biological phenomena such as the behaviour of insects in stored grains, and therefore multiphysics would be transformed into an omni-scientific approach. This is a scenario that could be well established in a couple of decades. It would have a profound effect on postharvest technology because it would be integrated with developments in a wide range of scientific disciplines, and it would help guide both research and practice. Postharvest laboratories are likely to be enriched by having visitors and recruits from a wide range of backgrounds. If postharvest technology is to take full advantage of the emerging possibilities that omni-science can offer it is essential that scientists and engineers remain steeped in their disciplinary knowledge. However, they should also be aware of the contemporary tools available to integrate their expertise into the wider field of postharvest technology.

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Research on the space temperature control of grain bins with groundwater and heat exchanger

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Abstract

As the grain temperature in a grain depot increases in spring and summer, deterioration of stored grain by mildew and pests can become serious. To solve these problems, a temperature control technique for the storage bin using a heat exchanger, fan coil units (FCU) and the abundant groundwater resources in Hangjiahu Lake in southern China was studied. The results indicated that the head-space temperature (HST) of the treated bin was controlled effectively; the maximum HST was less than 26°C. The maximum grain temperature of the surface layer (GT-SL) of the grain mass was less than 25°C in the treated bin, the increasing rate of GT-SL was stabilized. The average grain temperature (AGT) in the whole grain mass was less than 18.3°C. In the control bin, maximum HST was about 36°C, maximum GT-SL reached 28.7°C peak temperature, meanwhile, the rate of temperature increase in grain layers was rapid, with the AGT of 21.0°C. Using heat exchanger technology for 11 wks, the electricity cost was 1.175 RMB/t of grain. Therefore, a quasi low temperature grain storage (QLTGS) has been achieved in the experimental bin with this new method in Zhengjiang Province, China. It will have an important significance for safe grain storage.

Keywords: Grain storage; Low-temperature Grain storage; Groundwater; Heat exchanger; Fan coil unit

1. Introduction

The State Grain Depot (SGDD) of Deqing, Zhejiang is a mesothermal grain storage area of high humidity, which is located in the north of Hangjiahu Plain in Zhengjiang Province, China, and was the fifth of seven grain storage eco-regions of China. Under such conditions, it is easy to cause deterioration of stored-grain quality and serious insect damage during more than two years of storage, which makes it difficult to store grain safely. Therefore, the focus of this storage work is to develop a system to prevent deterioration and insect damage to the grain in this region.

An effective solution to the above problems is to reduce or delay the rise of grain temperature within the bin as much as possible. The SGDD commonly uses the low temperature of autumn and winter for natural ventilation of stored grain, which can drop the average grain temperature (AGT) to 5°C. The grain surface is then covered with a layer of bags filled with rice hulls to insulate the grain surface and delay the effect of outside and storage head space temperature (HST). This can keep the grain bulk in a low temperature state for an extended period of time. In practical application, it is found that though the grain surface is insulated with rice hull bags, the temperature of the bins ascends slowly due to the high outside temperature in the spring and summer. After the summer in SGDD, the AGT rises from 5 to about 25°C; the grain temperature of surface layer (GT-SL) was about 30°C, and the bin HST average was about 36°C. This leads to a c real problem of year-round grain storage of “cooling down is easy, keeping it cool is difficult”. Without grain cooling and maintenance technology, depot managers can not achieve the standard requirement of Zhengjiang Province on the low temperature grain storage (LTGS), which allows accelerated deterioration and insect damage of grain.

In grain depots in southern China, a variety of grain storage technologies are studied, such as mechanical refrigeration cooling (Yun, 1998), air-conditioning cooling (Meng et al., 1994; Hu et al., 1997), grain-chiller cooling (Li, 2002) and so on, to focus on resolving the problems of LTGS in summer, or achieving quasi low temperature grain storage (QLTGS). As a result of large investment cost in equipment and high operating costs, the promotion and application of these technologies are limited.

In a large flat bin filled with 1890 t of late paddy in the Central Grain Depot of Huzhou. An improved cold water and heat exchanger within the circulation plumbing was used to achieve a balanced temperature and humidity in the grain mass. During August to September 2006, the average of GT-SL was 25.8-28.1°C, so the system failed to meet the standard of QLTGS (Lu and Zhang, 2007) But, by using the abundant groundwater resources in Hangjiahu Lake and the selecting of a highly efficient heat exchanger, SGDD could develop a temperature control technique with groundwater and fan coil units (FCU). This new technique, with low capital investment, low operating cost and high efficiency, aimed to achieve the QLTGS in bins of SGDD is described.

2. Materials and methods

2.1. Principle

According to the heat exchange principle of fan coil units, deep groundwater of 45 m in SGDD was used moved by water pumps through FCUs placed in the bin head space. The warm air of the head space was ventilated through the fan coil units to reduce air temperature in the test bin. Reducing the head space air temperature reduced the heat conduction into the grain surface and, thus, the grain mass. Thus, AGT rise was slower in the test bin than the control bin.

2.2. Materials

2.2.1. Shape of the experimental bins

The No. 2 and No. 12 bins located in the northern part of SGDD were chosen to be the depot test sites; No. 2 was the test bin, and No. 12 was the control bin. Both bins were horizontal warehouses, with a length of 42.95 m, width of 18 m, eave height of 6.5 m, roof height of 12 m, grain depth of 3.5 m, designed capacity of 2000 t with a volume of 3286 m^3.

2.2.2. The storage conditions of the grain

The basic design factors of the test and control bins and grain being stored are shown in Table 1.

Table 1 Basic instance of test and control bins and the grain

Storage case of grain		**North part No.2 (control bins)**	**North part No.12 (test bins)**
Variety		wheat	wheat
Origin		Henan	Shandong
Quantity (T)		2024	2063
Density (kg/m^3)		792	781
Impurity (%)		0.7	1.0
Moisture (%)		11.5	11.6
Store mode		bulk storage with circuit bags	
Join time (year)		2007	2007
	Maximum grain surface temperature (°C)	24.4	23.4
June 22	Average grain surface temperature (°C)	19.5	18.1
	Average grain temperature (°C)	15.5	15.1

2.2.3. The experimental equipments

Acetone	0.97	—	—	—
4-hydroxy-4-methyl-2-pentanone	0.01	—	—	—
2-heptanone	—	0.95	—	—
6-methyl-2-heptanone	0.16	0.48	—	—
heptene-dione	—	0.286	—	—
6-methyl-5-ene-2-heptanone	1.28	—	—	—
3,5-heptadiene-2-one	0.21	0.15	—	—
2-octanone	—	0.35	0.91	0.71
2,3- octandione	—	—	0.32	0.88
3-octene-2-one	—	0.46	0.45	—
3-octdiene-2-one	—	—	—	0.06

6-methyl-5-heptanone	—	—	0.691	1.83
5-ethyl-6-methyl-2- heptanone	—	—	0.08	0.39
6-methyl-3,5-heptadiene-2-one	—	—	0.39	0.76
2-nonanone	—	0.169	—	—
3-nonene-2-one	—	—	0.25	—
2-undecanone	—	0.32	0.24	—
6,10 -dimethyl-2-undecanone	0.67	—	0.51	0.72
6,10-dimethyl-5,9-undecandione	0.291	—	—	—
2-dodecanone	—	0.56	0.55	0.12
2-tridecanone	—	—	0.17	0.19
2-pentadecanone	0.26	—	—	0.11
6,10,14-trimethyl-2-pentadecanone	2.60	0.41	0.79	1.99

1. FCU was produced by Yuli Air Conditioning Equipment Corporation in Jingjing, Jiangsu with a size of 140 cm x 45 cm
2. 23 cm, air volume of 350 m^3/h-2000 m^3/h and power of 60 W/h;
3. A well with a depth of 45 m was used for groundwater;
4. The deep well water pump was supplied by Minyi Pump Corporation, Shanghai, with a power of 1100 W/h, flow capacity of 5.5 t/h.
5. In/out pipes and accessories.

2.3. Methods

2.3.1. The control method of space temperature in grain bins

Twelve FCUs were connected in series, and installed along the center of the test bins at a height of 2 m above the grain surface; The groundwater deep well pipe was connected with the 12 FCUs in a series flow loop; the detailed connection process is shown in Figure 1. The groundwater of 18°C was pushed into the FCU by the deep well water pump. The water temperature pumped into the first FCU was 19.5°C, then after cooling water passed through the heat exchangers, the temperature of the effluent from the last FCU was 24°C. The cooling water flow was 5.5 m^3/h.

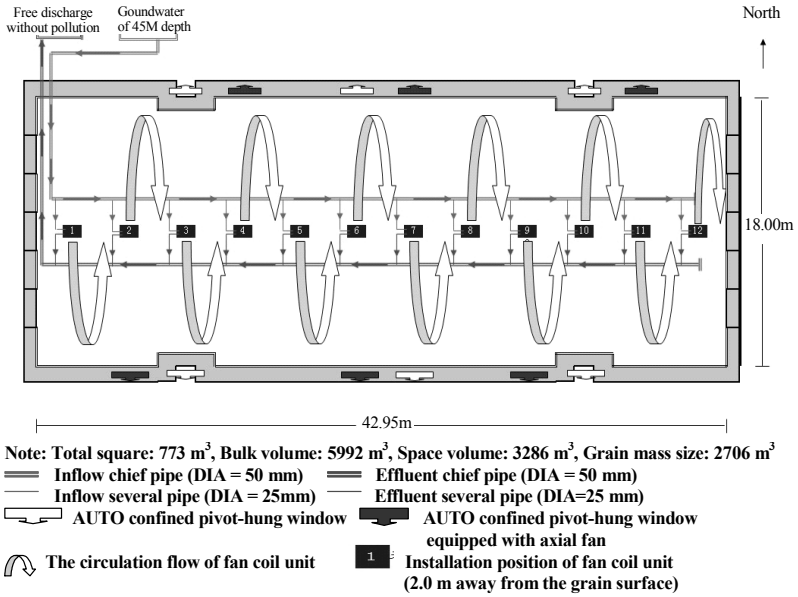

Figure 1 Plan sketch on design and installation of fan coil unit in test bins

2.3.2. The testing methods

1. Measurement of the space temperature: a psychrometer was placed in the centre of headroom above the grain surface; temperature was measured at 8:00, 13:00 and 15:00 daily;
2. Measurement of the grain temperature: the measuring and controlling system of Beibo Electronics Corporation, Zhengzhou, Henan was used. Fifty temperature measurement points were installed for each floor, 0.3 m from the grain surface, the bottom and walls of the bins. It was three-tier with upper, middle and lower layers having a point-to-point horizontal distance of 4.71 m, a column and vertical distance of 4.35 and 1.45 m, respecitively;
3. Measurement of electric energy consumption: a three-phase, four-wire watt-hour meter installed with the device to record data from beginning to end of the test period.

3. Results

3.1. The AGT in treated bin changed slowly with air temperature (AT)

The weekly variation of AGT in the test bin was significantly less than the control (Fig. 2). During the period of HST controlled by water cycle, the daily AGT in the test bin was maintained close to 24.0°C. The test bin AGT changed slower than the control bin, and the daily average AT. During the period of 15-29 d and 57-69 d, when the daily mean AT was about 35.0°C and similar to the control bin, daily AGT in the test bin only reached 25.0°C. The daily AGT in the control bin varied with the change of the daily mean AT.

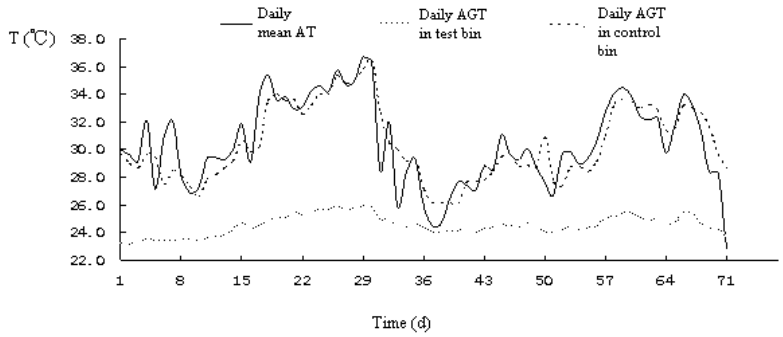

Figure 2 Changes of the average grain temperature (AGT) of the test and control bin, and daily mean air temperature (AT)

From the 29 to 38 d, AT dropped sharply to 26 and 24.3°C, respectively, due to continuous rain; the daily AGT in the control bin quickly dropped to 26°C during the same time. When the AT rose again, the control bin AGT also increased rapidly, and at 45 d, the AT and AGT both reached 29°C. However, the temperature change in the test bin was distinct from that of the control bin. When the AT dropped sharply to 24.0°C, the daily AGT in the test bin dropped slightly from 26 to 24.0°C. When the AT rose again, there is little change in the test bin, and until the 45 d, the daily AGT was still maintained at about 24.3°C.

3.2. The AGT in test bin rose slowly to achieve a QLTGS

The grain temperature in the test bin and control bins were compared continuously for 11 wks, from 22 June 2009 to 31 August 2009 (Table 2). There was a slight variation of the GT-SL and AGT in the test bin. From beginning to end of the test, maximum and mean GT-SL increased from 24.4 and 19.5°C to 24.9 and 21.8°C, an increase of 0.5 and 2.3°C, respectively.

The AGT increased 2.8°C from 15.5 to 18.3°C. However, temperature in the control bin was influenced largely by AT. From beginning to end, the maximum and mean GT-SL increased from 23.4 and 18.1°C to 28.7 and 24.3°C, a growth of 5.3 and 6.2°C, respectively. The AGT increased 6°C from 15.1 to 21°C. Over the whole storage period, AGT in the test bin did not exceed 20°C, and the maximum grain temperature did not exceed 25°C.

Thus, the QLTGS had been achieved in the test bin but failed to meet the standard of the LTGS (Grain and Oil Storage Technical Specifications, LS/T 1211-2008).

Table 2 Comparison of grain temperature surface layers (GT-SL) and average grain temperature (AGT) of the test and control bin.

Time	Temperature (°C)							
	Control bin				Test bin			
	GT-SL			AGT	GT-SL			AGT
	MAX	MIN	MEAN		MAX	MIN	MEAN	
The 1st week	23.4	14.3	18.1	15.1	24.4	15.4	19.5	15.5
The 2nd week	24.4	14.7	18.7	15.5	24.2	16.6	20.1	16.0
The 3rd week	24.5	15.6	19.5	16.2	24.5	17.1	20.4	16.5
The 4th week	26.9	16.7	20.6	17.2	24.8	17.1	20.8	16.7
The 5th week	28.2	17.6	21.7	18.0	25.2	17.7	21.2	16.7
The 6th week	27.3	18	22.3	18.5	24.9	17.8	20.7	16.8
The 7th week	26.8	19.2	22.7	19.2	24.7	18.4	21.6	17.5
The 8th week	27.2	18.2	22.8	19.5	24.8	18.6	21.7	17.7
The 9th week	26.7	19	23	19.8	24.9	19.2	21.7	17.9
The 10th week	28	20.5	23.7	20.4	24.6	19.2	21.8	18.1
The 11th week	28.7	21.2	24.3	21.0	24.9	19	21.8	18.3

The objective of the LTGS is that AGT not exceed 15°C, and maximum grain temperature does not exceed 20°C; the objective of the QLTGS is that AGT not exceed 20°C, and maximum grain temperature does not exceed 25°C.

Both of the weekly AGT of the test and control bin kept increasing throughout the experiment, but that of the test group was slower than that of the control (Fig. 3). Growth of the AGT in every layer of the test bin was significantly slower than in the control (Fig. 4).

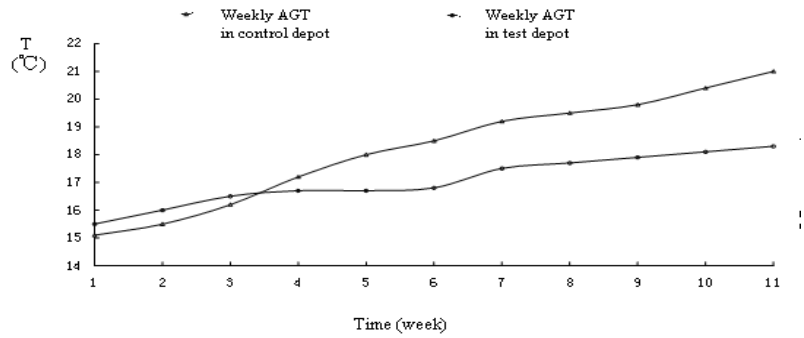

Figure 3 Changes of weekly average grain temperature (AGT) in the test and control bin

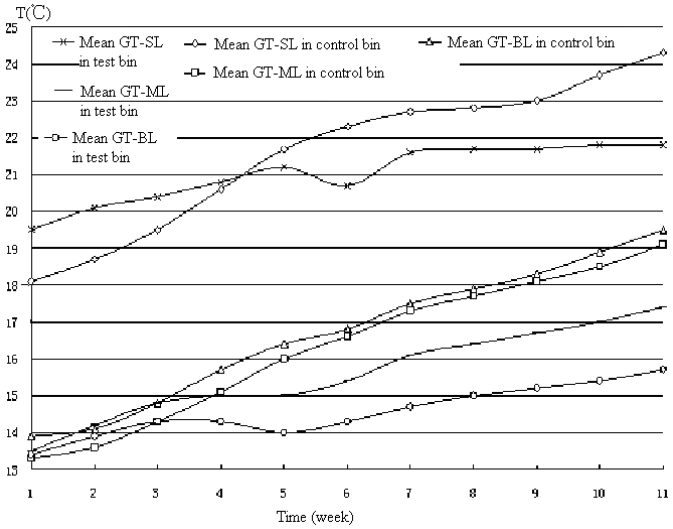

Figure 4 Changes of weekly average grain temperature (AGT) of different layer in test bin

3.3. Energy consumption of temperature control groundwater and fan coil cooling units

The experiment was conducted for 71 d, 24 h/d. The daily energy consumption was computed as (1.1 kW + 0.72 kW)/h × 24 h = 43.68 kW, and the average daily consumption for each tonne of grain was 0.021 kW. According to the price calculation, operating costs of equipment every day was 34.14 RMB. During the experimental period, the total operation cost was 34.14 RMB/d x 71 d = 2423.94 RMB; cost per tonne of grain was 2423.94 RMB/2063 t = 1.175 RMB/t.

4. Conclusions

It was effective to control the HST of the bin with a temperature control technique using groundwater and fan coil head space cooling units. By using this technique, the temperature transmission of the head space air heat was reduced, and the increase of grain temperature in each layer was delayed. The GT-SL was controlled to 22°C, and the AGT of the whole bin was controlled to 18.3°C. Therefore, the QLTGS has been achieved in the experiment bin with the new method. It will have an important significance for safe grain storage in Hangzhou.

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Study on in-store drying technology of paddy in China

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Abstract

In China, with accelerating growth of agriculture economy structure and rapid development of farming mechanization, the intensity and scale of grain planting has increased considerably. However, the drying of freshly harvested grain is a big problem needing to be resolved. In-store drying technology has good ability for large scale drying. Low energy consumption and successful application of this technology in Australia and America has attracted much attention by the Chinese government. Therefore, study of in-store drying started in China from the cooperative study of 'In-store drying of paddy in China' with Australia in 1997. This paper will introduce the main projects of in-store drying in China since 1997, different periods of the technology development, key problems solved, major achievements and the drying technology distribution within China. After about 13 years development, in-store drying of paddy in China includes technology and related equipment specifically for China, such as technology and equipment for mobile ventilation, mold prevention with ozone, heaters for drying grain moisture, and computerized cooling systems. The initial moisture content of paddy allowed for in-store drying increased from 16% to 25%, the bulk allowable depth of paddy increased from 1.8 m to 6 m, and was competitive with paddy drying throughout the world. The main tasks to advance in-store drying in China in the future will be integration of two stage drying technology for paddy and development of relevant equipment, wireless control ventilation technology and development of relevant equipment, study and application of in-store drying technology in new varieties such as wheat and rapeseed.

Keywords: China, In-store drying, Paddy, Moisture content

1. Introduction

Grain is the basic food source for human survival. Grain is related to, not only national economy and people's livelihood, but also to global development and security. China is a major grain-producing country as well as a large grain-consuming country. Chinese traditional agriculture comprises mainly small-scale operations relying on individual farmers. After hand-cultivation and harvest, farmers will mainly dry their grain by exposing them on tarps on the ground directly in the sun. This drying method depends heavily on weather conditions, and requires adequate drying space. With China's urban and rural economic and social development and application of urban-rural integration strategy, arable land has been utilized by gradually increasing scale of farm size. "Grain bank", "order agriculture", "grain storage on behalf of farmers" and other services for "three rural issues" has been carried out gradually. In these situations, a bottleneck of grain drying has emerged with large-scale cultivation, and the problems about grain drying have become more pressing. How to handle high-moisture grain is not only the concerns of China's agricultural sector and farmers, but also seriously concerns the government at all levels, this is then focused back on grain administrations, grain storage companies, and grain research institutes.

For high-moisture grain drying, the main method is drying in the sun, or treating grain for emergency in grain-producing enterprises and storage companies equipped with large or small special external high temperature grain dryers. However, the existing drying equipment has some apparent shortcomings such as high investment, high operating costs, serious food quality losses after drying and low utilization, which will restrict its wide usage. In addition, China's grain production and consumption characteristics need a longer storage period. Nearly 90% of the grain storages are warehouses; the special type of flat horizontal storehouses and limitations of existing transporting equipment are the main reasons leading to high costs of external high temperature drying equipment.

In-store drying is a technical measure allowing treatment of large volumes of grains directly in storehouses (according to storehouse size, the quantity of grain is from 100 t to several thousand tonnes) by use of specially equipped high-air-volume ventilation facilities with in-line air heaters, and the dry air conditions (temperature and humidity) controlled by computers and drying engineers. This in-store drying technology reduces stored-grain moistures to safe water activity (grain moisture content) levels in storehouses.

Since the 1980s, China began to study in-store drying of grain, especially with cooperation of Australia experts supported by the Australian Centre for International Agricultural Research (ACIAR) who promoted the rapid development of in-store drying of grain in China in the mid-1990s.

2. Study history

Chengdu Grain Storage Research Institute, Sinograin (CSR), is the only national research institute specializing in grain-storage technology, including grain drying. Since undertaking the Sino-Australian cooperation project "in-store drying of grain in China" in 1997, China has carried out a comprehensive and systematic study of in-store drying of grain and has spread the technology. The main work and the major technological achievements obtained in in-store drying of grain in China at different periods are shown in Table 1; the technical application example is showed in Table 2.

Table 1 The main research periods of In-store drying for grain in China

No.	Project name	Time	Main works	Major technological achievements
1	Sino-Australian cooperation project "In-store drying of grain in China"	1997 — 2001	1, personnel training; 2, data collection; 3, the drying tests of ground cage; 4,the in-store drying equipment research and development;	1, automatic control system for in-store drying; 2, rice ergosterol rapid detection method; 3, finished the tests of in-store drying for rice and maize;
2	Research and Development Project supported by Ministry of Science and Technology: research and development on upgraded in-store drying technology and equipment;	2002 — 2004	1, Basic technical conditions research on in-store drying for high-moisture grain; 2, Design and develop the movable combined vertical ventilation system; 3, Research and develop intelligent automatic detection and control systems; 4, Develop mobile dry air source equipment; 5, drying test in field;	1, obtain the safe storage period of high-moisture paddy and maize under different storage conditions; 2, developed the movable combined vertical ventilation system; 3, realize intelligent control system for in-store drying of paddy; 4, Technology Regulation of Application of in-store drying" (Draft)
3	Subproject of Science and Technology Project of Grain Production supported by Ministry of Science and Technology: the new technology development and demonstration on in-store drying of paddy in the Yangtze River middle and lower reaches;	2004 — 2006	1, to develop the complete sets of equipment for in-store drying of paddy; 2, to do process optimization and technology integration on in-store drying of paddy; 3, to carry out application demonstration of in-store drying of paddy in field; 4, to research and develop in-store drying for farmer storage;	1, Improved the movable combined vertical ventilation system and operation mode; 2, Efficient energy-saving auxiliary heating equipment; 3, ozone anti-mold equipment; 4, optimized the intelligent control system of in-store drying; 5, technology and equipment of in-store drying for farmer storage; 6, "technical regulation on the in-store drying of high-moisture paddy for farmer storage" (Draft)

Table 2 The technical application examples of in-store drying of grain in China

Place	Time	Grain	Initial moisture (%)	Termination moisture (%)	Depth of grain (m)	Amount (ton)	Energy consuming kW.h/1%.t
Jiangxi Nanchang	2003-1-25 to 2003-8	paddy	17.4	14.5	4.8	1533	4.30
Henan Anyang	2004-4-14 to 2004-6-20	maize	17.5	13.7	3.8	2830	2.32
Sichuan Deyang	2006-9-25 to 2006-11-7	paddy	16.1	13.5	5.0	1500	3.71
Shang hai	2007-4-23 to 2007-5-19	maize	15.6	13.8	5.0	5422	3.82
Shanxi Shangluo	2008-6-9 to 2008-7-20	maize	16.3	14.2	6.0	5280	3.72
Sichuan Chongzhou	2008-9-3 to 2008-11-10	paddy	23.1	13.1	2.5	963	4.18
Shanxi Weinan	2009-4-20 to 2009-6-12	maize	16.8	14.2	3.4	6280	3.36

3. Present situation

Through the above three national research projects, in-store drying of grain in China has developed technical systems and supporting equipment with its own characteristics, solved the problem of grain moisture content (m.c.) difference in different layers of grain and uneven drying throughout the grain bulk. These projects have promoted the use of in-store drying for about 10 million t of grain in many provinces such as Jiangxi, Shanxi, Ningxia, Hubei, Hunan, Sichuan, Shanghai, Henan, Yunnan and other provinces and cities. The bulk grain depth treated has exceeded 3 m, which is an internationally recognized depth limit. Particularly after the serious earthquake of 12 May 2008 ("5.12"), in-store drying technology has been used for paddy with from 18% to 23.5% m.c., with grain bulk depth from 2.5 m to 3.5 m in three earthquake-damaged locations (Qionglai, Chongzhou, Dujiangyan). This was undertaken with strong support of the local government, which had made important contributions to safe storage of grain after the disaster.

3.1. Technical procedure

The integrated procedure from purchasing to storage of new harvested paddy is shown in Figure 1. Depending on the actual situation of each storehouse, different drying methods such as external drying machines, flexible containers for drying (Figure 7) and other quick drying methods can be adopted to handle moist grain at harvest. This integrated procedure enables the whole process for paddy harvest, transport, drying and storage to operate continuously in which paddy needn't touch the ground, which reduces contamination to grain and saves the cost of purchasing additional storage.

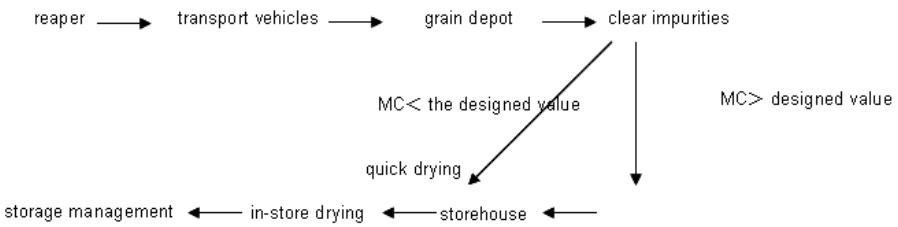

Figure 1 Integrated line of purchasing and storage of paddy

The initial moisture content of paddy treated by in-store drying is different according to style of the storehouse and the specific condition during ventilating. At present, the storehouse suitable for in-store drying of grain is mostly flat storehouse in China, and the depth of grain bulk includes 2.5, 4.5 and 6 m,

based on the initial storage moisture content of the paddy. The initial moisture designed for in-store drying of paddy with different height is showed in Table 3.

Table 3 The designed initial moisture of paddy for in-store drying.

Height of grain bulk (m)	2.5	4.5	6
Designed moisture (%)	≤25	≤21	≤18

3.2. Grain quality security system

The security system to maintain high grain quality includes six major sections: 1) in-floor aeration duct and pressure fan up-flow cooling ventilation system, 2) movable combined vertical ventilation system, 3) heat pump heating or dehumidification system, 4) intelligent (computerized) ventilation control system 5) ozone anti-mold system, and 6) professional design and service team.

3.2.1. In-floor aeration duct and pressure fan up-flow cooling ventilation system

Generally, grain storehouses are equipped with this system in China. The amount of grain treated by in-store drying commonly reaches 1000 t and it often takes about 2 wk to load grain into the warehouse. The pressure fan grain cooling ventilation system is mainly used to control the temperature of grain in the process of loading, thereby prolong the safe storage term of high moisture grain, which keeps fresh air flowing through the moist grain and will provide enough preparation time for in-store drying.

3.2.2. Movable combined vertical ventilation system

This system is mainly composed of stainless steel vent-pipe, special UPVC vent-pipe, circle vent-pipe, flexible vent-pipe with branches, which is easy to install and allows grain managers to use the system multiple times among several storehouses during the storage season. The vertical vent-pipes can been pulled part way out after finishing drying of that layer, which can effectively solve the moisture stratification problem during in-store drying for deep grain bulk. The system had been shown in Figures 3, 4, 5 and 6.

3.2.3. Heat pump heating or dehumidification system

Natural air drying with supplemental heating is the major measure to increase drying speed and overcome adverse weather conditions. The COP showing the energy-efficiency of the heat pump developed and produced by CSR is over four. The heat pump uses energy efficiently; it has a dual function to provide heat to grain drying in the fall and spring or provides cooling to reduce the grain temperature in summer, which provides highly efficient utilization of the machine.

3.2.4. Intelligent ventilation control system

Intelligent ventilation control systems consist of computer hardware and software systems. The system can automatically estimate the ventilation condition, automatically control the cooling fan and heat pump based on collected real-time data about temperature and humidity in the grain by an acquisition module. The system is multifunctional because it predicts ventilation requirements, controlling temperature and humidity, provides warnings and other features.

3.2.5.Ozone anti-mold system

The system effectively prevents the mould from rapidly growing and reproducing under hot and humid environment, thereby it prolongs the safe storage term of grain and protects the quality of high-moisture grain during in-store drying.

3.2.6. Professional design and service team

As the leading role in in-store drying technology research, CSR has formed a professional technical service team after 10 years of continuous research and development. This team visits every site with in-store drying application projects to learn more about the situation and formulate the best program. They conduct technical training for the local technical staff in technology application sites to help them master key technology of in-store drying. Their meticulous support services guarantee grain quality security during in -store drying.

3.3 Technical features

In-store drying technology has the following characteristics:

1. Low investment: the one-time investment reduces one-third to one-half compared with the equipment required for low-temperature drying for paddy with the same moisture removal.
2. Low operation cost: the operation cost is reduced at least one-third compared with equipment required for low-temperature drying of paddy.
3. Large drying capacity - the amount of grain treated is based on capacity of storehouse.
4. Maintains the grain quality well: the drying process is low-temperature low-humidity natural air ventilation drying, and utilizes ozone with high concentration to restrain mold, which effectively protects grain from damage of mycotoxin in the process of in-store drying while maintaining high grain quality.
5. Low grain loss: the newly harvested grain is directly transported into storehouses from harvest fields so the whole drying process has no grain transport delivery expense.
6. A high degree of automation: the operation process is controlled intelligently by a computer software cooling and drying algorithm;
7. energy-saving and environmental protection: high efficiency heat pumps, heating combined with cooling function, with no emissions of waste liquid or gas in drying makes this process efficient and environmentally friendly.

4. The trend of research and development

The existing in-store drying technology still has deficiencies such as drying moisture removal restricted by the grain bulk depth, high one-time investment of automatic control with cable, and technical requirements for automatic installation and debugging. With the acceleration of agricultural industrialization and intensification, China will carry out the following studies to solve the growing demand for paddy drying.

4.1. Application test and demonstration of in-store drying with stratified ventilation

The movable combined vertical ventilation system (Figures 3, 4, 5 and 6) effectively solves the moisture stratification problem of in-store drying of deep grain bulks. However, labor intensity of pushing in or pulling out the vertical ventilation pipes increases with increased grain depth. So a new procedure has been proposed to solve grain moisture stratification by establishing a stratified ventilation piping network. Main research includes carrying out the application test on in-store drying with stratified ventilation network, determining the ventilation conditions, researching and validating the characteristic index that can reasonably reflect the uniformity of mechanical ventilation, establishing the uniformity evaluation methods of ventilation and developing the protocol of technical operating guidelines on paddy in-store drying with the stratified ventilation.

4.2. Research and development on new technologies and equipment for in-store drying

The research and development on new technologies and equipment for in-store drying mainly includes new ozone anti-mold technology and parameter studies, research and development of efficient installation and distribution on movable combined vertical ventilation system technology, and research and development on remote wireless control technology and equipment.

4.3. Integrated application test and demonstration of two-step drying technology

Taking the internationally common technology of drying by several steps as reference, it's proposed to integrate the advantages of drying by steps and traditional in-store drying to develop new technologies and equipments on intensive drying of paddy. The main research and implementation program is showed in Figure 2.

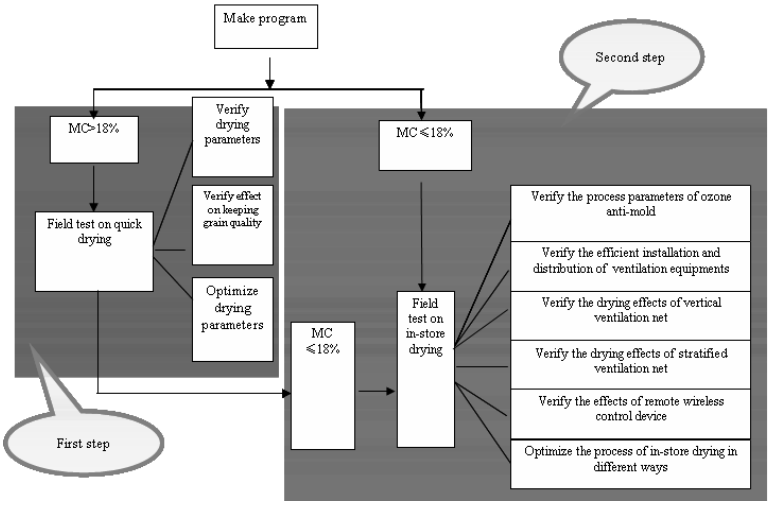

Figure 2 Integrated application test and demonstration of two-step drying technology

Figure 3 Combined vertical ventilation system blowers and air supply hoses

Figure 4 Ventilation system vertical aerator pipes spaced in grain

Figure 5 Solid and perforated piping components for one vertical aerator assembly.

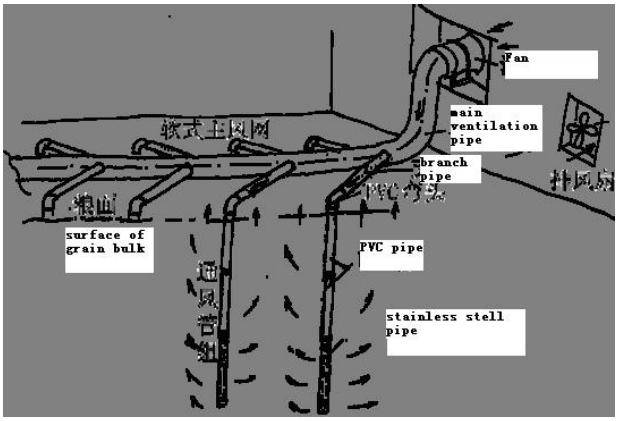

Figure 6 Drawing of combined vertical aerator pipe ventilation drying

Figure 7 Flexible container attached to fan and duct assembly for drying.

4.4. Research on in-store drying of wheat, rapeseed and other kinds of grains

In recent years, the research of in-store drying technology in China mostly focused on high moisture paddy and maize. However, wheat in-store drying technology applications are very few and the initial moisture content of wheat treated is below 16%. There are no research reports about rapeseed in-store drying technology applications. Therefore, application research on in-store drying of wheat, rapeseed and other kinds of grains will be carried out in future.

Section: Fumigation, Modified Atmospheres and Hermetic Storage

Quo Vadis the fumigants?

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Abstract

Fumigation is the most widely used procedure to control stored product pests to prevent economic and quality losses by providing various application methods and penetration capability into the treated commodity. However, most of the available fumigants have limitations in use due to various reasons. Methyl bromide (MB), according to Montreal Protocol, is scheduled to be phased out worldwide by 2015. Sulphuryl fluoride (SF) is known to have limited efficiency on egg stages of insects. Reportedly, it also contributes to the greenhouse effects, which may jeopardize its future use. Currently, phosphine (PH_3) is the most widely used fumigant worldwide due to its low cost and ease of application, though resistance observed in major pest species threatens the continued use of PH_3. Recent studies in beetles reveal that phosphine resistance is governed through two genes on separate autosomal chromosomes. Research on new fumigants continue to be restricted due to concerns over the adverse effects of fumigant residues in food and the environment that led regulatory agencies to take actions by imposing strict limitations on fumigant registration. On the other hand the phase out of MB and resistance to PH_3 has stimulated significant interest on development of alternatives like modified atmospheres (MAs), thermal disinfestation and irradiation. In Turkey, MAs applications are preferred for the treatment of organic food products and are performed mostly in transportable PVC structures (Cocoons or Volcani Cubes). In museums high nitrogen or SF applications are the only procedures to eradicate structural pests. Under the given constraints of registration of new fumigants, it is anticipated that more attention will be devoted to develop novel alternative treatments that are economically feasible, sustainable, user friendly and environmentally benign.

Keywords: Fumigants, Modified atmospheres, Insect resistance, Stored products pests, Fumigant alternatives

1. Introduction

Fumigation plays an important role in the protection of stored products against insects, mites, and rodents. It is a practical approach targeting or benefiting from respiration, an obligatory and continuous process in energy production during the lifetime of insects. So far, a variety of fumigants have been used and in the meantime all of them have been faced with restrictions of varying degrees due to some adverse effects either to environment or to human health, or to some materials. The most recent example is the worldwide ban on the use of methyl bromide (MB) due to its detrimental effects on stratospheric ozone layer. According to the Montreal Protocol in 1987, with some exceptions, such as quarantine and pre-shipment use and critical use exemptions, MB has already been banned in developed countries in 2005. The ban on MB will be put into practice in developing countries in 2015 (UNEP, 2002). Besides the lost of such a valuable fumigant, the use of phosphine is also threatened by the development of high-level resistance in a number of pest species (Schlipalius et al., 2006; Aurelio et al., 2007; Lilford et al., 2009). Sulfuryl fluoride, apparently the most promising fumigant to replace MB, has already declared as a potent greenhouse gas (Andersen, 2009). In the future, with the increase of its use and depending on its concentration in the atmosphere, it may be subject to ban like MB was banned.

The future of the fumigants will thus be determined by their impacts on environment, human health, and pest resistance. Development of effective, available and cost-efficient alternatives will also affect the fate of the fumigants.

2. Current status of fumigants

During the course of combating with stored products pest, there have been at least a dozen of materials used as fumigants (Rajendran, 2001; Navarro, 2006). The questioning of the future of the fumigants has commenced with the strict ban on the use of MB.

2.1. Methyl bromide (MB)

The Montreal Protocol, an international treaty signed by 175 countries in 1987, is globally phasing out MB, which has average ozone depleting potential of 0.4. According to the protocol, MB production and use will be banned totally in a progressive manner till 2015. However, the ban on MB bears some exceptions, such as quarantine and pre-shipment (QPS) treatments, emergency uses and certain critical uses where no alternatives have yet been available (TEAP, 2000). MB is very important in pest control in durable and perishable commodities and particularly in quarantine treatments. One of the main advantages of MB as a commercially desired fumigant is its action speed. Moreover, it has a lot of additional features such as worldwide preference by quarantine authorities, broad registration for use, good penetration ability, and the rapid aeration of the commodity after exposure. However, it has also some disadvantages as a highly toxic, odourless gas that adversely affects of a number of durables in terms of loss of viability, quality changes, taint and residues (Navarro, 2006).

There is a possibility that MB exemptions regularly reviewed in international meetings might not continue in the future. This has led researchers to develop technologies that allow the recovery of MB to recycle or destroy instead of release it to the atmosphere. Such technologies have found some chance to be implemented currently in North America and Europe but still are complex, expensive and needs technical assistance (Navarro, 2006).

2.2. Phosphine (PH_3)

Currently, phosphine is the only available fumigant registered worldwide to control stored and structural pests. It is mainly used in solid preparation of aluminum or magnesium phosphide. To maintain rapid and continuous phosphine gas during the fumigation, on-site phosphine generators (e.g; Quickflo-R®) were developed. To ensure more uniform and continuous gas concentrations, advanced application technologies such as "Closed Loop System" in USA, SIROFLO®, SIROFUME® and SIROCIRC® in Australia and PHYTOEXPLO® in Europe have been developed for application in different storage conditions. Metal phosphide formulations with slow or altered rates of phosphine release have been developed in Australia (QuickPHlo-C) and in India. Degesch Phosphine Generator/Degesch Granules system is a device using Magtoxin® granules for the rapid production of phosphine gas. Degesch Speedbox, another phosphine fast-generating device uses magnesium phosphide plates to generate phoshine.

Cylinderized gas formulations of phosphine such as Phosfume, ECO_2FUME®, FRISIN®, and $VAPORPH_3OS$® are also commercially available to a lesser extent. Phosfume and ECO_2FUME® are the mixture of 2% phosphine and 98% carbon dioxide. While FRISIN® is a mixture of 1.7% phosphine in nitrogen (98.3%), $VAPORPH_3OS$® is mainly composed of phosphine gas (99.3%) and designed to be applied for on-site blending with carbon dioxide or dilution with the surrounding air. This blending or dilution is needed the use of specialized equipment for the generation of a 2% mixture of phosphine in carbon dioxide, making it essentially the same as ECO_2FUME®. The Horn Diluphos System® that is currently in use in Argentina, Australia, Chile, New Zealand, Uruguay, and U.S.A, dilutes pure cylindered phosphine directly with air.

2.3. Sulfuryl fluoride (SF)

Sulfuryl fluoride is an inorganic, nonflammable, odorless, colorless, and broad spectrum gas that is used to fumigate buildings, transport vehicles, wood (Cox, 1997), flour mills, food factories, dried fruits and tree nuts and cereal grains (Navarro, 2006). SF was first developed by Dow Chemical about six decades ago as a structural fumigant against termites. Today, it is produced in the USA under the trade names of Vikane (99.8% SF 0.2% inert materials) and Profume (Navarro, 2006) and in China under the trade name of Xunmiejin (Guogan et al., 1999). SF seems to be the most promising fumigant to replace MB in terms of similar exposure time of 24 h at normal conditions. Moreover, it has some advantages over MB such

as faster diffusion rates than MB in the treated commodities (Navarro, 2006). However, the potential of SF acting as a greenhouse gas may restrain its use in the future as a fumigant.

Sulfuryl fluoride has a complex mode of action. After entering the body of an arthropod, through the stigmas in postembryonic stages or through the diffusion in egg stage, sulfuryl fluoride breaks down to fluoride and sulfate. Insecticidally active fluoride anion interferes with the metabolism of stored fats and carbohydrates by disrupting glycolysis and the citric acid cycle that the insect needs to maintain a sufficient energy for its survival. The fluoride ion is thought to bind to calcium (leading to spasms and seizures) as well as potassium and magnesium. Enzymes requiring a magnesium ion for their normal function are thus inhibited by SF. Among these enzymes are enolases (used to metabolize sugar) and ATPase, an enzyme that is important in cellular energy metabolism. The insect using protein and amino acids to produce energy cannot increase the metabolic rate sufficiently, and dies (Cox, 1997).

The egg stage is more tolerant to SF than any other stages. To overcome the failure in the control of egg stages of the pests during SF fumigation, Reichmuth and Klementz (2008) proposed the use of SF in combination with other fumigants such as hydrogen cyanide (HCN), CO_2, phosphine, or heat. They stated that in Germany, a combination of 2 g m^{-3} of HCN and about 30 g m^{-3} of SF within 40 hours has successfully been applied. According to the different research works in the literature, the same authors concluded that combining of SF also with heat would provide the complete control of egg stages of main stored products pests in the course of disinfestations. Sulfuryl fluoride can also be applied under low pressures yielding with much shortened exposure times (Zettler and Arthur, 2000).

There is a tremendous infestation by wood boring insects in historical palaces of Turkey. Disinfestations of museums in Turkey had been based on using MB at a concentration of 60 g m^{-3} (Ferizli and Emekci, 2008). The historical palaces were fumigated with SF in 2008 in Turkey due to the complete ban of MB in 2005. With the help of 12,000 m^3 h^{-1} capacity gas mixture circulating fans, a closed gas circulating system was used to maintain uniform gas concentrations throughout the palaces. Dosage was calculated using ProFume Fumiguide system.

2.4. Propylene oxide (PPO)

Beside its use in chemical industry as an intermediate to produce commercial and industrial products, including polyether polyols, propylene glycols, propylene glycol ethers; etc., Propylene oxide is commonly used in the sterilization of packaged food items against bacteria, mould and yeast contamination. It has also been registered in the USA since 1984 as a fumigant for the control of stored-product insects in processed spices, cocoa and processed nutmeats (except peanuts). In contrast to MB, PPO is not an ozone depletor and degrades into nontoxic, biodegradable propylene glycol in the soil and in the human stomach. Since PPO is flammable from 3 to 37% in air, it has to be use under low pressures or in CO_2-enriched atmospheres to avoid flammability (Isikber et al., 2006). A complete mortality for all life stages of *P. interpunctella* followed by 4-h laboratory fumigations with PPO at 30°C at 100 mm Hg were reported by Isikber et al. (2006). Thus, commercial scale application of PPO with low pressure can replace MB in quarantine and pre-shipment (QPS) conditions where low pressure treatments are technically and economically available and feasible (Isikber et al., 2006).

2.5. Carbonyl sulfide (COS)

A major sulfur compound naturally present in the atmosphere at 0.5 (± 0.05) ppb, Carbonyl sulfide, is a colorless gas (Wright, 2000) and is present in foodstuffs such as cheese and prepared vegetables of the cabbage family. Traces of COS are naturally found in grains and seeds in the range of 0.05-0.1 mg kg^{-1} (Wright, 2000; Navarro, 2006). The use of COS as a fumigant for the fumigation of durable commodities and structures was patented worldwide in 1992 by CSIRO, Australia. COS has also been trademarked in Australia as COSMIC®. BOC Limited has an agreement with CSIRO for its manufacture and worldwide distribution (Ducom, 2006).

COS at reasonable concentrations from 10 to 40 g m^{-3} was shown to be effective on a wide range of postharvest pests in all stages, including mites at exposure times between 1-5 days at temperatures above 5°C (Desmarchelier, 1994). Amongst the tested pests, *Sitophilus oryzae* (L.) was found to be the most tolerant species to COS and could be controlled at 20 g m^{-3} for 5 days of exposure. Researches on COS in Australia, Germany and the USA revealed that egg stage was highly tolerant to the fumigant, however, the effective exposure period was half that of phosphine at temperatures above 5ºC (Rajendran, 2001).

There has not been found any adverse effect on quality of bread, noodles or sponge cake (wheat) (Desmarchelier et al., 1998), on malting and brewing characteristics of barley or on oil content/color of canola (Ren et al., 2000). Seed germination in wheat, oats, barley and canola was not affected by COS fumigations (Wright, 2000). However, there are contradictory reports in the literature on negative effects of COS on germination of cereals except sorghum and barley, off odors in walnuts, in milled rice, and color change in soybeans (Navarro, 2006).

COS did not show any reaction with a variety of materials including hard and soft timbers, paper, iron, steel and galvanized sheet, PVC, polyethylene, and brick applied with high concentrations at high temperature and r.h. (Wright, 2000). However, avoiding of corrosion on copper, Ren and Plarre (2002) suggested that COS for direct use as a fumigant must be manufactured to eliminate contamination (to <0.05%, v/v), or the fumigant scrubbed of H_2S before application on site.

2.6. Ethyl formate (EF)

Ethyl formate is known as a solvent and is used as a flavoring agent in the food industry (Rajendran, 2001; Navarro, 2006). EF is a volatile compound that occurs naturally in a variety of products, including beef, cheese, rice (Desmarchelier 1999), grapes and wine and is generally recognized as a safe (GRAS) compound.

EF is a volatile, highly flammable liquid at normal ambient temperatures, boils at 55°C, and vaporises readily at normal grain temperatures. Ethyl formate was previously registered in the USA for control of several stored-product pests, including *Tribolium confusum* Jacqueline du Val and *Ephestia figulilella* Gregson. Registrations for the use of EF in the United States of America have expired. Currently in Australia, EF has been used for the fumigation of dried sultanas, in particular. Studies in Australia indicate that, unlike phosphine, ethyl formate is rapidly toxic to storage insects (Annis and Graver, 2000). Effective commodity dosage ranged from 300 to 400 g m^{-3} with 72 h exposure period (Rajendran, 2001). BOC Limited has recently developed and registered Vapormate®, a cylinderised formulation of 16.7% (w/w) ethyl formate in liquid carbon dioxide (for use in Australia). It is a new cereal grain, stored-product and fresh produce fumigant for application by pressurized cylinders. It is a rapid-acting fumigant, effective in a range of from 4 to 24 hours. It is a safe fumigant since the TLV is 100 ppm for EF and 5000 ppm for CO_2 (Ducom, 2006). The CO_2 acts as both a solvent and propellant for the mixture and dramatically reduces any flammability risk due to ethyl formate. In tests with a highly phosphine resistant field strain of *Ryzopertha dominica* (F.); laboratory strains of *Tribolium castaneum* (Herbst) and *S. oryzae*, a single dose of 450 g m^{-3} of Vapormate was found to be sufficient to obtain high level mortality (> 99%) of all stages of *T. castaneum* and *R. dominica* when the grain is held for 24 hours and moderate control (86%) of *S.oryzae* (Haritos et al., 2006b). Forced flow application of ethyl formate and CO_2 vapor through the grain by means of a pump at a flow rate of 6 L min^{-1}., not only provides more even distribution of the fumigant but also causes very high levels of mortality of *S. oryzae* and *T. castaneum* mixed stage cultures (Haritos et al., 2006a).

EF can be used with methyl isothiocyanate (MITC). MITC, originally a soil fumigant against nematodes and fungi, can significantly reduce the dosage of EF to below the flammable level, additionally MITC increases the toxicity of EF. A mixture of EF and MITC (95% EF plus 5% MITC) has recently patented under the name of GLO2 (Ren et al., 2008). GLO2 was shown effective against all life stages of the major grain insect pests. It is fast acting (less than 24 hrs) and requires a short withholding period, about 8 days, but much less with aeration (Ren, et al., 2008).

2.7. Hydrogen cyanide (HCN)

Hydrogen cyanide is currently registered in India and New Zealand (Navarro, 2006). HCN has previously been used to fumigate mills in various countries, including France, Germany, and Switzerland (Rambeau et al., 2001). It is a colorless liquid and smells of bitter almonds. It is lighter than air and has a boiling point of 26°C. HCN is flammable, but in fumigation conditions, concentrations are far under the explosion limits. HCN is very toxic and extremely quick-acting on most living organisms. Due to high degree of sorption at atmospheric pressure, it does not have the quick effective penetration that MB has. It is easily dissolved in water and thus will bind with moisture and can be difficult to ventilate. HCN may be used for the fumigation of many dry foodstuffs, grains, and seeds. Although HCN is strongly sorbed by many materials, this action is usually reversible when they are dry, and, given time, all the fumigant

vapors are desorbed (Navarro, 2006). Rambeau et al. (2001) reported that all stages of major mill and food factory pests, including *T. confusum, T. castaneum*, *Plodia interpunctella* (Hübner), and *Sitophilus granarius* (L.) could be controlled at a Ct product of 10 gh m^{-3}, though to ensure HCN penetration and kill insects at a depth of about 10 cm in flour heaps, the prevailing Ct product should be around 60 gh m^{-3}. In the presence of minor leaks, initial HCN concentration was proposed as 5 g m^{-3}.

2.8. Carbon disulfide (CS_2)

Carbon disulfide, an old fumigant, is used for on-farm fumigation of bulk grains in grain silos, bagged grain stored in sheds and bulk gains stored in sheds in Australia and to a limited extent in China (TEAP, 2000). High flammability, long exposure period, persistence in the treated commodity, adversely affected baking quality lack of residue limits set by Codex Alimentarius and high human toxicity were mentioned among the limitations of the fumigant (Rajendran, 2001; Navarro, 2006).

2.9. Methyl iodide (MI)

Methyl iodide, a patented pre-plant soil fumigant against soil inhabiting pests and structural fumigant against termites and wood rotting fungi is also known to be very effective as a space fumigant, being most toxic to eggs and least toxic to adults of *S. granarius*, *Sitophilus zeamais* Motschulsky, *T. confusum*, and *P. interpunctella* (Goto et al., 2004). Though MI is considered as a carcinogenic compound, The U.S. Environmental Protection Agency (EPA) registered MI as a soil fumigant on October 5th, 2007 (EPA, 2009).

2.10. Ethane Dinitrile (EDN)

Ethane dinitrile is also referred to as Cyanogen (C_2N_2). It is a broad range spectrum fumigant; effective against weed seeds, soil insects, nematodes, and fungi. It is an environmentally safe, colorless gas with an almond-like odor. It is a gas at ambient temperatures with a boiling point of –21.2°C. It is soluble in water. The threshold limit value (TLV) of 10 ppm (v/v) compares favorably with that of both methyl bromide (5 ppm) and phosphine (0.3 ppm). It is highly toxic to stored-product insects and is fast acting (except *Sitophilus* spp) (Ducom, 2006). It has a good penetration capability through the grain mass and it desorbs quickly. It also affects germination of treated seeds due to its phytotoxic properties. It is rather considered for space and flour/rice mill fumigations and disinfestations (Navarro, 2006). CSIRO's Division of Entomology, Australia currently holds patents for use of EDN as a fumigant in the major worldwide markets till 2014. BOC Limited has signed an exclusive global license agreement with CSIRO for EDN as a soil, timber fumigant and grain sterilant. EDN is marketed under the trade names of Sterigas 1000 Fumigant (Active Constituent 1000 g kg^{-1} Ethanedinitrile; Flammable) and Sterigas 200 Non-Flammable Fumigant (Active Constituent 200 g kg^{-1} Ethanedinitrile; EDN 20 wt% in liquid carbon dioxide) (Ryan et al., 2006).

2.11. Ozone

Ozone is a powerful oxidant that reduces or inhibits mold spore development and kills stored product insects. It can be used as an insect control agent in food commodities at levels less than 45 ppm (Rajendran 2001, Navarro, 2006). It can be generated on the treatment site without any residue on the treated product. A major disadvantage with ozone is its corrosive property towards most of the metals (Mason et al., 1999). Moreover, it quickly transforms or decays into two molecules of oxygen within less than an hour. Therefore, a special ozone air delivery and return system is needed for an effective ozonation treatment of a storage facility (Campabadal et al., 2007). Ozonation experiments carried out at a popcorn facility yielded 100% mortality for *S. zeamais* and *T. castaneum*, placed 0.6 m below the grain surface (Campabadal et al., 2007).

2.12. Volatile essential oils of botanical origin

There are many research articles in the literature confirming the fumigant toxicity of different volatile essential oils of botanical origin on stored-product insects (Shaaya et al., 1997; Stamopoulos et al., 2007; Isikber et al., 2008; Korunic et al., 2008). These researches were mostly carried out in empty fumigation chambers and thus may not reflect the actual fumigation situations where penetration of the plant extracts into deep layers fails, due to the strong absorption by the commodity. Moreover, aromatic scents of the essential oils permit them only to be applied in empty premises or to commodities such as seeds where the scent of the volatile essential oil would not present a restriction after the treatment. Another important

constraint for the use of botanical extracts is that such alternatives of plant origin also need toxicological and safety data for registration for use as fumigants (Navarro, 2006). Physical properties of essential oils such as high boiling point, high molecular weight and very low vapour pressure prevent the use of essential oils for large-scale fumigations. Thus, essential oils are believed to have the potential for small-scale treatments and space fumigations (Rajendran and Sriranjini, 2008).

2.13. Modified atmospheres (MAs)

The use of MAs has dated back to ancient Egyptians in the form of hermetic storage of grain by which grain itself through respiration creates an atmosphere rich in carbon dioxide and low in oxygen (White and Leesch, 1996). Today, MAs have increasingly gained attention as pesticide-free organic food demands have increased. It aims to create an atmosphere lethal to pests in stored commodities rich in CO_2 or low in O_2 by using CO_2, N_2 or their mixtures at normal or altered atmospheric pressure within the storage facility. So far, MAs composed of either CO_2, N_2 or inert gases have classically been used in different parts of the world for the fumigation of a variety of commodities including grains, pulses, tree nuts, dried fruits, coffee and cocoa beans, spices, medicinal herbs, geophytic bulbs and historic artifacts (Adler et al., 2000)

Turkey is one of the leading countries in the world in exporting dried fruits and hazelnut. MB has a critical importance in the production of dried figs, in particular. Each year, Turkey exports some 40 thousand tonnes of dried figs. Dried figs are harvested between mid of August and late September. There are some 50 dried fig-processing plants in Turkey, most of them family sized plants that run 3 months during the fig-processing season. Processing of 40 thousand tonnes of dried figs in a three-month period in small sized plants needs fast fumigation procedures to eliminate *Carpophilus* spp and *Cadra cautella* (Walker), which are the main pests of dried figs infecting the crop in the orchards. Thus, due to time limitation, exposure time lasting more than a day is not tolerated. This makes MB unique for dried fig production in Turkey. Research carried out to find alternatives to MB in Turkey showed that MAs applications using high CO_2 in flexible PVC units (Volcani Cubes) were effective and could be used against the main pests of dried figs, however, the exposure time of 5 days was needed to attain a complete kill (Emekci et al., 2007). Thus, MAs applications currently in use in Turkey are mostly restricted to organic food products of various kinds.

Museum objects were disinfested by means of MAs applications due to the complete ban on the use of MB in Turkey in 2005. High nitrogen gas treatments of historical artifacts in PVC cubes of 30 m^3 volume led to a complete mortality of all life stages of Khapra beetle, lesser grain borer, confused and red flour beetles after 30 days of exposure at ambient temperatures. Nitrogen gas was obtained from a gas generator of 4 $Nm^3 h^{-1}$ outlet flow capacity to maintain a low oxygen atmospheres around 1% O_2, a PLC Scada system was set up to restore nitrogen levels in different cubes when the oxygen level increased above 1% (Emekci and Ferizli, 2008).

MAs applied in combination with positive pressure or elevated temperature increase the performance of MAs. Significant reduction in exposure time to a few hours can be obtained with the use of high carbon dioxide under high pressures ranging between 10-37 bars. Generally, increase in pressure decrease the lethal exposure time. Eggs, especially in early stages of development were known to be less sensitive to high pressure carbon dioxide treatments than other stages (Adler et al., 2000; Navarro, 2006). The cost of high pressure chambers limits the use of this method only to the treatment of valuable commodities such as dried fruits, nuts, spices, herbs and cocoa beans (Adler et al., 2000; Navarro, 2006). Elevated temperatures also help MAs to decrease the lethal exposure time significantly (Donahaye et al., 1994).

2.1.4. Vacuum treatments

Low O_2 atmospheres can be mechanically obtained by vacuum. The primary mechanisms of action of vacuum treatment on insect survival are shown to be lack of oxygen and dehydration due to removal of water vapor (Navarro, 2006). The need for massive, rigid and expensive structures withstanding the low-pressures was the main barrier in using low pressures at the large-scale commercial level. With the development of flexible plastic units, GrainPro Cocoons®, sufficiently low pressures (25-50 mmHg absolute pressure) to kill the insects can be obtained using a commercial vacuum pump and maintained for indefinite periods of time (Finkelman et al., 2003). This technology, known as vacuum-hermetic

fumigation (V-HF) is currently in use at commercial level for pest treatment of organic soybeans and flours in Israel (Navarro, 2006).

2.1.5. Resistance to phosphine and its management

Resistance, as in the case of phosphine, is the inevitable result of the continuous use of the fumigants in leaky conditions, improper applications and exposures. Although stored-products insects can develop resistance to the fumigants and modified atmospheres, resistance in field conditions is currently limited to phosphine. The number of pest populations showing resistance to phosphine has been increasing worldwide since it was first shown by a global FAO survey on pesticide susceptibility in 1972/1973 (Champ and Dyte, 1976).

Zuryn et al. (2008) proposed that phosphine targets the mitochondria in vivo and direct alteration of mitochondrial function may be related to phosphine resistance. They believed that multiple factors that influence metabolism, and specifically mitochondrial function, have a direct influence on both phosphine toxicity and resistance against its toxic effects. Mitochondrial membrane potential, rate of electron flow through the mitochondrial respiratory chain (Chaudhry, 1997), ATP levels, metabolic supply versus demand, and mitochondrial generated oxidative stress are all thought as metabolic factors that may contribute to phosphine sensitivity or resistance. There is a significant interrelation between respiration rate and phosphine resistance. The populations with lower carbon dioxide production showed a higher resistance ratio, suggesting that the lower respiration rate is the physiological basis of phosphine resistance by reducing the fumigant uptake in the resistant insects (Aurelio et al., 2007). The reduced uptake of the phosphine in resistant insects might be due either to the presence of a phosphine insensitive target site or to a membrane-based efflux system that excludes phosphine gas in resistant insects (Chaudhry, 1997).

In terms of phosphine resistance, there are three types of phenotypes: susceptible type, weakly (mildly) resistant type and highly resistant type. Phosphine resistance was found to be governed by multiple genes in both mildly and highly resistant phenotypes, at least one of which contributes a major factor to resistance in each type (Collins et al., 2002; Ebert et al., 2003; Athie and Mills, 2005; Lilford et al., 2009). Ebert et al. (2003) using molecular genetic techniques found that two major genes primarily control resistance in highly resistant strains of *R. dominica*. The first gene is responsible for 'weak' resistance, whereas, insects with a stronger level of resistance have the gene responsible for 'weak' resistance plus another gene. The second gene has little effect on its own but strongly enhances the effect of the first gene. Resistance is characterized by incompletely recessive alleles on these major genes. Thus heterozygous individuals show a limited expression of a lower level of resistance similar to susceptible insects. Schlipalius et al. (2002) showed that strongly resistant strain carrying resistance alleles has two loci on different chromosomes. Both Collins et al. (2002) and Schlipalius et al. (2002) conclude that one of the genes determining resistance in the strongly-resistant strain is also present in the weakly-resistant strain. Schlipalius et al. (2006) proposed that the gene (rph_1) shared between the weakly-resistant strain and the strongly-resistant strain was responsible for the initial emergence of phosphine resistance in Australia. Selection of the recessive allele for rph_1 under fumigation subsequently caused the selection of the recessive allele at an additional, secondary resistance gene (rph_2). Thus, strong level of resistance is depending on the presence of resistance alleles on both rph_1 and rph_2 (Collins, 1998). For *R. dominica* resistance for those homozygous with both copies of the sensitive gene has been determined to be well over 250X those with no copies of the resistance genes, whereas there is a resistance factor of 2.5X to 30X if the resistance genes are present in only one of the two locations, depending on which location (Lilford et al., 2009).

Collins (2006) predicts that in about 10 years weak resistance in *R. dominica* will reach a frequency of 100% all over Australia and strong resistance to phosphine will become a major problem in a few years time when weak resistance frequency reaches 80% throughout the country unless resistance is managed. Beside the increasing frequency of resistance, Collins (2006) also worries about its potential increase in strength. A strain of rice weevil imported under quarantine from southern China was revealed to be about 50% more resistant to phosphine than the most resistant strain of any species in Australia (Nayak et al., 2003). This situation may lead in the future that phosphine will be either ineffective, or almost useless because effective fumigations will require long fumigation periods and very high concentrations of gas (Collins, 2006). Moreover, Ebert et al. (2003) concluded that the resistance alleles would be completely

persistent in the field, even without the selective pressure of phosphine fumigation. Thus, rotation of control chemicals would not, of itself, lead to a reduction in the frequency of phosphine resistance alleles in the field. Thus, to combat resistance, fumigations must be fully effective.

The most of the phosphine-resistant populations exhibits reduced developmental and population growth rates than the susceptible counterpart populations. This implies that phosphine resistance is associated with fitness cost, which can potentially compromise the fixation and dispersal of the resistant genotypes (Pimentel et al., 2007). However, some phosphine-resistant populations did not show a fitness cost. Therefore, resistance management strategies based on minimizing phosphine use aiming at eventual reestablishment of phosphine susceptibility and subsequent reintroduction of this fumigant will be useful only for insect populations exhibiting a fitness cost associated with phosphine resistance. Therefore, recognition of the prevailing phosphine-resistant genotypes in a region is important to direct the management tactics to be adopted (Sousa et al., 2009).

For the management of phosphine resistance, early detection of the resistance and its strength is very important. There are several test methods for checking resistance in the field or in the laboratory. Of these, the use of resistance testing kits to test the pests before the application of the fumigant, and varying the phosphine concentration and the exposure time respectively, so that resistant beetles are also treated successfully with phosphine dosage accordingly can be useful. Reducing the selection pressure by limiting the application number against the populations exhibiting fitness cost or by applying non-chemical alternatives such as cooling and hygiene is recommended. Resistant insects must be totally eradicated only using approved rates of phosphine that are researched and known to control resistant insects, and by using alternative fumigants or protectants where available (Collins, 2006).

3. Conclusions

Worldwide ban on MB is no doubt threatening the future of stored-products protection against insect and mites. Currently, phosphine is the fumigant of choice regarding its low cost, availability, versatility in application, ease of use, and global acceptance as a residue free treatment. However, major stored-product insects have already developed strong resistance against phosphine and unfortunately resistance is spreading throughout the world. Sulfuryl fluoride, the most promising alternative, has not yet been available worldwide. There are other alternative fumigants, each of which is suitable only for treating a particular commodity or for application in specific situations. Thus, proper use of fumigants, particularly phosphine, is very important for protecting stored products against pests and for extending the use of phosphine. The monitoring and rapid detection of resistance and its strength is also vital for the safe use of fumigants. In case of the resistance, necessary actions must be taken immediately according to the nature of the resistance, i.e. whether it is weak, strong or is associated with fitness cost or not.

Use of non-chemical IPM tools, such as pest exclusion, hygiene, MAs, natural enemies, physical factors are also very important and efficient in pest controlling without the use of the fumigants. It is shown that by the implementation of an IPM program based on insect monitoring to detect infestation loci to intervene timely, there was no need to make regular annual fumigation with MB in a flour mill in Italy (Savoldelli and Panzeri, 2008). Similarly, heat disinfestation treatments are successful for the control of insect pests in flour mills or in empty grain bins. MAs including high CO_2, N_2 applications, vacuum treatments (Navarro, 2008), or hermetic storage (Rodriguez et al., 2008) provide excellent alternatives that is economic, sustainable, available, safe and environmentally benign to the use of traditional chemical fumigants in different situations, including bulk storage of grain and pulses, protection of organic food, protection of historic artefacts', museums and libraries, and modified atmosphere packaging for the food industry.

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Cost comparison of Methyl Bromide and Sulfuryl Fluoride (ProFume®) for fumigating food processing facilities, warehouses, and cocoa beans

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Abstract

Costs of fumigating a food processing/warehouse facility using methyl bromide and sulfuryl fluoride (ProFume®) were compared using an economic-engineering approach. The two fumigants were also compared for fumigating cocoa beans. Sulfuryl fluoride is economically feasible for cocoa beans. It is economically feasible for some, but not all, applications in food processing facilities and warehouses.

Keywords: Economics, Cost, Benefit, Fumigation methyl bromide, Sulfuryl fluoride, ProFume®

1. Introduction

Methyl bromide (MB) has been used for more than 50 years to control insects, nematodes, pathogens, and weeds. However, the parties to the Montreal Protocol classified it in 1992 as an ozone-depleting substance, and agreed in 1997 to an international phaseout. Because of its importance, many U.S. users have been concerned that existing alternatives to methyl bromide will be less effective and cause financial losses (USDA, 2000).

Phillips et al. (2000) noted that fumigant insecticides are the best tools for quickly reducing insect pest infestations in food processing structures and bulk grain storages, and that methyl bromide is the fumigant of choice in most flour mills and other food processing facilities in North America. Thus, its phase-out is critical in the food processing industry.

Because of concerns that alternatives to methyl bromide for certain applications were unsuitable or not economically feasible, The Report of the Technology and Economic Assessment Panel of the Montreal Protocol allowed for exemptions for use of methyl bromide where, among other criteria, "There are no technically and economically feasible alternatives or substitutes available to the user that are acceptable from the standpoint of environment and health and are suitable to the crops and circumstances of the nomination" (UNEP, 2005).

With few alternatives to MB available, National Pest Management Association (NPMA) has been able to obtain a Critical Use Exemption (CUE) under this provision, permitting its members to use up to a specified amount of methyl bromide each year to treat commodities, and food and feed processing plants. Beginning in 2004, a promising alternative to MB – ProFume® (PF), chemically known as sulfuryl fluoride – was approved by EPA and labeled for use with several cereal grains, dried fruits, and nuts. More recently, it was approved for use on cocoa beans. Although it has similar pest-control capabilities to MB, little independent information has been available to determine whether PF is an economically feasible substitute for MB in food processing facilities and warehouses, and for commodities. The purpose of this analysis was to compare the costs of fumigations using PF and MB, in both food processing facilities and in fumigations of cocoa beans.

2. Materials and methods

Statistical techniques such as econometrics would permit such a comparison if sufficient data with consistent measurements were available. In the short time since approval in 2004, though, the number of firms having used both fumigants is low, and the number of fumigations in which those firms that have used PF is very low and under widely varying conditions, so the usefulness of statistical comparisons is likely quite low.

Thus, an economic-engineering and partial budgeting approach was used, estimating costs using engineering and technical specifications. This approach provides estimates of costs that typical firms would face under typical scenarios, rather than costs particular firms might have experienced under unique situations.

As such, this approach permits comparisons between the two fumigants while holding other factors constant. Within reason, the economic engineering approach should provide useful cost predictions if underlying conditions change, by changing the parameter values used in the calculations. From the perspective of the fumigator, the profitability using either fumigant is Revenue Received from Fumigation minus Cost of Fumigation, where Revenue = Amount received from the fumigation customer (Client), and Cost = Labor Cost + Training Cost + Equipment Cost + Cost of Chemicals Used. It is assumed that a fumigator's revenue will be the same regardless of which chemical is used (this assumption was verified by the fumigator interviews), so the focus here is on costs. Although there may be public relations benefits from using a non-ozone depleting chemical, those benefits would depend on individual firms' marketing efforts.

2.1. Data sources

A potential limitation of an economic-engineering approach is that, because it is based on technical and engineering specifications, it may not reflect realities of use in actual fumigation situations. To provide confidence that the individual cost components reflect the realities of actual fumigations with these products, the economic-engineering estimates were calibrated using several data sources. The most important source was a set of telephone interviews with six fumigators who have used both MB and PF. These hour-long interviews focused on similarities and differences between MB and PF in costs of a typical fumigation. Since, as the fumigators noted, there really is no typical fumigation, a hypothetical fumigation of a 28,317 m^3 food processing/warehouse facility with a fumigant half-loss time of 12 hours was considered. The data determined were: labor needed for a fumigation (setup, fumigation, ventilation, and takedown), wages paid for labor, training needed for workers, differences in dosage and chemical costs, differences in equipment cost and facility preparation, differences in power use, typical pests targeted, and other relevant differences as determined by the fumigators.

A second set of interviews was conducted with wholesale distributors of MB and PF. The primary information obtained from these interviews was the wholesale price differences between MB and PF. The interview data from fumigators and wholesale distributors was supplemented with information from Dow Agrosciences – hereafter Dow – (cost of equipment used for PF, Fumiguide® dosages for PF, and field trial data on amount of PF needed for various fumigations), from National Pest Management Association (recommended dosages for MB and data condensed from several journal articles on efficacy of several fumigants on four stages of various insect species), and from journal articles on the effects of temperature on efficacy of methyl bromide for several species of insects (Vincent et al., 1980; Bell, 1988). Table 1 shows the parameters used in the core model. These parameter values reflect industry data at the time of this study. Changes in the industry or economy, including changes in underlying industry structure or changes in regulations or their interpretation, would likely affect these values.

Table 1 Parameters used to calculate costs of fumigating a 28,317 m^3 food processing facility using methyl bromide (MB) or ProFume (PF). Values in USA $.

Parameter	MB	PF
Labor Rates ($/hr)		
Setup	$18	$18
Supervisory	$50	$50
Fumigation	$18	$18
Aeration	$18	$15
Overtime	$28	$28
Hours/Worker		
Setup	5	5
Supervisory	15	15
Fumigation	24	24
Aeration	12	8
Overtime	20	16
# Workers		

Parameter	MB	PF
Setup	4	4
Supervisory	1	1
Fumigation	4	4
Aeration	3	3
Overtime	1	1
Worker Training		
hours per worker per year	5	5
yearly fee per worker	$150	$150
Interest Rate	**10%**	**10%**
Temperature (C)	29.4	29.4
Half-Loss Time (hrs)	12	12
Building Size (m^3)	28317	28317
Equipment Life (yrs)	4	4
Purchase Prices ($)		
Fumiscope	$1333	$1333
Interscan/electronic monitor	$1000	$3559
Draeger Tube Monitor	$215	$0
Heavy-duty hoses, fittings	$1500	$2480
monitoring hoses	$475	$475
high-capacity fans ($100/fan * 13 fans)	$1300	$1,300
Present Value Factor (PVIFA)	3.170	3.170
Cost of Fumigant ($/kg)	$15.00	$11 - $15
Fumigant Dose (g/m^3)	16, 24, 32	Fumiguide low (26), high (50); 40
Equivalent fumigations per year	50	50

2.2. Labor cost

Labor used in a fumigation includes a survey or analysis (screening) of the fumigation site (typically by a supervisor), then preparation of the facility by workers (including thorough sealing of vents and other openings; according to firms interviewed, this often can be done while the plant or warehouse is in operation), the actual fumigation, ventilation of the facility after fumigation, and removal of temporary sealing materials.

All of the fumigators surveyed indicated that these job components differed very little between the two fumigants. Some indicated that if there were any differences, PF required more attention to sealing because "…it is more volatile than MB" (technically, it has lower specific gravity (Thoms et al., 1990) and has a higher vapor pressure). In addition, they indicated that PF might require more setup time because hoses are inserted into the facility from outside, whereas MB tanks are brought into the facility itself. However, Thoms et al. (1990) note that methyl bromide can be introduced into a structure from the outside, and one of the fumigators interviewed reported capability of doing that.

On the other hand, the additional time and effort required for PF at these stages might be offset by reduced time needed for takedown (since PF tanks are already outside the facility and don't need to be taken out of the building). The similarity between MB and PF fumigations in these kinds of costs is supported by the reported experiences of Subramanyam (2006). Also, because PF has higher vapor pressure and lower sorption, most of the fumigators reported that aeration of the facility after fumigation would take less time.

Labor cost is specified as [(setup labor hours/worker x number of setup workers x operating wage rate) + (supervisory labor hours/worker x number of supervisors x supervisory wage rate) + (fumigation labor hours/worker x number of fumigation workers x fumigation wage rate) + (aeration labor hours/worker x number of aeration workers x aeration wage rate)]. If any of these workers must work more than eight hours per day, they are assumed to receive time-and-a-half pay for those hours.

2.3. Training cost

Training cost is specified as a combination of an annual training fee (assumed to be $150/worker) plus an hourly charge for each worker equal to his/her hourly wage rate x number of hours of training required per year (assumed to be five hours per worker). The total training cost for all workers is divided by the number of fumigation jobs per year (assumed to be 50) to express the training cost as training cost/job.

2.4. Equipment cost

There are some differences in equipment cost between MB and PF because PF requires more specialized equipment. Typically, a computer is needed to calculate dosage of PF using the Fumiguide®. Strong-walled hoses are needed to introduce PF into the facility. During fumigation, concentration of PF and MB is typically measured using a Fumiscope or similar device, and a device such as Interscan (PF) or color diffusion tube-type monitor (MB) is used to measure whether fumigant concentrations have decreased sufficiently to permit safe re-entry of the facility after ventilation. Since a tube-type monitor is the only approved device for determining re-entry clearance after a MB fumigation (Degesch America), the amortized cost of a tube-type monitor as well as the cost of two tubes at 12 USA$ per tube (assuming one test in each of two locations) is included for MB.

Each piece of equipment is amortized over its expected useful life using the formula Equipment Cost = [purchase cost of equipment]/$PVIFA_{ni}$, where $PVIFA_{ni}$ denotes present value interest factor for an annuity of n years at i percent interest. $PVIFA_{ni} = [1 - (1/(1 + i))^n]/i$, where n is the usable life of the machine and i is the interest rate on the loan. Dividing by $PVIFA$ allocates the investment cost, including interest cost, equally over each year of the equipment's useful life. The yearly equipment cost is divided by the number of fumigations per year to express equipment cost as equipment cost per fumigation.

2.5. Cost of chemicals used

Cost of chemicals used appears to be the main factor affecting fumigation cost differences, according to the interviewed firms. Chemical cost is measured for each chemical as dosage in g/m^3 x 1,000 x cost (USA$/kg) x 28,317 m^3. PF's Fumiguide has three choices for fumigators: a high dose, which should be nearly 100% effective for all stages (eggs, larvae, pupae, and adults) of the insect species for which it is approved for use, a user-defined rate, and Fumiguide's low dose, which should be effective for all post-embryonic stages and 50% effective for the egg stage of most species. MB is efficacious for most insects at all life stages at the recommended doses.

Dosage rates for MB are taken from reported experiences of the fumigators interviewed, as well as from label rates and other data sources. Dosage rates for MB are assumed to apply for temperatures from 21.1 - 37.8°C. The label rate for MB is 16 g/m^3 for processed food (up to 48 g/m^3 for spices and herbs, and 64 g/m^3 for dried peas). Half of the fumigators interviewed reported using 24 or 32 g/m^3 for a 24-hour exposure time, and half of the fumigators reported that their standard rate for MB was 16 g/m^3. This was especially true if they had previously fumigated a particular facility and repaired any leaks, or if they had monitored the concentration-time (CT) product and found that a lower dose provided effective exposure of insects to the fumigant. There is also a possibility that a fumigator may need to use a higher rate of MB.

In addition, Dow conducted 96 fumigations at food processing facilities and warehouses. Their average dosage for these facilities was 40 g PF/m^3, suggesting that as fumigators gain experience with PF, they may find that they will be able to reduce dosage from the Fumiguide recommendations, adapting it to specific facilities just as they have with MB. Although it is included here for comparison purposes, none of the fumigators interviewed used the low dose for fumigating processed food. Since the low dose is not sufficient to kill all eggs of some insect species (particularly red flour beetle, a typical pest reported by the fumigators), they reported concerns about the potential for insect population rebound and dissatisfied customers. However, Subramanyam (2006) reported effective fumigations using PF's low dose. He noted, though, that in two of three mills, insect populations reached pretreatment levels after two months, and that further research was needed to determine the reason for this population rebound (Subramanyam, personal communication). Here, the dosage of MB is varied from 16 g/m^3 to 32 g/m^3, and the dosage of PF is varied from Fumiguide's low dose to Fumiguide's high dose.

Fumigators and distributors reported that differences in price they paid for MB and PF ranged from zero (no difference) to PF 4.41 USA$/kg less expensive than MB. Because the number of observations is small, and the range of reported values is large, presenting the results based on the range of price/quantity observations rather than on an average of them provides greater confidence in the results. Thus, the wholesale cost of MB is set at 15.43 USA$/kg, and the wholesale cost of PF is varied from 11.02 USA$/kg to 15.43 USA$/kg (a relative difference of 0 USA$ to 4.41 USA$/kg, consistent with the range reported by fumigators and wholesalers).

3. Results

3.1. Labor and training costs

Labor costs for a hypothetical fumigation with each fumigant are shown in Table 2. The only difference between MB and PF is a reduced labor cost for ventilation for PF because its greater vapor pressure and lower sorption likely permits faster aeration of the facility after fumigation.

Table 2 Equipment cost per year and total per job for methyl bromide and ProFume fumigation. Values in USA$.

Amortized Equipment Cost per yr.	Methyl Bromide	ProFume
Fumiscope	$421	$421
Interscan/electronic monitor	$315	$1123
hoses	$473	$782
monitor hoses	$150	$150
tube-type monitor	$68	$0
monitor tubes ($24/job x 50 jobs)	$1200	$0
Fans	$410	$410
Total per job (50 jobs/yr)	$61	$58

3.2. Equipment costs

Equipment costs for each fumigant using the parameters specified in Table 1 are shown in Table 3, which reports the costs on a yearly basis and as cost per job, assuming 50 fumigations jobs per year.

Table 3 Labor cost per (hypothetical) fumigating job for methyl bromide and Profume. Values in USA$.

Labor Cost per job	Methyl Bromide	ProFume
Setup	$360	$360
Supervisory	$750	$750
Fumigating	$1728	$1728
Ventilation	$648	$432
Overtime	$864	$864
Total	$4350	$4134

3.3. Cost of chemicals used

These results indicate that PF has a very slight advantage in equipment cost, and a somewhat larger, though still small, advantage in labor cost. However, the cost of fumigant used in a typical fumigation is higher for PF than for MB for most scenarios. Although the price per kg of PF is the same or lower than that for MB, a greater quantity of fumigant is typically used for a PF fumigation than for a MB fumigation. Although the fumigating companies were not asked about profitability, one of them noted that because more PF fumigant is used in a typical fumigation, its revenues (and profits) are higher fumigating with PF than with MB. The markup it charges clients is the same for each chemical, but since the quantity of PF used is higher, the extra revenue received is correspondingly higher. The flip side, of course, is that the client faces higher costs.

3.4. Total fumigation costs

To show the fumigation costs under various combinations of fumigation dosage and fumigant price, Table 4 is arranged so that from left to right the dosage of MB varies from 16 g/m^3 to 24 g/m^3 to 32 g/m^3, and the dosage of PF varies from Fumiguide's low dose to Dow Agroscience's average field test dose of 40 g/m^3 to Fumiguide's high dose, while from top to bottom, the price of PF is varied.

Table 4 Cost of hypothetical 24-h fumigations of a 28,317 m^3 food processing facility for methyl bromide and ProFume. Values in USA$.

			Cost per Job ($)					
Cost MB/Kg ($)	**Cost PF/Kg ($)**	**Item**	**Methyl Bromide (16 g/m^3)**	**Methyl Bromide (24 g/m^3)**	**Methyl Bromide (32 g/m^3)**	**(low ProFume dose 26 g/m^3)**	**(ProFume Dow average - 40 g/m^3)**	**ProFume (high dose 50 g/m^3)**
15.34	15.34	Equipment	61	61	61	58	58	58
		Labor	4350	4350	4350	4134	4134	4134
		Training	19	19	19	19	19	19
		Fumigant	7000	10500	14000	11452	17500	22008
		Total Cost	11430	14930	18430	15663	21711	26219
15.43	13.22	Equipment	61	61	61	58	58	58
		Labor	4350	4350	4350	4134	4134	4134
		Training	19	19	19	19	19	19
		Fumigant	7000	10500	14000	9816	15000	18864
		Total Cost	11430	14930	18430	14027	19211	23075
15.43	11.02	Equipment	61	61	61	58	58	58
		Labor	4350	4350	4350	4134	4134	4134
		Training	19	19	19	19	19	19
		Fumigant	7000	10500	14000	8180	12500	15720
		Total Cost	11430	14930	18430	12391	16711	19931

For example, when PF costs 11.02 USA$/kg (4.41 USA$/kg less than MB), the dosage rate for MB is 24 g/m^3, and the dosage rate for PF is the Fumiguide® high dose, then the total fumigation cost for PF is 19,931 USA$. This is 33% higher than the 14,930 USA$ cost of a MB fumigation. The difference is smaller if the dosage rate of PF is lower. In the example above, when the dosage rate for PF is reduced to 40 g/m^3 (the Dow experiments' average), the cost of a PF fumigation is 16,711 USA$. This is 12% higher than the 14,930 USA$ cost of a MB fumigation. Table 5 shows these percentage differences.

Table 5 Percent by which ProFume fumigation cost exceeds methyl bromide fumigation cost for a 28,317 m^3 food processing facility*. Values in USA$.

		% by which PF fumigation cost exceeds MB fumigation cost		
Cost PF/Kg ($)	**MB (g/m^3)**	**26 ProFume (low dose g/m^3)**	**ProFume (Dow average - 40 g/m^3)**	**ProFume (high dose 50 g/m^3)**
15.43	16	37	90	129
	24	5	45	75
	32	-15	18	42
13.22	16	23	68	102
	24	-6	29	55
	32	-24	4	25
11.02	16	8	46	74
	24	-17	12	33
	32	-33	-9	8

*Negative numbers indicate that MB cost exceeds PF cost.

Conversely, the difference is larger if the relative cost of PF fumigant is higher or if the dosage rate of MB is lower. For example, if the cost of PF is only 2.20 USA$/kg less than the cost of MB, the dosage rate of MB is 24 g/m^3, and the dosage rate of PF is 40 g/m^3, the total cost of a PF fumigation is 19,211 USA$ and the cost of a MB fumigation is 14,930 USA$, so that the cost of a PF fumigation is 29% higher than that of a MB fumigation. If the dosage rate of MB is lowered to 16 g/m^3 (while holding other values the same), the cost of a MB fumigation drops to 11,430 USA$ and the cost of a PF fumigation is 68% higher than the cost of a MB fumigation. (These parameter values are consistent with those fumigators who reported using 16 g/m^3 for a MB fumigation and Fumiguide's high dose for a PF fumigation).

3.5. Fumigation of cocoa beans

The fumigators reported very little difference between MB and PF in labor cost and equipment cost, so those parts of the economic-engineering model are transferred directly from the previous section, assuming fumigation jobs comparable in size to that assumed in the previous section. However, fumigation of cocoa beans requires a lower dosage of ProFume than fumigation of food processing facilities because cocoa bean fumigation is commodity fumigation rather than space fumigation, and the commodity is taking up a larger proportion of the fumigated space.

The fumigators interviewed reported successful fumigation with 16 g/m^3 of MB, and 24 g/m^3 of PF, a dose which was recommended by Dow representatives for application to cocoa beans. Table 6 shows the cost of fumigating cocoa beans using these doses. When the two fumigants cost the same per kilogram, a PF fumigation costs about 29% more. When the costs differ by 2.20 USA $/kg, a PF fumigation costs 16% more, and when the cost difference is $4.40/kg, a PF fumigation costs 2% more.

Table 6 Cost of hypothetical 24-h fumigation for cocoa beans (MB dose at 16 g/m^3 and PF at 24 g/m^3). Values in USA$.

		Cost per job ($)						
MB dose (g/m^3)	**Item**	**Methyl Bromide @$15.43/kg**	**ProFume @$15.43/kg**	**% Diff.**	**ProFume @$13.03/kg**	**% Diff.**	**ProFume @$11.03/kg**	**% Diff.**
16	Equipment	61	58		58		58	
	Labor	4350	4134		4134		4134	
	Training	19	19		$19		19	
	Fumigant	7000	10500		9000		7500	
	Total Cost	11430	14711	29%	13211	16%	11711	2%
24	Equipment	61	58		58		58	
	Labor	4350	4134		4134		4134	
	Training	19	19		19		19	
	Fumigant	10500	10500		9000		7500	
	Total Cost	14930	14711	-1%	13211	-12%	11711	-22%

*Negative numbers indicate that MB cost exceeds PF cost.

However, if the dose used for MB is 24 g/m^3, the rate specified in the National Pest Management Association's application for Critical Use Exemption – the label for MB specifies a rate ranging from 1 - 2 lbs/1,000 ft^3 (16-32 g/m^3) for cocoa beans using a 16-24 h exposure, Table 6 shows that when the two fumigants cost the same per pound, a PF fumigation costs about 1% less. When fumigant costs differ by 2.20 USA$/kg, a PF fumigation costs 12% less, and when the cost difference is 4.40 USA$/kg, a PF fumigation costs 22% less. The key reason for PF being relatively more attractive economically for cocoa beans than for food processing facilities, compared to MB, is because the amount of PF needed for cocoa beans is substantially lower.

4. Discussion

The most important factor affecting relative profitability of the two fumigants is amount of PF required for effective fumigation relative to amount of MB required. Under typical assumptions and parameter values, a PF fumigation uses about two thirds more fumigant than MB. , Even though unit price of PF is

typically less than price of MB, a fumigation of a 28,317 m^3 warehouse using PF is 28% to 55% more expensive than the same fumigation using MB. The difference is less if the price of PF is reduced relative to the price of MB, or if dosage rate of PF can be reduced. PF can be less expensive than MB, however, for fumigating cocoa beans because the amount of PF needed for cocoa beans is substantially lower.

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Presentation of results of fumigation of different commodities and fumigation of resistant insect species with Quickphlo-R phosphine generator

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Abstract

Over the years fumigation is being done with conventional phosphine formulations like tablets, pellets and bags or Magnesium Phosphide formulation with all the built in inefficiencies, hazards and labor involved in applying the product. This has resulted in some insects developing resistance to phosphine or increased doses of phosphine. However, trials with QuickPHlo- R phosphine generator and the QuickPHlo- R granular formulation showed the merits of this technology over conventional formulations and application methods to achieve 100% kill of the so-called resistant species with phosphine gas.

The technology is operator and environment friendly with built-in scrubber for deactivation of spent formulation.

Storage of canola in hermetic plastic bags

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Abstract

Due to the small size of the seed, canola (*Brassica napus or Brassica campestris*) offers different challenges in the harvest and the subsequent post-harvest operations. Often, in Argentina, farmers do not have enough permanent storage capacity so they overcome this deficit with the use of hermetic plastic bags (silobags). The objectives of this work were: 1) Determine the feasibility of the bagging and extraction processes of canola. 2) Monitoring the condition of canola by periodic measurement of carbon dioxide (CO_2), temperature, moisture content (m.c.) and quality of the grain. Thirty tonnes of canola with initial m.c. of 6 % were stored in a silobag in the southeast of the Buenos Aires province, Argentina. The storage period was extended from November 2008 to November 2009. The variables measured every two weeks were CO_2 concentration, m.c. and grain quality parameters, such as foreign matters, fat acidity and fat content. The temperature and relative humidity (r.h.) of the interstitial air inside the bag and of the ambient air were also recorded with a frequency of one hour. It was observed that, even the size and characteristics of the canola seeds, it was possible to perform the bagging and extraction operations of canola seeds without problems. The r.h. in the interstitial air remained below 50% along the entire storage period. The temperature of the grain inside the bag followed the monthly average ambient temperature. The CO_2 concentration ranged from 1 to 8 %, indicating low to moderate biological activity in the grain mass. The m.c., foreign matters and fat values remained unchanged throughout the storage period. The fat acidity increased during storage in 0.7 % points, reaching a final value of 1.4 %, but did not represent a commercial quality loss. It was concluded that under the conditions of temperature and m.c. evaluated in this study it is possible to store canola in hermetic plastic bags without commercial quality deterioration.

Keywords: Silobags, CO_2 concentration, Interstitial air, Moisture content, Fat acidity.

1. Introduction

Canola (*Brassica campestris or Brassica napus*), is an oilseed widely spread in the world, that produces excellent quality edible oil.

The increasing demand of vegetal oils as renewable energy source (biodiesel) transformed the European Unión (EU) into the main importer of soybean, canola, sunflower and palm oil in the 2006, with canola oil the most desired one for the elaboration of biodiesel.

In Argentina, the Buenos Aires province is the area of greater diffusion of canola, with 14 thousand hectares. This crop was limited in the production mainly because it competes with wheat for the land use, and the wheat production used to be more profitable than canola. The lack of selective herbicides that would allow eliminating an extensively distributed weed (*B. napus*, called "Nabo") closely related to canola, is other reason that reduced its adoption in some areas. In the Argentine wheat region, canola constitutes a diversification alternative to enrich the rotation scheme, especially in the South of the Buenos Aires and Pampa provinces. Since canola is harvested earlier than wheat, allows anticipating the soybean planting a couple of weeks (two crops per year in the same land) with greater expected yield than planting soybean after wheat (SAGPyA, 2010). Under this production system (double crop), the combination canola-soybean is more profitable than the combination wheat-soybean.

The obtained edible oil from canola is one of the most appreciated and demanded by its excellent quality, and along with the olive oil, is considered one of the best for human consumption due to its contribution to the low cholesterol formation in the blood. The genetic improvement in the last 40 years, mainly Canadian cultivars, has allowed increasing the quality of this oil (SAGPyA, 2010).

Due to the small size of the seed, canola offers different challenges in the harvest and the subsequent post-harvest operations (Bartosik, 2008). Probably, the factor that affects the quality during storage of canola is the initial condition of the seed prior to storage. Harvested canola seed can maintain high respiration rates, up to 6 weeks, before turning quiescent. This process, commonly called "sweating", constitutes a very unstable condition for the stored canola (Thomas, 1984). During this stage a constant monitoring is required, since the high respiration rate of the seed produces conditions of heat and humidity that favor development of mold in storage. The mold growth produces more heat and humidity, and as a result the seeds of canola can be damaged. The effect of the "sweating" can be diminished by storing dry and cool grains (Thomas, 1984).

Due to the small size of the seed it is necessary to check and repair all the orifices, cracks and fissures of the storage facility, combines and grain handling equipment.

For many farmers who do not have permanent storage structures, storing grain in hermetic plastic bag (silobag) is a popular alternative solution in Argentina (Bartosik et al., 2008). Each silobag can hold approximately 200 tonnes of wheat (180 tonnes of soybean) and with the handling equipment currently available, the loading and unloading operation is fast, simple and totally mechanized. These silobags are 60 m long, 2.74 m diameter and the plastic liner is made of three layers (white outside and black inside) with 235 micrometers of thickness (Rodríguez et al., 2001 and 2002 a, b, c). A modified atmosphere is generated in the silobag, where the concentration of CO_2 increases and O_2 decreases.

Storage of canola in silobags has not been documented until this study. The objectives of this work were: 1) Determine the feasibility of the bagging and extraction processes of canola. 2) Monitoring the canola storage condition in the silobag by periodic measurement of carbon dioxide concentration, temperature, moisture content (m.c.), fat content and fat acidity.

2. Materials and methods

This study was carried out in a farm located in the South East of Buenos Aires province, Argentina, where 30 t of canola were harvested and stored in a silobag. The test started right after the harvest (25/11/2008) and lasted until the silobag was opened for selling the grain (23/11/2009).

Two sampling locations were established in the silobag. In each sampling location the gas composition was analyzed with a portable gas analyzer (PBI Dan Sensor, CheckPoint, Denmark), perforating the plastic cover with a needle at three levels at each sampling location: close to the top of the bag, at the middle and close to the bottom.

In each sampling location three samples of seeds were collected from three different levels of a 1.7 m tall bag (top = 0.10 m depth, middle = 0.75 m depth, and bottom = 1.6 m depth) using a standard torpedo probe. After probing the silobag the perforations were sealed with a special tape in order to restore the air-tightness. This sampling procedure was repeated approximately every 2 wk during the entire storage period. The samples were taken to the laboratory to determine the commercial quality. The analyzed parameters were m.c., foreign matters, fat acidity and fat content.

The temperature and relative humidity (r.h.) of the interstitial air inside the bag and of the ambient were recorded at a frequency of one hour with two data loggers (Hobo, H8, pro series, ONSET Computer Corporation). One of them was placed inside the silobag, inserted in the grain mass in the central zone, about. 0.5 m from surface, for monitoring the temperature of the grain and interstitial air r.h. The other was placed on the outside of the silobag, covered with a plastic film to minimize the effect of sun radiation.

3. Results

The bagging and extraction operations of canola were carried out without problems, even though the small size of the seed and the low repose angle. The holding capacity of a 2.7 m diameter silobag (standard of the market) was of 3100 kg per linear meter of bag (similar to soybean and maize). The first sampling location (R 1) had a maximum CO_2 concentration of 4.3%, minimum of 1.2%, average of 2.6% and a standard deviation of 0.9% (Figure 1).The second sampling point (R 2) had a maximum CO_2 concentration of 8%, minimum of 0.6%, average of 3.8% and a standard deviation of 2.2% (Figure 1). Figure 2 shows that, as expected, the percentage of foreign matters did not change along the storage time.

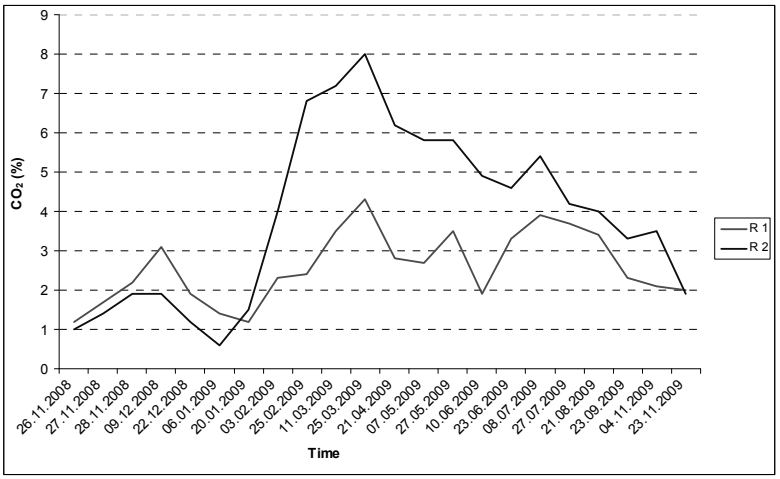

Figure 1 Changes in CO_2 concentration in two sampling locations (R1 and R2) of the canola hermetic plastic bag.

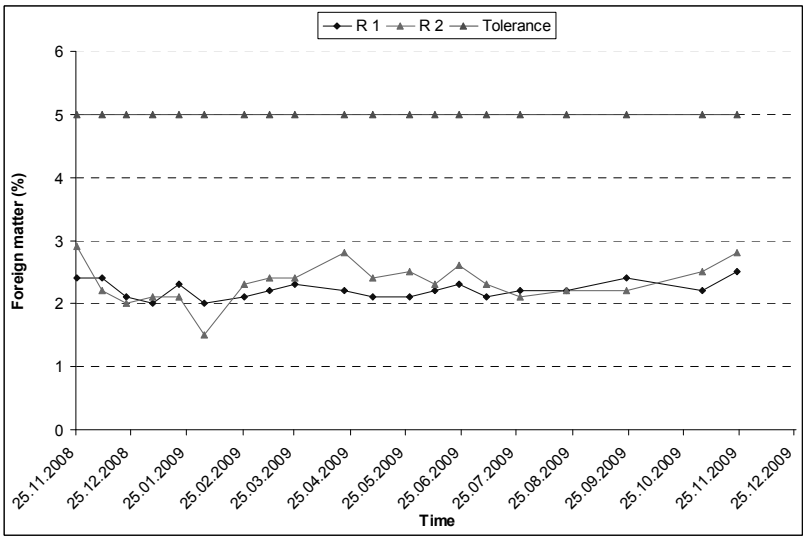

Figure 2 Percentage of foreign matters in two sampling locations (R1 and R2) and commercial standard tolerance of canola.

The percentage of fat acidity increased during storage from 0.7% to 1.4% at the end of the storage time. The increase in fat acidity started after 6 mo of storage, in May (Figure 3).

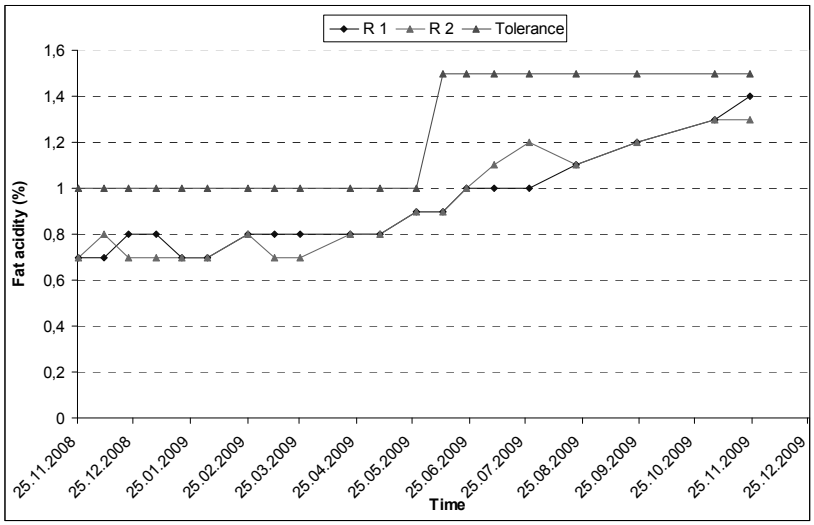

Figure 3 Fat acidity in two sampling locations (R1 and R2) and commercial standard tolerance of canola stored in bags.

Figure 4 shows that the fat content remained constant in the range between 44.5% and 45% during the entire storage period.

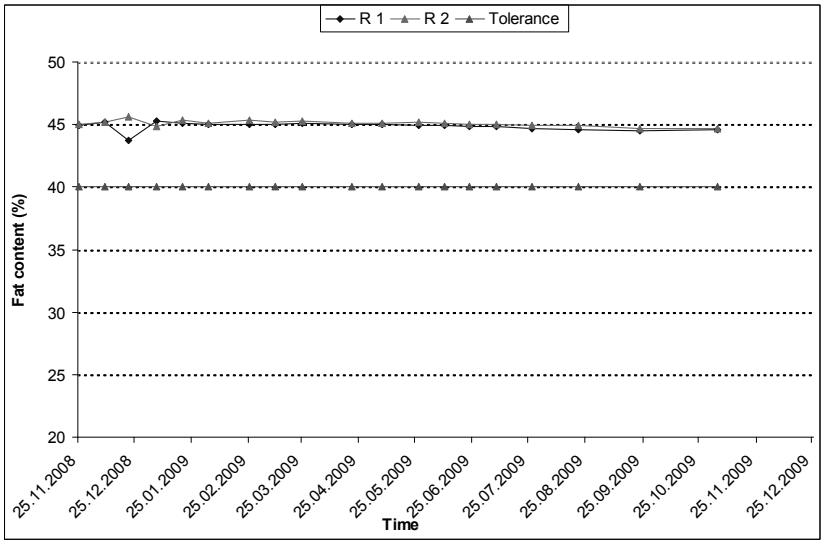

Figure 4 Fat content (%) of canola stored in bags in two sampling locations (R1 and R2) and commercial standard tolerance.

Figure 5 shows that the m.c. of canola seeds remained fairly constant during storage (between 5.3 and 6.2%).

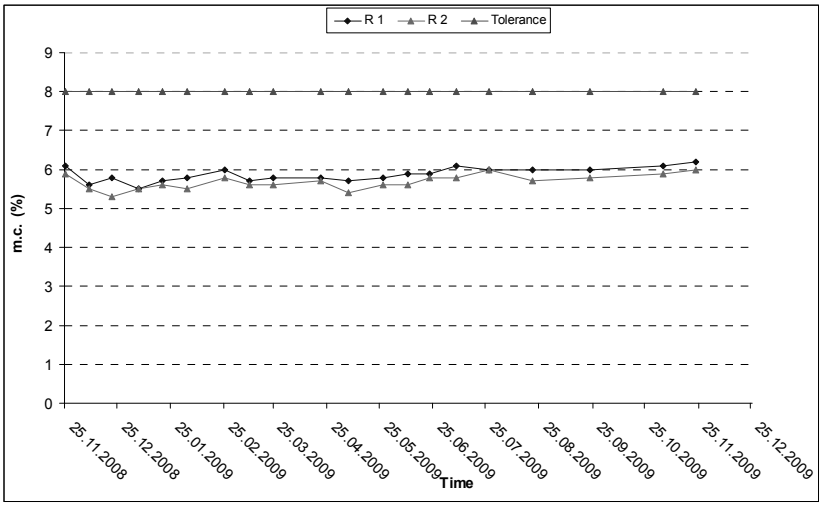

Figure 5 Changes in m.c. of canola based on the time of storage and the commercial standard tolerance.

The temperature of the canola seeds at the harvest time was of 37ºC (end of November, late spring). During storage, the seed temperature quickly decreased close to the daily ambient average temperature (25°C). The seed temperature decreased during the winter time to 5°C and then increased during the spring to almost 20°C (Figure 6).

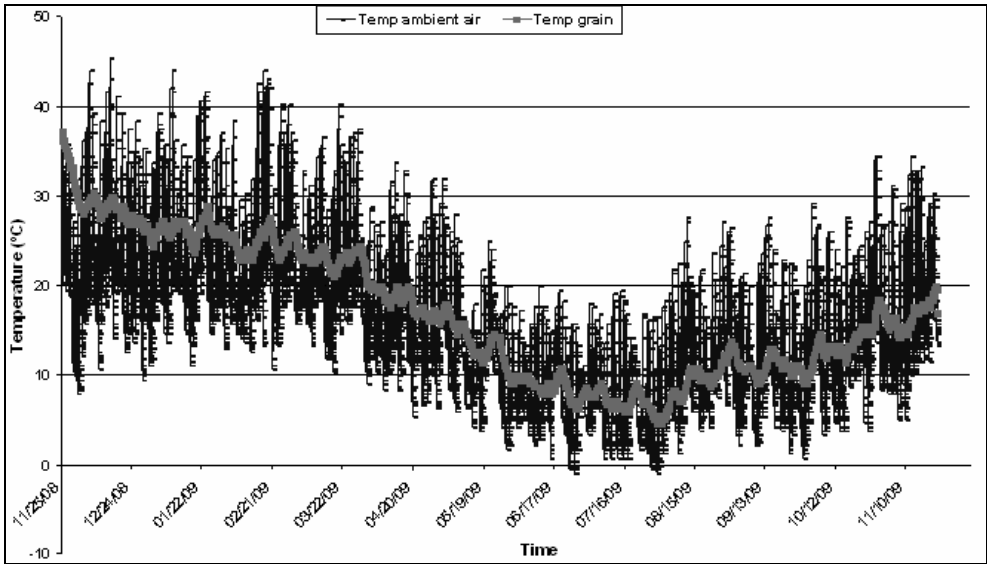

Figure 6 Temperature of the seeds and the ambient air over time of storage.

As shown in Figure 7, the r.h. of the interstitial air remained in between 40 to 50% throughout the storage period.

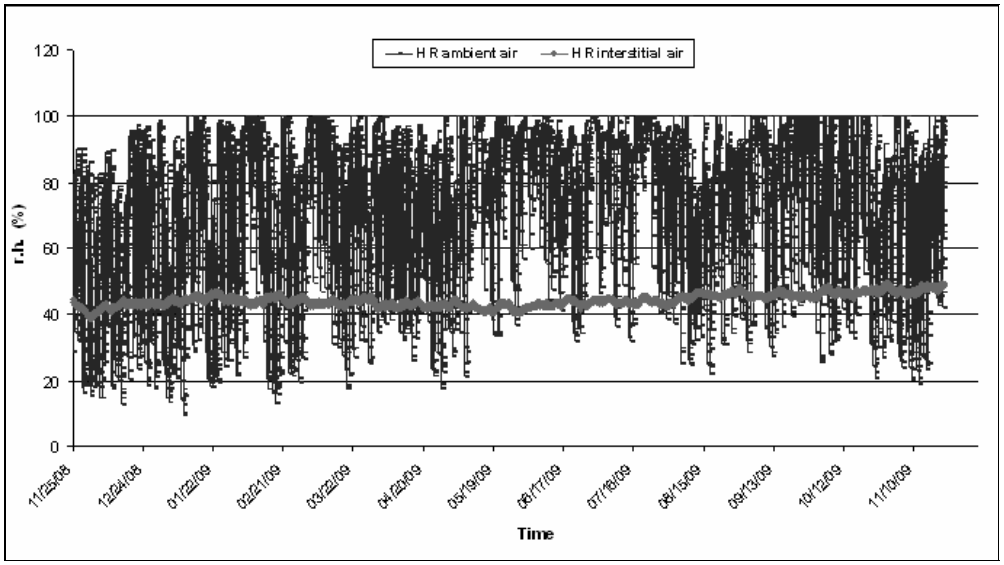

Figure 7 Relative humidity of the interstitial air and that of the ambient air over time of storage.

4. Discussion

The CO_2 concentration ranged between 1 and 8%, remaining at low levels to indicate low to moderate biological activity in the seed mass. Low biological activity was expected since the seeds had a m.c. below the safe limit of 8%. These CO_2 concentration values are in agreement with those observed by Bartosik et al. (2008) and Rodríguez et al. (2002 a, b, c) for other grains stored in silobags (wheat, soybean, sunflower and maize) in safe conditions (dry and clean). Additionally, these CO_2 concentrations agree with those reported by Darby and Caddick (2007) for canola stored at the same m.c. levels under Australian climate conditions.

In February and March, the CO_2 concentration of sampling location 2 increased from 1.5% to 8%. This increase in biological activity could be produced by the penetration of rain water through perforation that remained unsealed. However, no measurable damage was recorded in the bag. Unexpectedly, as storage time progressed the CO_2 concentration decreased down to 2%. It could be hypothesized that water penetrated the bag wetting a small proportion of grain in such a way that increased the biological activity. During the storage time, the localized moisture was slowly distributed by diffusion and air convective movements to the rest of the seed mass, eliminating the spot that could produce biological activity.

The foreign matter concentration was always below 3%, which is the tolerance of the commercial standard for canola in Argentina. It was not expected that this parameter would change during storage, but it is important to establish that to ensure that the bagging operation did not substantially increase the foreign matter, neither damaged the grain.

The fat acidity was always lower than the maximum commercial standard limit allowed in Argentina. The standard allows increase in fat acidity from harvest time until May to 1%, while the samples were below 0.8%. After May, the limit allows increase to 1.5%, while the samples had a maximum of 1.4% by November, one year after harvest.

The change in fat acidity observed in this study for canola was similar to those observed by Rodriguez et al. (2001) for sunflower seed (8.4 % m.c.) stored in silobag, which increased from 0.88% to 1.39% after 160 d of storage.

The fat content remained above the minimum limit (40%) established in the Argentina as commercial standard for canola (SAGPyA, 1994). The amount of fat remained unchanged, which is in agreement with the low biological activity observed in the bag.

These results agree with previous data (Bartosik et al., 2008; Rodríguez et al., 2002; Ochandio, 2008) that show that the average seed m.c. does not change during storage in the silobag, although some moisture stratification can be observed, particularly when seed is stored at relatively high m.c.

The temperature of the grain follows the variation of the monthly average ambient temperature, in agreement with the results found by Bartosik et al. (2008). The temperature had a minimum of 4.6ºC in the winter, by the end of June, and it began to rise during spring, with the increase of the ambient air temperature. Additionally, it was also shown that the core of the grain mass did not exhibit the daily temperature oscillation that could be observed in the ambient air temperature.

The r.h. (45 %) of the interstitial air was the expected equilibrium relative humidity for canola at 6 % m.c. Since the seed m.c. did not substantially change during storage, it was also expected that the equilibrium relative humidity remained constant.

In conclusion from this study it was observed that, in spite of the small size and the special characteristics of the canola seeds, it was possible to perform the bagging and extraction operations of canola seeds without problems, and that under the conditions of temperature and m.c. evaluated in this study, it is possible to store canola seeds in silobags without quality deterioration at commercial level for a relatively long period of time of 1 year.

Acknowledgments

The authors want to thank the Ribner family for facilitating their farm, grain and logistic for making the silobag used in this study. Special thanks to Leandro Cambareri for helping with the data collection. Thanks to the National Institute of Agricultural Technologies and the silobags manufacturing companies (IPESA and Plastar) for the financial support of this study.

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Storage of quality malting barley in hermetic plastic bags

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Abstract

The main destination of barley grown in Argentina is malt production. The main standard quality parameter for the malting industry is to maintain at least 98% germination percentage (GP). A typical operation is to harvest dry barley (around 12%) and store it in hermetic plastic bags, a temporary storage system of modified atmosphere, until end use in the malting industry. The objective of this study was to determine whether the typical Argentinean storage condition of malting barley in hermetic plastic bags produces a deleterious effect in its commercial and industrial quality. Two plastic bags filled each with 180 tonnes of malting barley were used for this experiment, one with 11% moisture content (m.c.) and the other with a range between 11 and 11.5% m.c. The experiment began immediately after harvest on December 27th (early summer) and lasted for five months. Carbon dioxide (CO_2) concentration, grain temperature, m.c., protein and GP were evaluated every 2 wk. GP did not substantially decrease during the entire storage period for both bags, but samples with higher m.c. had the lowest GP. The protein percentage remained stable throughout the entire evaluation period for both bags. The maximum value of CO_2 in the bag with 11% m.c. was 4.4%. The bag with the higher range of m.c. had a maximum CO_2 value of 13%, and this high concentration was associated to a small portion of spoiled grain, presumably due to rain water entering the bag through perforations in the plastic cover at the bottom of the bag. It was concluded that it is safe to store quality malting barley with 12% m.c. or less in hermetic plastic bags for five months.

Keywords: Silobag, Grain, CO_2, Germination

1. Introduction

The annual production of barley in Argentina is currently estimated in 2 million tonnes, of which 90% is used for malt production (Cortesse, 2009). The malting process requires a high and uniform germination of barley seeds (Savio and Cattaneo, 2008). Consequently, the commercialization standard includes specific parameters for determining the industrial performance of the grain, such as grain size, maximun and minimun protein values (between 11 and 13%) and a high percentage of viability or germination (basis of 98%, tolerance of 95%) (SAGPyA, 2009).The length of the storage period could be critical for some of the barley quality parameters listed above, for example, seed viability, and thus for the performance of the malting process. Moisture content (m.c.) and temperature of grain during storage can affect enzymatic processes for the production of malt (Darby and Caddick, 2007).

In Argentina, barley is stored in bins and flat storage structures by industry and grain elevators 489,000 t, according to the Cámara Industrial de Cervecería Argentina (2007), although a significant fraction of the harvested grain is retained by farmers in temporary storage structures made of hermetic plastic bags (silobags). This is a self-modified atmosphere storage system, with limited exchange of gasses between the interstitial atmosphere and outside atmosphere. Each plastic bag is 60 m long, 2.74 m in diameter and 235 microns thick. Bags are made of a plastic material with three layers, black on the interior and white on the exterior side. Each bag can store about 180 t of barley, soybean or corn.

There are few references to barley storage in plastic bags. Recently, Ochandio, et al. (2009) found good quality preservation of barley (high germination percentage and stable protein content) after 12 months of storage at around 12% m.c. (marketing limit). Darby and Caddick (2007) mentioned that barley stored in plastic bag under Australian conditions, even with 11% m.c. or less, can result in a peripheral layer of damaged grain with general deterioration and loss of quality for malting. Simulation studies calculated an area of 20 cm near the surface of the bag (20% of stored grain) is susceptible to deterioration (Gaston, *et*

al., 2007) for wheat stored in a plastic bag in the same season as barley. The objective of this study was to determine whether typical Argentine storage conditions in hermetic plastic bags affect industrial and commercial quality of malting barley.

2. Materials and methods

Tests were conducted on a farm "Mitikily", in the district of Tres Arroyos (Buenos Aires, Argentina). Two plastic bags (A and B) were filled with approximately 180 t of malting barley each. The barley in bag A had about 11.0% m.c., while in bag B it ranged between 11.0 and 11.5% m.c. The experiment began immediately after harvest on December 27th (early summer) and lasted during five months. Carbon dioxide (CO_2) concentration, grain temperature, m.c., protein and germination percentage (GP) were evaluated.

Three sampling locations were established for each plastic bag, the first one at 5 meters from the beginning of the bag (S.I), the second sampling location was at the central part of the bag (S. II) and the third location at 5 m from the end of the bag (S. III). The sampling procedure in each location consisted in measuring the CO_2 concentration with a portable gas analyzer (PBI Dan Sensor, CheckPoint, Denmark), perforating the plastic cover with a needle. A wood stick with three temperature sensors was then inserted into the grain mass (diagonally, from top and side to bottom and center of the silobag) to measure grain temperature at approximately 0.1; 0.7 and 1.4 m from the grain surface. The temperature readings were obtained between mid-morning and noon. Later, in each sampling location, a grain sample was collected using a standard torpedo probe from three different levels (0.10; 0.75 and 1.6 m depth, corresponding to the top, middle and bottom layer, respectively, being the total height of the bag was 1.7 m). Material from each of the three sampling locations was segregated by level (surface, middle, and bottom). The grain samples were stored in sealed plastic bags and brought to the Grain Postharvest Laboratory (GPL) of Balcarce Experimental Station of the National Institute of Agricultural Technologies (INTA) for testing. After probing the plastic bags, the openings were sealed with a special tape in order to restore the air-tightness. The described sampling procedure was repeated approximately every two weeks during the entire storage period.

Grain samples were analyzed for m.c. (GAC 2100, Dickey-John). Protein grain analysis was done by the Kjeldahl method (realized by Maltería Quilmes, Tres Arroyos), as it is regulated by the Argentine barley quality standard (SAGPyA, 2009). Germination testing was carried out following the recommendation of ISTA (2008): pre chilling for 48 h and then placing samples to germinate for 7 d at 20°C in light conditions; there were four 4 replicates of 50 seeds for each level of each sampling site. During extraction of the grain at the end of the storage period the presence of spoiled grain was also documented via visual inspection.

3. Results

Figure 1 shows the average m.c. of each sampling point during storage. The initial m.c. was from 11.5 to 12%. There was a decreasing trend over time of about 0.5% m.c., in all locations. The maximum m.c. standard deviation between layers was 0.26% (Figure 2) and occurred in the third sampling date, and remained below this value during the remainder of the experiment.

The initial GP in both bags was near 100%. In bag A (11% m.c.) the GP were above 98% at the end of the experiment (Figure 3). On the other hand, some locations of the bag B (Fig. 4) it showed a slight decreasing trend. In the S. I site (initial 11.5%m.c.) the GP reached a level of 97.4% at the end of storage. The final GP values in S. II of bag B (11.3% m.c.) was 98.6%, while S.III (11.1%m.c.) finished with a GP of 97.8%.

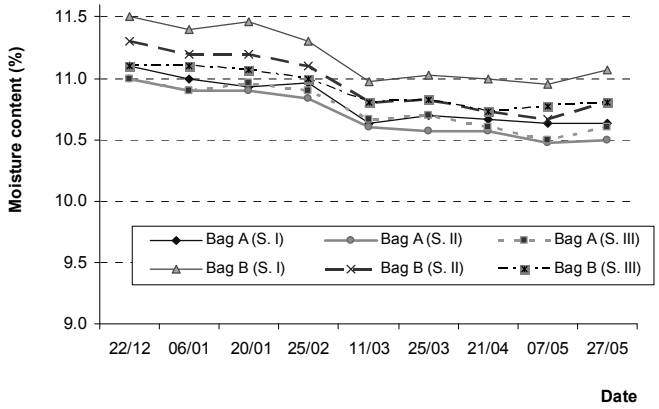

Figure 1 Evolution in time of average moisture content (%) for three sampling points of the A and B bags.

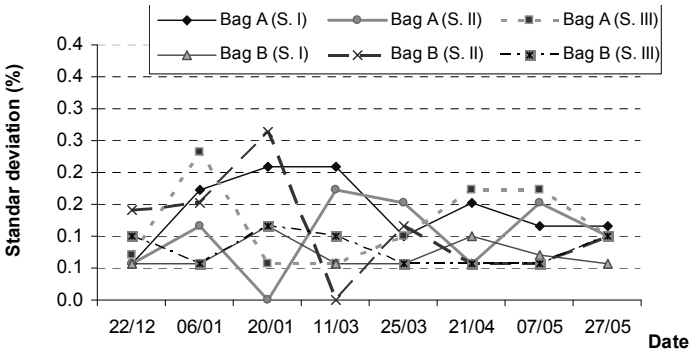

Figure 2 Evolution in time of moisture content standard deviation (%) for three sampling sites (S. I, S. II and S. III) of A and B bags.

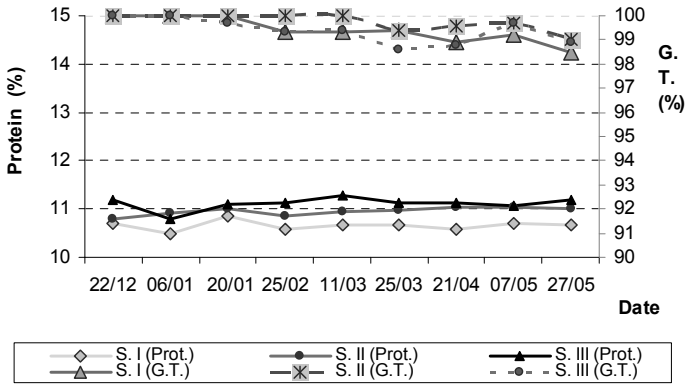

Figure 3 Evolution in time of average protein content (%) and germination percentage (GP (%)) for three sampling sites (S. I, S. II and S. III) of the bag A.

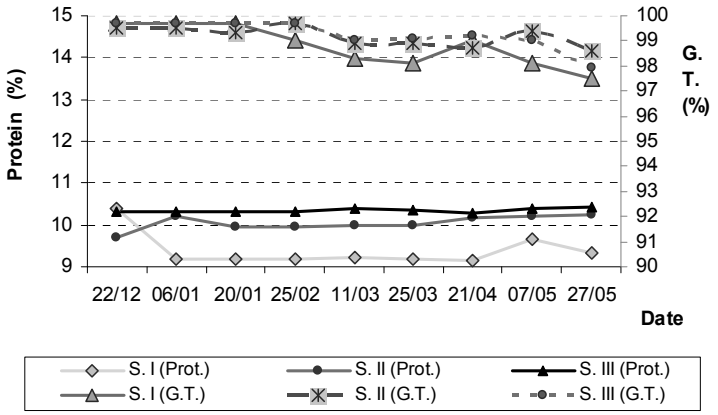

Figure 4 Evolution in time of average protein content (%) and germination percentage (GP (%)) for three sampling sites (S. I, S. II and S. III) of the bag B.

In general, it was observed that values of GP in the upper layer remained above the average value, and more values below average were found in lower layers (Table 1).

Table 1 Germination percentage (GP) values (%) for each sampling level (top, middle and bottom) and average value for each sampling site (S. I, S. II and S. III) for bag A and B at the end of the storage time.

Silobag	Site	Level	GP (%)	Average GP (%)
Bag A	S. I	Top	99,5	98,5
		Middle	97,5	
		Bottom	98,5	
	S. II	Top	99,5	99,0
		Middle	98,5	
		Bottom	99,0	
	S. III	Top	98,5	98,8
		Middle	98,5	
		Bottom	99,5	
Bag B	S. I	Top	97,5	97,4
		Middle	97,7	
		Bottom	97,0	
	S. II	Top	99,5	98,6
		Middle	100,0	
		Bottom	96,2	
	S. III	Top	99,0	97,8
		Middle	96,0	
		Bottom	98,5	

The initial protein content in bag A varied between 11.2 and 10.8%, which fall within the range established by commercialization standard of malting barley (minimum 10%, maximum 12%), and remained stable throughout the period (Fig. 3). In S. I of bag B (Fig. 4) there was a decrease in protein content (from 10.3 to 9.2%) after January 6th. The protein level in S. II of bag B was stable during the 5 months of storage with values close to 10%. The S. III was always with protein values above the limit of commercialization.

Figures 5 and 6 show changes in the average temperatures for each grain layer for bags A and B, respectively. The temperature of the grain in bag A at the beginning of the storage time reached 30ºC in all levels of the bag. Over the course of storage time, temperature decreased steadily until the end of February, and remained relatively stable until the end of summer with grain temperature near 20ºC.

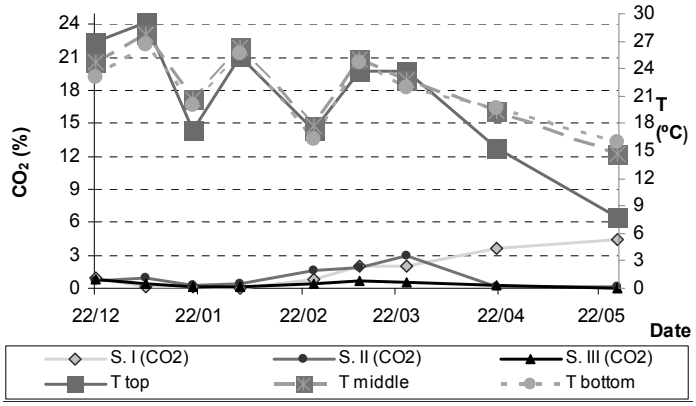

Figure 5 Evolution in time of CO_2 concentration (%) for three sampling sites (S. I, S. II and S. III) and average temperature (ºC) for three different levels (top, middle and bottom) of the bag A.

During the fall, temperature of the top layer declined to 9.5ºC in late autumn. The middle and lower grain layers presented similar values of grain temperature throughout the summer and early autumn, showing a tendency to decrease towards the late autumn. Temperature of the grain in bag B showed a similar trend as in bag A (Fig. 6).

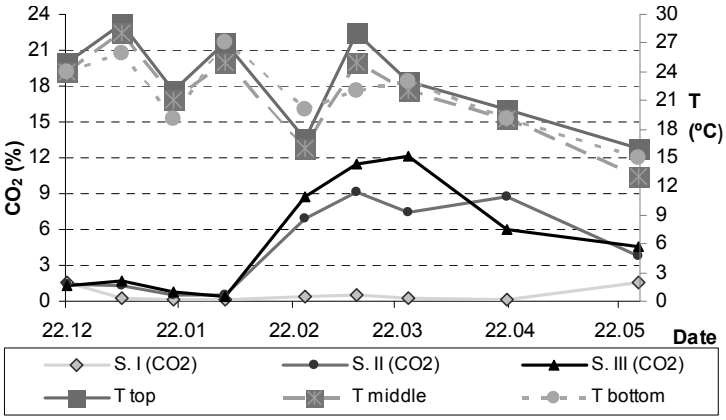

Figure 6 Evolution in time of CO_2 concentration (%) for three sampling sites (S. I, S. II and S. III) and average temperature (ºC) for three different levels (top, middle and bottom) of the bag B.

Figure 5 also shows the evolution of CO_2 concentration in Bag A during the 5 months of storage. The area close to the end of this bag (S. III) showed a minor modification of atmosphere (values below 0.5%) throughout the test. In the middle sector of the bag (S. II), the CO_2 concentration started very low, with a peak of 3% in March that slowly decreased towards the end of the storage period. Area S.I also showed low biological activity during the first 3 months of the experiment, having similar values to the rest of the bag. During the last two months of storage a steady increase of CO_2 was observed, peaking at 4.4% on May 22th. In bag B, CO_2 values were below 2% during the first one and half months of storage (Fig. 6). After February 2, the values rose indicating increasing biological activity at the center and end of the bag. Level peaked at 12 and 9% by the end of March for locations S. II and S. III, respectively. The S. I sector of the bag maintained with low and stable CO_2 values throughout the study. During the extraction of grain, isolated areas with damaged grain were observed (between 10- and 15-cm thick) on the low layer of the sectors S. II and S. III of the bag B.

4. Discussion

Even though Figure 1 shows a decreasing trend in m.c. over time, the magnitude of difference between maximum and minimum values of different sampling dates from the same sampling location was only 0.5%. Similar variations have been reported in studies with barley (Ochandio *et al.*, 2009) and other grains (Azcona et al., 2009, Bartosik et al., 2008a). These authors suggested that the low variation in m.c. values could be contributed to the precision of the moisture meter used or to experimental error during sampling. In both bags, m.c. values between layers of the same sampling site were similar (Fig. 1), and there was no increase in the variability of the m.c. over time (Fig. 2), indicating no substantial stratification in moisture level.

Since the silobag is made of a hermetic plastic cover, no moisture variation should be expected during storage, unless rainwater enters the bag through openings. However, Gaston et al. (2009) mentioned that a temperature differential between the top layer and the rest of the bag caused migration of moisture from the core of the grain mass to the top layer, and to a lesser extent the bottom layer. Moisture migration can lead to m.c. rise, increasing the risk of grain spoilage (and malting quality deterioration) in localized areas of the silobag.Until now, the magnitude of the moisture stratification process during storage in the silobag was not clean. On the one hand, Darby and Caddick (2007) reported moisture stratification during storage of dry barley (≤ 11% m.c.) under Australian conditions in non-punctured silobags. This stratification increased m.c. in the peripheral layer up to 13% over winter, but remained dry over summer with temperatures above 30°C, indicating that the grain could be stored in perfect condition for up to 6 months. On the other hand, Ochandio et al. (2009) did not find m.c. stratification in 12% m.c. barley silobags, even after 1 year of storage.

Gaston et al. (2009) considered that grain m.c., grain temperature, grain temperature fluctuation magnitude and storage time affect the magnitude of m.c. stratification.

As shown in Figures 5 and 6, while grain temperature at the beginning of storage was approximately 30ºC (possible higher in the upper layer), this value decreases rapidly. Accordingly, Bartosik, et al. (2008a) collected hourly temperature data in different layers of a wheat silobag. Their results indicated that temperature of the grain mass in the bottom and middle layers followed the average monthly temperature (decreasing steadily during the end of summer, autumn and winter), while the upper layer underwent constant changes, following the daily variation of temperature.

The low initial m.c. of grain, the decreasing temperature during storage and the absence of localized m.c. deposition prevented formation of layers of spoiled grain in the periphery of the grain mass. As a result, protein values remained constant in both bags in all sampling points, with the exception of S. I in bag B. The GP was above the industry requirements when m.c. was near 11%. However, where m.c was higher (11.3 to 11.5%) a tendency for GP to slightly fall below 98% was observed, at the end of the storage time. Contrastingly, Ochandio, et al. (2009) did not find changes in the protein levels or GP during storage of 12% m.c. barley.

Low biological activity, as indicated by a minimum atmospheric modification, was observed in bag A and the S. I sector of bag B. Rodriguez *et al.* (2008) pointed out that changes in the atmospheric composition in a silobag containing wheat was mostly explained by grain m.c. The equilibrium relative humidity (r.h.) for barley at about 12% m.c. and 20°C is about 50%, much lower than the r.h. required for storage fungi development (70%). Bartosik *et al.* (2008b) stated that silobags with sectorized spoiled grain had a substantially higher atmospheric modificaton in compared to silobags or portions of the silobag, with grain in good condition. These authors proposed CO_2 monitoring as an early indicator of a spoiling process of grain in silobags. Based on these observations, it could be hypothesized that the increase in CO_2 concentration observed in sectors S. II and S. III of bag B could be related to an area of spoiled grain producted by rain entering the bag through non visible perforations at the bottom side. Supporting this hypothesis, it localized spoiled grain were obsevered on the floor of the bag close to sampling locations S. II and S. III. This spoiled grain resulted from gastightness problems with the bag rather than unsafe storage conditions.

Generally, it can be concluded that storing malting barley under typical conditions for Argentina would result in grain temperatures above 35°C in the peripheral layer only 2 to 3 h during hot summer days, decreasing at night to 18-20°C. Temperature of the grain mass will follow the average ambient

temperature through the season, decreasing during fall and winter. The quality deterioration of malting barley increases if the storage m.c. is above 12%. Storing malting barley at high m.c. values can also lead to moisture stratification, with localized moisture deposition in the grain upper layers of grain. This could end with localized spots of spoiling grain, or reduction in the GP. However, storing dry malting barley in silobags (less than 12% m.c.) can be considered safe for a period of up to 5 months.

Acknowledgments

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Non-chemical on-farm hermetic maize storage in east Africa

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Abstract

Hermetic post-harvest maize storage can effectively control maize weevil, *Sitophilus zeamais*, which can be responsible for up to 50% damage to stored maize grain. Its use eliminates the need for toxic and expensive chemicals. Laboratory experiments were conducted on hermetic storage systems to evaluate the effects of temperature (10^{o} vs. 27°C) and maize moistures (6.3 to 16%) on maize weevil biology and mortality rate, and to quantify weevil oxygen consumption. Ten-day weevil mortality was higher in hermetic vs. non-hermetic storage, in 6.3% moisture maize vs. 16%, and at a 27°C storage temperature vs. 10°C. Oxygen depletion results allow estimation of daily weevil oxygen consumption as a function of storage temperature and maize moisture for East Africa conditions.

Keywords: Maize storage, Hermetic storage, *Sitophilus zeamais,* Maize weevil control, Maize deterioration

1. Introduction

Maize (*Zea mays* L. ssp. Mays; corn) uses and accounts consumption by humans in East Africa far exceeds other for more than 50% of total caloric intake in local diets (Sinha, 2007). Hand harvesting is carried out after physiological maturity, followed by drying and storage. Drying maize to below 14% moisture is recommended for preservation in East Africa. Drying to 8% moisture is possible with sun drying or drying by means of wood fire or solar dryers.

Tropical heat, moisture and open-air storage promote rapid insect multiplication and mold formation in stored maize (FAO, 1994). Rapid insect development occurs when temperature is within 5 to10°C of optimal temperature, which for most storage insects, is in the range of 25 to 35°C (FAO, 1994). The maize weevil (*Sitophilus zeamais* Motschulsky) is the principal deterioration insect of stored maize, sorghum, and other grains in the tropics (Longstaff, 1981; 1986; Jacobs and Calvin, 2001). About 96 million of the 140 million ha of maize grown globally is in the tropics, where the vast majority of the maize is stored without chemical protectants on-farm (Lindblad and Druben, 1980). Consequences include direct food losses and reduced future maize production for farmers, since 70% of all maize seed planted in Eastern and Southern Africa is sourced directly from previous year's harvest (Dhliwayo and Pixley, 2003).

Overall, 20-30% of Ethiopian stored maize is lost to *S. zeamais* infestation, while 100% damage has been found in maize stored for 6 to 8 months in the Bako region of the country (Demissie et al., 2008a). Mulungu et.al., (2007) also found about 18% of shelled maize with weevil damage in stored maize in Tanzania, while Demissie et al. (2008b) found levels of 11-59% weevil infestation in husk-covered maize stored at Bako, Ethiopia, after one month of storage.

Hermetic storage isolates the storage ecosysytem from the external environment while respiration within the storage ecosystem causes O_2 reduction and CO_2 accumulation, leading to suffocation and dehydration of weevils (Navarro et al.,1994). A study by Moreno-Martinez et. al. (2000) utilized 150 g samples of maize grain of hybrid AN 447 infested with 20 unsexed *S. zeamais* and stored within 250-mL glass containers, fitted with an oxygen analyzer. The jars were stored at 26^{O}C, 16% moisture, 70% r.h., and 18h: 6 h L:D photoperiod. Maize weevil mortality was recorded at 3-day intervals, by checking 12-jar replicates of hermetic as well as non-hermetic samples. They found that oxygen was depleted to 0% in 6 to 9 days in the hermetic treatments, while it decreased to 8.4% after 30 days in the non-hermetic treatment.

The experiment also included treatments with fungus spores added and treatments without weevils. The rate of oxygen depletion in treatments containing weevils was more rapid than those containing fungi and maize alone, while treatments with maize alone had much lower oxygen utilization rates compared to fungi and maize.

Plastic bagging employs layers of air-tight and water-tight PVC and polyethylene bags, within which grain is hermetically stored. Triple bagging, which involves tying three bags separately within each other is currently employed by Purdue researchers in the hermetic preservation of cowpeas, in Central and Western Africa. With a one-time cost of $3 per household, this storage system has the potential to increase household income on average by about $150 per year (Murdock et.al., 2003; Carroll and Fulton, 2008). Studies are under way to determine plastic bag life (Murdock, 2010).

Steel containers are excellent candidates for low-cost hermetic storage on farms in the East African sub-region. Lindblad and Druben (1980) and Adhikarinayake (2005) described the use of recycled steel oil drums, filled with maize, for hermetic storage and simultaneous mechanical isolation from rodents, while Murdock, et al. *(*2003) described bruchid-infested cowpea stored for 6 months in sealed drums with minimal losses. Such containers may be contaminated by petro-chemicals or something else, and need to be properly cleaned to prevent cross contamination of maize stored within them. Common methods for determining types of petro-chemicals present, and for measuring the level of contamination involve methanol extraction followed by gas chromatography (Turriff, et al., 1998). The use of locally available soaps for cleaning is also common practice, although the efficacy of this method of cleaning is not well documented.

Further development of effective hermetic storage systems for maize requires more extensive information on the oxygen requirement of weevils within maize stored over a range of moistures and temperatures. The objectives of this research were to determine the effects of oxygen level, maize temperature, maize moisture and their interaction on the survivability of maize weevils over time in hermetic containers.

2. Materials and methods

A laboratory scale hermetic storage system was used, where products of weevil, mold, and maize respiration serve as an effective pest control strategy in stored maize. The research employed instrumentation for the quantification of oxygen levels. Treatment conditions of temperature (10 and 27°C) and moistures (6.3, 8.0 and 16%) were selected as appropriate minimums and maximums of typical maize storage conditions in East Africa. The treatment assignment to jars and chambers was done using PROC GLM (SAS Institute Inc.,100 SAS Campus Drive, Cary, NC 27513).

Maize grain of the commercial hybrid Fontanelle *6T672* at about 16.5% moisture was harvested using a 4420 Deere combine. Following harvest it was cleaned to remove broken maize and foreign material and stored at 4°C until use. Experimental maize was dried to target moistures, using a laboratory drier for drying to 16% and air at 45°C for drying to 8% or 6.3%. Moistures were confirmed using the oven method (103°C oven for 72 h) (ASABE, 2008).

A stock culture of 100 adult *S. zeamais* (unsexed) obtained from the Iowa State University Entomology Departmental laboratory were placed in five unsterilized 3.74-L glass jars, with screen lids, containing 16.5% moisture Fontanelle 6T672 maize. The weevils were allowed to oviposit on the maize to develop a colony. This was achieved by placing jars in a rearing chamber at about 27°C and at interstitial relative humidity determined by maize moisture, for two months (Arannilewa, et al., 2006). Two chambers at 10 and 27°C were utilized in the experiments. They were model 13-988-126 GW Fischer Scientific Isotemp refrigeration chambers (Thermo Fisher Scientific Inc., Waltham, MA 02454), with temperature controls.

One-pint (473 mL) Kerr canning jars (Mason Jar 61000, Jarden Home Brands, 14611 W. Commerce Road, Daleville, IN) were utilized in both the weevil mortality and oxygen quantification experiments. In the weevil mortality experiment, each canning jar was loaded with 350 g of maize and 30 adult weevils, while 90 weevils were loaded into each canning jar along with about 185 g of maize at the appropriate moisture levels in the oxygen quantification experiment. Hermetic tests utilized canning jars, as is, while non-hermetic tests utilized jars fitted with aluminum screens which allowed air passage but not weevil escape.

2.1. Weevil mortality study

The experimental design consisted of four factorials (days, maize moisture, temperature, and replication), with weevil mortality being the dependent variable. Days had five levels (2, 4, 6, 8, and 10 days), maize moisture had two levels (6.3 and 16%), temperature had two levels (10 and 27°C), and four replications were used. These conditions approximate those employed by Moreno-Martinez et al., (2000), although test conditions were based on results of preliminary laboratory tests. Each replication had a total of 16 treatments (10 hermetic and 6 non-hermetic) assigned to each of the two chambers (Wohlgemuth, 1989; Evans, 1987). The hermetic jars had five levels of days and the non-hermetic had 3 levels of days (2, 6 and 10), while both had two levels of maize moisture (6.3 and 16%). Each of the 128 treatment jars contained 30 weevils and 350 g of maize.

The criteria for determining weevil mortality relied on a combination of observed rigor mortis features (Gullan and Cranston, 2000). Weevils that were curled up and/or had outstretched legs; lying on their side or back; immobile; unattached to maize kernels; found to flow with kernels when jar was tilted; and hard to the touch even when exposed to ambient air were assumed dead. To determine mortality, each jar from the 16 treatments (T_1-T_{16}) was examined for dead weevils on the day on which it was randomly assigned. The hermetic treatment counts were done on days 2, 4, 6, 8, and 10, while the non-hermetic treatment counts were done on days 2, 6, and 10. The number of dead weevils was recorded from the counts and utilized in the statistical analyses, and for testing the hypotheses of differences in weevil mortality for different temperatures and moistures, under hermetic and non-hermetic conditions.

2.3. Oxygen quantification study

To determine oxygen depletion under different maize moisture and temperature, ninety weevils were loaded into each of the Kerr hermetic canning jars along with about 185 g of maize, at 8 or 16% moisture. The jars, which were connected to the two model 65 oxygen sensors (AMI, 18269 Gothard Street, Huntington Beach, CA 92648), a PMD 1408FS DAC system and a computer, were randomly assigned to the two environmental chambers, for oxygen monitoring. Liquid-in-glass thermometers, mounted on rubber stoppers were used to monitor chamber temperatures (10°C and 27°C), and recorded oxygen levels from each sensor were corrected to the average of the two sensor output values.

3. Results and discussion

The first study consisted of testing the hermetic and non-hermetic 10 and 27°C temperatures, and maize at 6.3 and 16% moisture, under hermetic and non-hermetic conditions, with replication. At 27°C, weevil mortality reached 100% in six days for both 6.3 and 16% moisture maize (Fig. 1). At 10°C, weevil mortality increased over time, but only reached 28 and 5% for 6.3 and 16% moisture maize, respectively (Fig. 2). Decreases in mortality from day 2 to day 4, and from day 6 to day 8 came about because three different jars were opened and discarded after a mortality count on each sampling date. These results show that mortality increases more rapidly at higher maize storage temperature, and more rapidly at lower maize moisture levels.

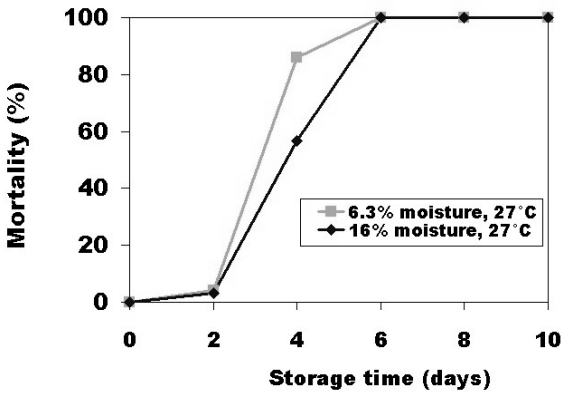

Figure 1 Mortality of *Sitophilus zeamais* hermetic storage at 27°C (averaged over three replications).

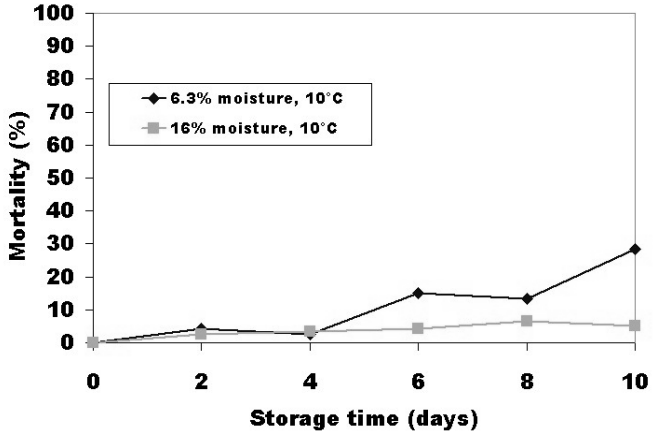

Figure 2 Mortality of *Sitophilus zeamais* hermetic storage at 10°C (averaged over three replications).

Based on Figures 3 and 4, weevils seem to have a natural mortality rate, which is dependent on natural mortality factors, irrespective of the treatment combination. The maize weevil is a vector of some predatory fungi, and has other natural enemies capable of reducing its population in open air storage (Sétamou, M. 1999; Imamura, et al., 2004; Hansen and Steenberg, 2006). Hence, a higher level of variability in mortality is associated with non-hermetic treatments.

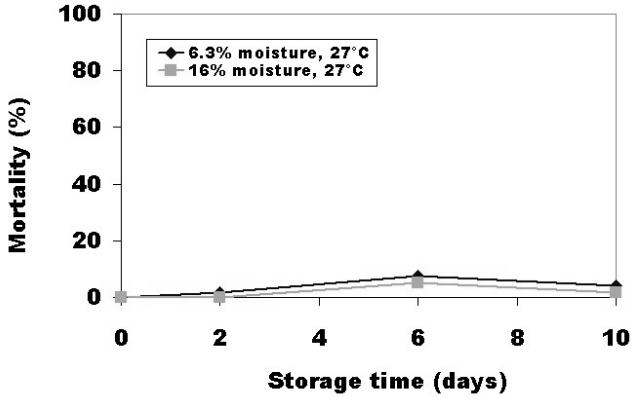

Figure 3 Mortality of *Sitophilus zeamais* non-hermetic storage at 27 °C (averaged over three replications).

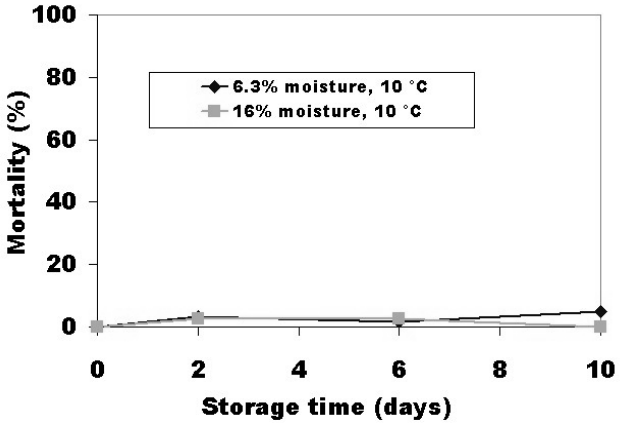

Figure 4 Mean mortality of *Sitophilus zeamais* non-hermetic storage at 10°C (averaged over three replications).

Table 1 shows mean percent mortality rates at day 10, along with standard deviations. Looking at the rows of Table 1, the three-factor (temperature by moisture by day) mean difference of differences (interaction) is more significant at 10°C than at 27°C, in both hermetic (23.3) and non-hermetic (5.0) treatments. And a comparison of the columnar differences indicates higher level of interaction at the 16% (95, 1.7) maize moisture than at 6.3% (71.7, 0.8), for both types of treatment. Interaction occurred because the mean percent mortality was not the same for the different levels of day, temperature and maize moisture.

Table 1 Mortality of *Sitophilus zeamais* after 10 d under different conditions and the differences between row and columns.

	Mortality ±SD (%)					
	Hermetic			**Non-hermetic**		
	Grain moisture			**Grain moisture**		
Temperature (°C)	**6.3%**	**16%**	**Difference**	**6.3%**	**16%**	**Difference**
10	28.3 ± 8.8	5.0 ± 10.0	23.3	5.0 ± 6.4	0.0 ± 0.0	5.0
27	100 ± 0.0	100 ± 0.0	0	4.2 ± 8.3	1.7 ± 1.9	2.5
Difference	71.7	95		0.8	1.7	

Wohlgemuth (1989) suggested that insects and fungi of stored products are inactive at 10°C and below, but cause substantial damage at temperatures up to 35°C. Our results show low levels of mortality after 10 days at 10°C, especially in 16% maize. This suggests that there continues to be considerable activity at that temperature.

Oxygen concentrations indicate that 100% weevil mortality is achievable at both 10 and 27°C and both 8 and 16% moisture content (Fig. 5, 6). Following the trends observed in the mortality study, oxygen depletion was faster and 100% mortality is achieved sooner for higher maize temperatures and lower maize moistures. Figure 6 shows results obtained at 27°C for 16% and 8% moisture.

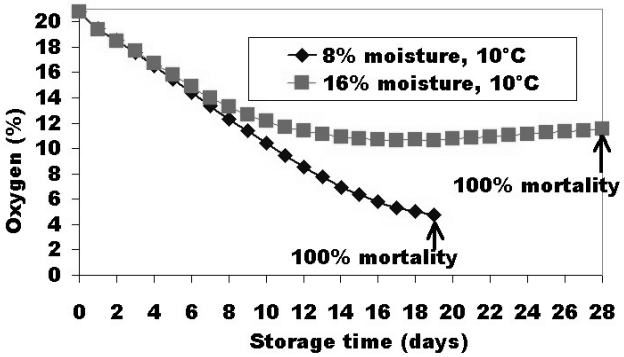

Figure 5 Average percentage oxygen for three replications at 8 and 16% maize moisture and 10°C.

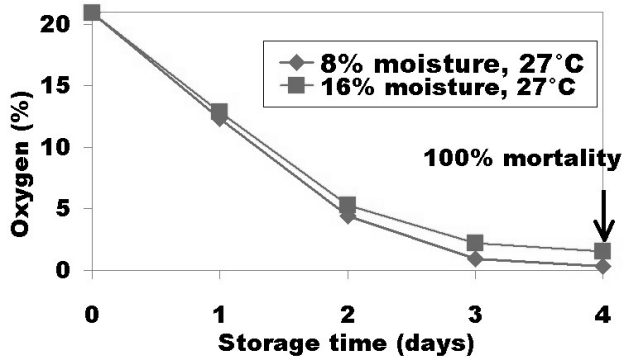

Figure 6 Average percentage oxygen for three replications at 8 and 16% maize moisture and 27°C.

Maize kernel density values were needed in order to calculate gas volumes within a mass of maize. Kernel densities of triplicate samples of test maize were measured using an Accupyc model 1330 pycnometer (Micromeritics, Gosford, New South Wales, Australia). Kernel densities were adjusted to 8 and 16% moisture using the procedure described by Dorsey-Redding et al. (1989). Adjusted values are shown in Table 2.

Table 2 Conditions to achieve 100% mortality of *Sitophilus zeamais* and maize densities.

Storage Time (days)	Moisture (%)	Temperature (^{O}C)	Kernel density, (g/cm^3)
28	16	10	1.26
19	8	10	1.24
4	16	27	1.26
4	8	27	1.24

Table 2 also shows average time to 100% weevil mortality at each of the test conditions. Data from studies on respiration jar volume, maize bulk and kernel densities and weevil counts were used to calculate weevil oxygen consumption for each of the four maize moisture-temperature combinations (Fig. 7). Data from the Moreno-Martinez *et al*. (2000) allows calculation of one point on Figure 7.

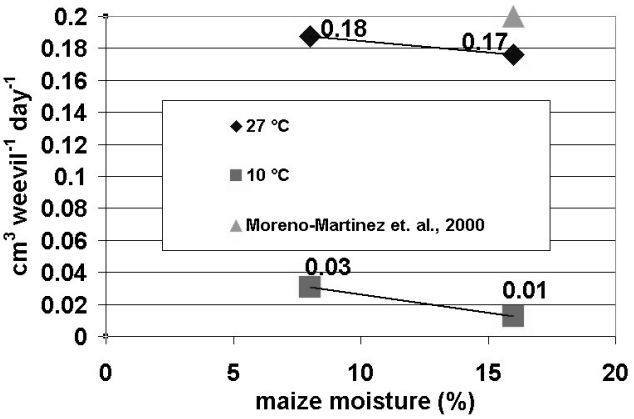

Figure 7 Average oxygen consumption of maize weevils in shelled maize.

At 16% maize moisture and 26°C, weevil oxygen consumption was 0.20 cm^3 $weevil^{-1}$ day^{-1}. This shows good agreement with the present study. Equations for the two lines on Figure 7 are:

27°C: Y=-0.00141x+0.199; 10°C: Y=-0.00234x+0.0496.

The area within the four points on Figure 7 (10°C to 27°C, 8 to 16% moisture content) includes most maize storage conditions on farms in East Africa. The graph may be used to predict the time to 100% mortality in any hermetic storage container.

3.1 Prediction example

A 225-L (55-gal) barrel contains 162 kg of maize at 10% moisture stored at 20°C. The maize contains 100 weevils per kg. Interpolating between points on Figure 7 predicts an oxygen utilization value of 0.114 cm^3 $weevil^{-1}$ day^{-1}.On average, weevils die when oxygen level reaches 4%. Using container and maize information, along with the calculated oxygen utilization value, the predicted time to 100% mortality is calculated to be nine days.

4. Conclusions

The hermetic storage and oxygen quantification studies show that hermetic storage is effective for weevil control in stored maize. Weevil oxygen consumption data allow prediction of the days to 100% mortality in a hermetically sealed storage container as a function of container volume, weevil numbers, maize moisture, and storage temperature.

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Continuous ozonation treatment systems as other alternative more efficient grain protection technologies

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Abstract

Previous static bed ozonation scale-up and demonstration trials have proven the use of ozone as an effective technology for grain protection without affecting its end-use quality. Due to the lack of current availability of high capacity ozone producing generators, grain treatment through static bed ozonation systems are limited to be used in metal silos of capacities smaller than 644-t. Also, the trials have shown that treatment time has to be of no less than 4 d during application in order to be effective for pest control. Therefore, more efficient ozonation treatment systems are needed for proper ozone usage for stored product protection. The primary objective of these research studies was to design and test a semi-continuous counter-flow ozonation and a continuous ozonation flow treatment systems in order to ozonate grain at faster rates based on the concentration-time product (CTP) of ozone required to achieve 100% insect mortality and effective mold reduction in grain. The procedure of the counter-flow semi-continuous ozonation system consisted of removing each grain layer inside a metal silo with a tapered unloading auger after each layer reached the desired ozone CTP. The treated grain is subsequently transported to a storage or shipping silo. The continuous ozonation flow system involves applying high ozone concentrations through a modified grain loading screw conveyor where ozone and grain are moving continuously in the same flow direction. The counter-flow semi-continuous ozonation system was successfully tested and proved to be a technically feasible tool for pest control and mold reduction. The continuous ozonation flow system was proved as an effective tool for treating grain during handling while achieving 100% insect mortality, and effective mold reduction.

Keywords: Ozonation, Continuous treatment, Grain, Pest control, Molds.

1. Introduction

Previous fixed bed ozonation scale-up and demonstration trials have been conducted in grain storage structures and have proven the use of ozone as an effective technology for grain protection without affecting its end-use quality. This fixed bed ozonation system aimed at achieving an initial sterilization phase by reaching a target concentration of 50 ppm at the opposite side of the ozone inlet and maintaining it for 72 h. Ozone is artificially produced by a generator and introduced to the grain storage structure at a constant rate. For this treatment setup, usually a recirculation or exhausting system is needed so ozone is not wasted once the leading front exits the grain mass and is not just exhausted into the environment. Among the advantages of ozonation compared to traditional phosphine fumigation for pest control are that it can be generated at the treatment site and no residue is left on the treated product (Strait, 1998). Due to the lack of current commercial availability of high capacity ozone producing generators, grain treatment with ozone through fixed bed systems are limited to be used in metal grain silos of capacities smaller than 644-t. Also, the trials have shown that treatment time has to be of no less than 4 d during application in order to be effective for pest control. Therefore, more efficient ozonation treatment systems are needed for proper ozone usage for stored product protection. The ozonation treatment alternatives were built on knowledge gained from previous fixed bed ozonation trials and focused on the development of a semi-continuous counterflow ozonation system and a continuous ozonation flow system in order to treat grain for fungal spores sterilization and insect eradication at a

continuous rate that will result in a faster treatment time compared to the current fixed bed treatment method.

The semi-continuous counterflow ozonation system was designed based on an in-bin continuous flow dryeration system used for on-farm grain drying in metal round silos (Marks et al., 1993). These drying systems have tapered unloading augers in the grain silo floor that are capable of unloading the silo's bottom grain layer which can result in a removal of a depth of 15.2 to 30.4 cm of grain in the silo after completion of one full revolution. The procedure for the semi-continuous counterflow ozonation treatment involves removing the bottom grain layer after it reached the desired ozone concentration to achieve insect mortality, mold reduction, and/or off-odor removal. The treated grain is subsequently transported to a semi-truck trailer, grain wagon or second storage silo. At the same time, untreated grain from another storage silo is added on top of the grain surface in the same amount as the treated grain that was removed by the tapered unloading auger in order to maintain a constant grain depth during the treatment process. The system control variables are; airflow, ozone mass flow and exposure time. Airflow is controlled by a variable speed fan that is sized up to deliver at least the minimum air velocity of 0.03 m/s to move ozone through a grain mass (Mendez et al., 2002) and a maximum that will not cause too great of an ozone dilution effect in the bottom grain layers. The ozone mass flow is controlled by the ozone producing generator. The only safe current method to control this variable is by shutting down one or more of the four chambers of the ozone generator. The exposure time of the grain to ozone is controlled by determining the CT product as a function of the ozone concentration at each grain depth needed for the desired treatment effect (odor removal, fungi sterilization and insect mortality). Once the desired CT product is achieved and maintained, the unloading tapered auger is programmed to remove the bottom treated grain layer.

The continuous ozonation flow system consists in applying high ozone concentrations through a modified screw conveyor in the same flow direction that the grain is moving continuously during a handling operation in order to achieve the desired CT product for insect eradication. The treated grain at the end of the modified grain loading screw conveyor falls through a flow directed pipe into a receiving chamber. At the same time, untreated grain is continuously moved into the modified screw conveyor at a constant rate. The control variables of the system are: grain residence time and ozone mass flow. The grain residence time is controlled by the rotational speed of the modified screw conveyor auger which is defined as the time it takes the first kernel introduced into the modified screw conveyor to come out at the other end after it has been treated with ozone. The ozone mass flow is controlled in the same way as in the semi-continuous counterflow ozonation system. The primary objective of these research studies was to design and test a semi-continuous counter-flow ozonation based on achieving a constant concentration-time product CTP throughout the treatment in the bottom layer of the grain mass inside the silo and to quantify any possible mold reduction and to design and test a continuous ozonation flow treatment system in order to ozonate grain at faster rates based on the CTP of ozone required to achieve 100% insect mortality and effective mold reduction in grain.

2. Materials and methods

In both semi-continuous counterflow and continuous ozonation flow treatment trials, ozone was produced using the corona discharge technology by a four chamber generator (OzoBlast) manufactured by O3Co. (Aberdeen, ID) that has a rated capacity of producing 1,000 g/h.

2.1. Semi-continuous counterflow ozonation treatment trial

The semi-continuous counterflow ozonation treatment was conducted in a corrugated metal grain silo (Fig. 1) of a capacity of 250 t with the following characteristics of 9.1 m diameter with a sidewall height of 6.7 m and a centrifugal fan powered by a 2.2 kW motor, a propane burner, a grain distributor, and a tapered sweep unloading auger system of a diameter of 9 m (Shivvers, Corydon, IA) which rotates around the silo diameter removing 12 t of grain for each full rotation. During the treatment trial, the grain drying system fan was replaced by a 0.35 m diameter and 3,500 rpm axial fan manufactured by Sukup Manufacturing Company (Sheffield, IA) powered by a 0.75 kW motor that was controlled by a variable frequency drive (VFD) in order to control the airflow that pushed the ozone into the grain mass. The airflow values produced by the axial fan were quantified by previously measuring them using a fan performance test (ANSI/AMCA 210, 1999) for different fan rotational velocities (rpm) and at different fixed pressures.

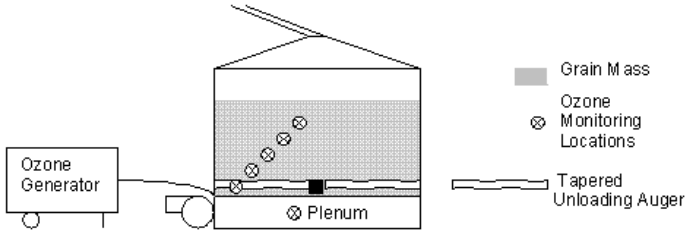

Figure 1 Semi-continuous counterflow ozonation treatment system in dryeration grain silo with ozone monitoring setup.

Ozone concentration was quantified with a monitoring system that measured the concentration at different depths of the grain mass (Fig. 1) using multiple monitoring lines connected to an ozone analyzer model IN-2000 made by IN USA Inc. (Boston, MA). Ozone produced by the generator was discharged from each of its four chambers out through 2.54 cm Teflon supply lines into the grain silo. The four ozone supply lines were positioned to empty into the plenum of the grain silo through the fan transition in order to achieve uniform distribution of ozone below the silo's perforated drying floor.

The trial was conducted by treating 190 t of maize in cycles of a constant grain mass of 63 t. In each treatment run the bottom grain layer of 12 t was exposed to the desired CTP. After treatment, each bottom grain layer was removed and an untreated grain layer of 12 t was added to the grain mass.

Mold colonies from each bottom grain layer inside the grain silo before and after treatment were quantified by determining the number of colony forming units (CFU) on the grain surface. Samples of 25 g of corn was added to 50 mL of 0.05% Triton X-100 solution and mixed for 2 min. The wash was serially diluted and plated onto malt salt medium. Plates were incubated at 28ºC for 3 to 5 d.

2.2. Continuous ozonation flow treatment trial

The continuous ozonation flow system was designed as a modified grain loading screw conveyor (Fig. 2) by LynnTech (College Station, TX). It was made from stainless steel (SAE grade 316) with a length of 6.3 m and a diameter of 0.102 m. The internal shaft had a length of 6.3 m, a diameter of 0.038 m and a pitch of 0.102 m. Attached to the base of the conveyor was a hopper of a 22.7 kg capacity used to feed grain into the system. At the exit of the conveyor was a drop shoot made of 0.102 m PVC pipe ending in a collection bucket covered with a carbon fiber filter to destroy any ozone exiting the system. Along the length of the screw conveyor was a manifold that distributed the ozone through six injection points separated every 1.5 m. A 0.75 kW motor with an inverter connected through a variable frequency drive manufactured by Maraton Electric (Wausau, WI) was used to control the rotational speed of the screw conveyor. Throughout the trials, the screw conveyor was set at a fixed inclined angle of 35 degrees.

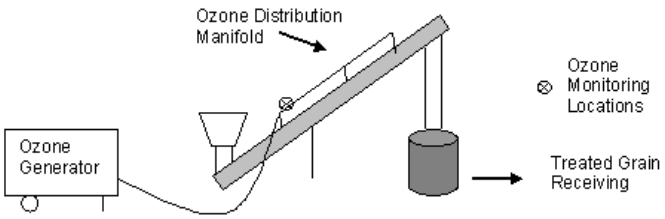

Figure 2 Continuous ozonation flow treatment system with ozone distribution manifold and monitoring setup.

Ozone concentration in the distribution manifold was determined with an ozone analyzer model H1 manufactured by IN USA Inc. (Boston, MA). Ozone produced by the generator was discharged from each of its four chambers through 2.54 cm Teflon supply lines into a 5.08 cm Teflon main supply line that was connected to the ozone distribution manifold along the modified screw conveyor.

The trial was performed by treating 15.9 kg samples of maize at different CTP in the modified screw conveyor with dry ozone produced directly from the ozone generator and humidified ozone. The

humidified ozone was obtained by producing dry ozone from the ozone generator and introducing it into a water tank to absorb moisture after it is passed through 90 L of water. The humidity level of this mixture was not determined.

Insect mortality was used as the main parameter and it was determined using five laboratory-made insect bioassays for each of the two insect species. These insects were; adults of the red flour beetle [not used at a later stage] *Tribolium castaneum* (Herbst) and the maize weevil [not used at a later stage] *Sitophilus zeamais* Motschulsky. Each insect bioassay contained 10 insects and were placed and mixed on each 15.9 kg grain samples before each treatment and control trial runs. Mold reduction was measured using the same procedure mentioned for the semi-continuous counterflow ozonation trial.

3. Results and discussion

3.1. Semi-continuous counterflow ozonation treatment trial

During the semi-continuous counterflow ozonation trials in the bottom treated grain layer, an approximate constant ozone concentration value of 90 ppm was maintained throughout the treatment process (Fig. 3) and the treated grain layers during the full cycle had an average CTP of 425 ppm.h. Three different phases can be identified during the treatment process. The first phase involves buildup of ozone concentration during which ozone reacts with the available reactive sites in the bottom grain layer. The second phase involves maintaining a steady state of one hour between the grain and ozone at its maximum concentration. The third phase ozone concentration decreases due to unloading of the bottom grain layer. The airflow rate affected the ozone concentration during the three phases. During grain layer removal, airflow was increased to avoid ozone leakage through the unloading system. Airflow remained mainly constant through the steady state of the treatment. Ozone after it reached its maximum concentration produced a sterilizing effect in the bottom grain layer causing it to move to the subsequent grain layers. Therefore, this treatment effect was accounted to the CTP quantification of each layer during a full treatment cycle starting when they enter the grain silo all the way to their exit. Mold reduction after treatment of each grain layer varied from 15 to 77%. No pattern was found between CTP increase and mold reduction increase.

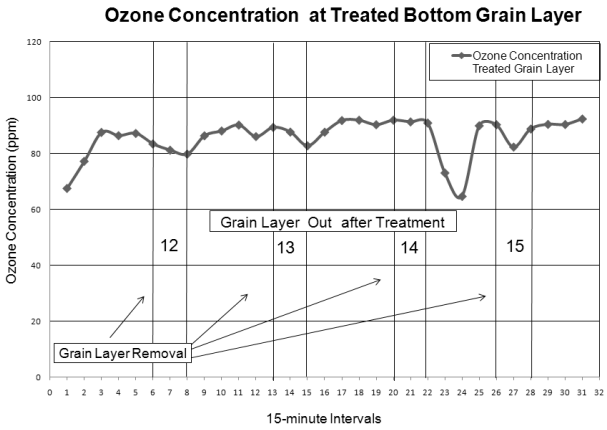

Figure 3 Ozone concentration at four different subsequent treated bottom grain layer in silo during semi-continuous counterflow ozonation treatment during several cycles of constant grain mass.

3.2. Continuous ozonation flow treatment trial

During the treatment trial, the average residence time it took the grain sample to move from the bottom of the modified loading screw conveyor to its end (run) was 2 min for both applications of dry and humidified ozone. Based on theoretical calculations and ozone quantification in the distribution manifold, for each run of the grain samples through the system, the ozone concentration was 47,820 ppm.min. Therefore, the concentration-time product during each run was 95,640 ppm.min (47,820 ppm x 2 min).

Based on previous research work, the CTP for 100% insect mortality is 3,600 ppm.h that equals 216,000 ppm.min. At the continuous ozonation flow system 216,000 ppm.min is achieved at 4 min and 34 s between the second and third run. Therefore, it was determined that the 3 passes through the system was the minimum number of runs to try to achieve 100% insect mortality for both applications.

The average residence time for the 3-runs for both humidified and dry ozone showed a higher value of 6 min and 31 ± 0.10 sec during dry ozone application compared to humidified ozone that had an average of 5 minutes and 30 ± 0.20 sec. The control runs for humidified and dry ozone applications showed an average residence time of 5 min and 21 ± 0.22 sec and 5 min and 31 ± 0.13 sec, respectively.

The insect mortality results for maize weevil and red flour beetle (Fig. 4) were 100% for the 3 runs tests using both humidified and dry ozone. The control 3-runs tests showed an average insect mortality of 18% for RFB and 30% for MW. The insect mortality in the control runs was caused by mechanical movement of the auger and the grain samples. The mold reduction results for the 3-runs treatments for both applications showed a considerable decreased from 11,646 to 6 CFU/g count for humidified ozone and 10,622 to 68 CFU/g count for dry ozone. Based on these results, it was shown that there is no difference between dry and humidified ozone application.

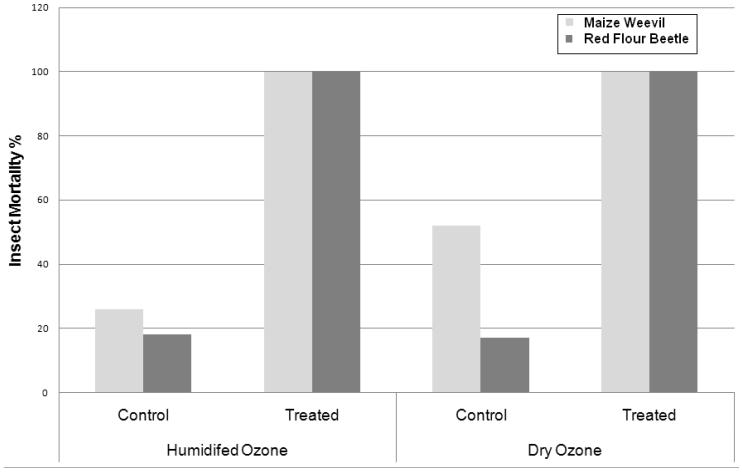

Figure 4 Insect mortality (%) in bioassays of adult maize weevil and red flour beetle placed in grain samples for 3-runs treatments of continuous ozonation flow trial using humidified and dry ozone.

The insect mortality in the insect bioassays during 6, 4, and 2 min residence time for maize weevil resulted in 100, 82 and 64%, respectively. In the control runs for 6, 4, and 2 min residence time were 32, 24 and 7%, respectively. For red flour beetle, the insect mortality in the insect bioassays for 6, 4, and 2 min residence time resulted in 100, 57 and 79%, respectively. The insect mortality during the 4 min residence time treatment resulted in a lower value compared to the 2 min residence time treatment. No explanation can be found for this issue since in the 4 min residence time treatment the insect bioassays are exposed to almost double the ozone CTP than in the 2 min treatment. The control runs for 6, 4, and 2 min residence time, resulted in 18, 12, and 8%, respectively. Also, the insect mortality for 2 min residence time had a low value of 8%, therefore, it can be assumed that no other external factor caused the 1-run treatment to have a higher insect mortality than the 2-run treatment.

The mold reduction results (Fig. 5) showed a difference in CFU/g of maize mold count of almost 50% reduction between no treatment and ozone treatment. During treatment, the lowest mold count was obtained in the 3-runs treatment (6 min residence time) with a value lower than 5,000 CFU/g of maize due to the higher exposure time the grain was treated in the continuous ozonation flow system. Based on the standard deviation, there is no difference in mold count for the 1-run and 2-runs treatments or 2 and 4 min residence time. Therefore, it can be assumed that in order to have a more effective mold reduction, grain has to be exposed to at least a CTP of 300,000 ppm.min.

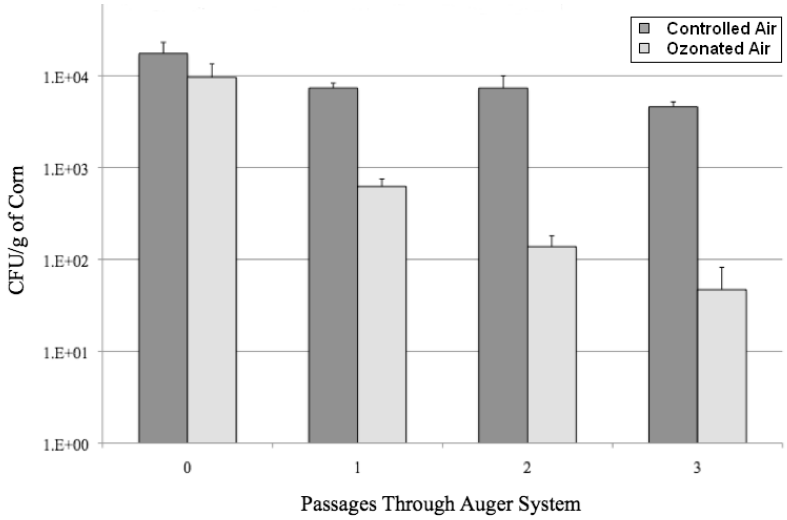

Figure 5 Colony forming units per gram of maize (CFU/g) for mold count on grain for 3-runs, 2-runs and 1-run treatments and control run of continuous ozonation flow trial.

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Structural fumigation efficacy against *Tribolium castaneum* in flour mills

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Abstract

Structural fumigations of food processing plants to manage stored-product insects have been a major component of pest management programs, but limited information on field efficacy is available. Efficacy, based on pheromone trapping data, consists of initial reduction in captures after treatment and recovery in trap captures over time after treatment (i.e., rebound). Patterns of *Tribolium castaneum* reduction and rebound were evaluated after 21 fumigations in two flour mills. Influence of time of year fumigation occurred, environmental conditions, and impact of other pest management tactics on efficacy was determined as well. Information generated can be used to guide fumigation decisions, including the development of risk thresholds for levels of pheromone trap captures.

Keywords: *Tribolium castaneum,* Fumigation, Flour mills, Efficacy, Methyl bromide

1. Introduction

It is challenging to evaluate the effectiveness of pest management tactics in food facilities such as mills, processing plants, and warehouses. Pest infestations are difficult to identify and sample because they are spatially and temporally patchy and typically in cryptic locations (Campbell, 2006). Pheromone trapping programs are widely used to determine temporal and spatial patterns of pest populations in food facilities, but since these traps primarily capture dispersing individuals trap captures are not necessarily related to actual population density and distribution (Arbogast and Mankin, 1999). To evaluate field efficacy, bioassay insects can be placed in facilities in areas where insects are thought to be located, but bioassay efficacy does not necessarily reflect the impact on the hidden pest population (Fields, 2007). Evaluation of efficacy is also complicated by the difficulty in replicating treatments given the many differences among facilities and within a facility through time, and in isolating the effects of single pest management tactics given the many other ongoing operations in commercial food facilities that can also impact pest populations. Small-scale laboratory studies that can be replicated and controlled more easily often do not adequately simulate the spatial and temporal patterns of exposure to treatments that occur under more real world conditions (Toews et al., 2009).

As a consequence of these issues, pest management in the food industry often relies on calendar-based application of pesticides or other control tactics rather than using monitoring information to guide management decisions. The lack of information on efficacy has also hampered the adoption of new technologies and more effective integrated pest management (IPM) programs. A major management tool for stored-product insects in food processing plants has been periodic structural fumigation with methyl bromide, although there has been little published research on its effectiveness in the field (Fields and White, 2002). Use of methyl bromide as a structural and commodity fumigant is being phased out worldwide under the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer (UNEP, 2000). The phase-out has triggered new research into the efficacy of methyl bromide and alternative treatments for food facilities (e.g., Campbell et al., 2002; Roesli et al., 2003; Campbell and Arbogast, 2004; Toews et al., 2006; Fields, 2007; Small, 2007). However, it is often difficult to draw firm conclusions from this research given the challenges of evaluating efficacy as described above and the limited number of replicate treatments involved in each study. To move forward, it will be necessary to develop larger data sets for commercial food facilities from which general patterns in efficacy can be determined and the impact of different factors on treatment efficacy evaluated.

Assessment of treatment efficacy in a food facility using pheromone traps has two components: immediate reduction in insect captures and rebound in captures over time following treatment. There are a number of factors that can influence efficacy, in addition to the characteristics of the treatment itself, including pest population abundance and distribution, environmental conditions during and after treatment, other management tactics being conducted concurrently. Here we summarize research which is presented more fully in Campbell et al. (2010a,b) that used data sets generated by long-term monitoring of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), in two commercial mills to evaluate the impact of fumigation on the number of beetles trapped and how pest abundance, environmental conditions, and changes in management programs impact both the immediate reduction and rebound of beetle captures. Using these data, we also developed beetle capture thresholds that might be used to assess the risk of rapid increases in abundance, as measured using pheromone traps.

2. Materials and methods

Mill #1 was a five-floor flour mill in which eleven structural fumigations were performed (nine with methyl bromide and two with sulfuryl fluoride (ProFume, Dow AgroSciences, Indianapolis, IN, USA)) between July 2002 and December 2008. Methyl bromide concentration ranged from 20 g/m^3 to 26 g/m^3 for 24 h and the two sulfuryl fluoride concentrations were 32 g/m^3, 19 h exposure, and 111 g/m^3, 18 h exposure. An improved management program was instituted after November 2004, which included the integration of regular aerosol applications of either 1% or 3% synergized pyrethrins (Entech Fog-10 or Entech Fog-30, Entech Systems, Kenner, LA, USA) (1.0 mL/m^3) and methoprene (Diacon II, Wellmark International, Schaumburg, IL, USA) (0.01 mL/m^3) at 2-4 week intervals, with enhanced sanitation and targeted insecticide treatment or cleanup of hot spots (located by trapping). This change provided a unique opportunity to evaluate changes in pest populations achieved by aerosol applications and sanitation over a period of several years as well as its impact on fumigation efficacy. Mill #2 was a five-floor wheat processing facility which underwent twelve structural fumigations between March 2003 and December 2008. This mill was fumigated twice a year with methyl bromide at a typical concentration of 24 g/m^3 and ~20 h exposure time. Both mills were in the same geographic area and had a pest management and sanitation program in place throughout the study.

Tribolium castaneum was monitored using Dome traps baited with pheromone lures for *Tribolium* spp. and a kairomone attractant (Trécé Inc. Adair, OK, USA). There were 55 trapping locations in Mill #1 and 32 in Mill #2. Traps were checked approximately every two weeks and the mean number of beetles captured per trap per standardized two week period (beetles/trap/period) and the proportion of the traps that captured one or more beetles per standardized two week period (i.e., probability of capture) were calculated.

Air temperature on each floor of Mill #1 and Mill #2 was recorded hourly using data loggers (HOBO® H8 family, Onset Computer Corp., Pocasset, MA, USA) placed 1.5 m above the floor and used to calculate mean daily temperatures inside each mill. Outside daily air temperatures were obtained from local weather stations. Mean temperatures during fumigation were calculated using hourly temperature data collected during the period when the fumigation was performed.

3. Results and discussion

3.1. Seasonal patterns

Mean number captured and probability of capture of *T. castaneum* varied considerably among sampling periods at both mills, but generally tended to increase over time, unless a fumigation occurred between monitoring periods. There was an average increase of 52.7±8.2% (n=286) in mean *T. castaneum* capture and 24.8±4.7% (n=285) in probability of capture between monitoring periods when no fumigation was performed, and in Mill #1 neither mean trap capture (General Linear Models (GLM) Procedure: F=2.24; d.f.=1,153; P=0.14) nor probability of capture (GLM: F=1.73; d.f.=1,153; P=0.19) was changed significantly by institution of the enhanced IPM program. This pattern of relatively consistent increase over time differs from other species in these mills, such as *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), the Indian meal moth, and *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), the warehouse beetle, that followed seasonal patterns of increase and decrease in abundance (Campbell et al., 2002; Toews et al., 2006).

Favorable interior temperatures for *T. castaneum* development and fumigation effectiveness were maintained most of the year, but there was seasonal variation in temperature. Temperature inside the mill tended to follow outside temperatures during the warm season (April to September), although it was always warmer than outside, and to stabilize at a temperature warmer than outside during the cool season (October to March). For example, at mill #1 the mean temperature in the warm season was 29.6±0.1°C inside and 21.0±0.2°C outside (8.3±0.1°C warmer) and in the cool season it was 24.0±0.1°C inside and 5.3±0.2°C outside (18.5±0.2°C warmer). The mean air relative humidity inside Mill #1 was relatively low year round at 32±16%. The change in mean interior temperature between seasons could impact population growth rates (Sokoloff, 1974), and the differences between interior and exterior temperatures could impact immigration.

3.2. Initial reduction following fumigation

Mean number of *T. castaneum* captured per trap following fumigation was reduced by 84.6±4.6% (n=23 fumigations): an average of 11.4±3.5 beetles/trap/period captured in the period immediately before compared to 0.8±0.2 beetles/trap/period in the period immediately after fumigation. Fumigation reduced the probability of capture by 70.9±5.1%: an average probability of capture of 0.58±0.07 and 0.20±0.05 for the monitoring periods immediately before and after fumigation, respectively.

Capture of adult *Tribolium* in traps immediately after fumigation has been reported previously (Campbell and Arbogast, 2004, Toews et al., 2006, Small, 2007) and could result from survival within structure and/or movement into structure after treatment. If survival inside the mill were the primary mechanism behind the presence of adult beetles in traps immediately after fumigation, then we hypothesize that the number of beetles captured after fumigation should be positively correlated with the number present before fumigation. And, the number of beetles per trap and probability of capture after fumigation were positively correlated with the mean number captured and the probability of capture immediately before fumigation (Pearson Correlations, $P>0.05$). If movement into structure after fumigation was the primary cause of adult beetle capture immediately after fumigation, then we hypothesize that trap captures after fumigation should be greater after warm season fumigations and less after cool season fumigations. Fumigations were sorted into two seasons: spring (April - June) (n=9) and fall (October – December) (n=11) periods: which differed in outside temperatures (11.8±1.8°C during fall and 18.9±1.2°C during spring fumigations (GLM: $F=8.90$; d.f.=1,16; $P=0.001$)), but not in inside temperature (24.4±0.6°C; GLM: $F=0.03$; d.f.=1,16; $P=0.86$). There was no difference between seasons in reduction immediately after fumigation in either mean number of beetles captured (GLM: $F=2.86$; d.f.=1,18; $P=0.11$) or probability of capture (GLM: $F=0.59$; d.f.=1,18; $P=0.45$). There was also no difference between the spring and fall fumigations in the mean number of beetles captured (GLM: $F=0.05$; d.f.=1,18; $P=0.82$) or the probability of capture (GLM: $F=0.34$; d.f.=1,18; $P=0.56$) immediately after a fumigation. These findings do not support the hypothesis that the presence of beetles in these mills after fumigation is due to immigration, as has been observed for other stored product species (Campbell and Arbogast, 2004); rather it involves survival of treatment within the mills.

3.3. Rebound after treatment

Rebound refers to the recovery or increase in trap captures following a reduction due to application of a treatment, and in this particular analysis refers to time to reach a particular capture threshold. Rebound results from individuals surviving treatment, including eggs and early instars not detectable in pheromone traps immediately after treatment, individuals that immigrated into facility, and the progeny of the survivors and immigrants; as modulated by environmental conditions and management tactics. Rebound rate in mean trap capture and probability of capture varied considerably among the fumigations and no linear or non-linear regression model significantly fit the combined data from all fumigations. Therefore, we developed threshold values and analyzed the time to reach the first monitoring period that matched or exceeded those thresholds as a measure of rebound. The mean trap capture threshold used was 2.5 beetles/trap/period, which corresponded to the median value immediately prior to fumigation in these mills. Time to rebound to the mean trap capture threshold was 174±33 d (n=21; censored observations=8), which is a biased mean since it is likely shorter than the actual rebound time because some times mills were fumigated prior to reaching threshold (i.e., censored observation). It took a significantly longer time to reach the threshold after fall fumigations than spring fumigations (survival analysis using Kaplan-Meier log-rank test, $Z=4.122$, $P=0.042$): 248±50 d (n=9, censored events=5)

compared to 104±21 d (n=9, censored events=3). The probability of capture threshold was 0.50 of the traps with captures of one or more beetles, which also corresponded to the median value prior to fumigation. For rebound to the probability of capture threshold, the mean time was 120±21 d (n=21, censored observations=4) and there was not a significant difference between seasons (Kaplan-Meier analysis: Z=3.752, *P*=0.05).

Rebound patterns measured in this study were highly variable, which probably reflects the impact of these many diverse factors on population growth and also that pheromone traps are imperfectly correlated with actual population levels. Clearly, management practices, including timing of fumigation, can impact rebound pattern, and reducing the rate of population increase can reduce the need or frequency of fumigation or other structural treatments.

3.4. Impact of management program on fumigation efficacy

Improvement of management practices at Mill #1, which started in the fall of 2004, provided an opportunity to evaluate the impact on trap captures and fumigation efficacy over a period of four years. After change in management strategy, there was a nine-fold reduction in mean *T. castaneum* capture and two-fold reduction in probability of capture in the mill. In Mill #2, which did not have the same change in management program, there were no differences in these measures over the same time periods. Reduction in mean trap capture immediately after fumigation was not significantly effected by change in management program (92.2±2.8% before versus 91.2±4.0% after management change (GLM: *F*=0.04; d.f.=1,9; *P*=0.84)). However, probability of capture immediately after fumigation was significantly effected by change in management (46.2±9.3% before versus 82.8±9.3% after change, GLM: F=7.59; d.f.=1,9; *P*=0.02).

After the pest management changes it took longer to rebound to the mean capture threshold (Kaplan-Meier analysis: Z =4.874, *P*=0.03); 49±15 d (n=5, censored events=0) before and 246±71 d (n=5, censored events=2) after change. It also took longer to rebound to the probability of capture threshold (Kaplan-Meier analysis: Z =5.801, *P*=0.02); 38±14 d (n=5, censored events=0) before and 165±46 d (n=5, censored events=0) after change. A consequence of these changes in pest abundance was that the number of fumigations was reduced from two or more per year before the management change to one per year afterward. The single fumigation per year after change in management program was in the fall.

Reduced rebound time following fumigation under the improved program could be explained in part by a smaller starting population following fumigation, reduced population growth rate due to lower interior temperatures and reduced immigration due to lower exterior temperatures associated with fall fumigations, or because the management program is increasing the mortality rate within the population. There was a negative correlation between mean beetle capture immediately after fumigation and time to rebound to mean trap capture threshold (Pearson Correlation: *r*=-0.626, *P*<0.01, n=21) and probability of capture threshold (Pearson Correlation: *r* =-0.596, *P*<0.01, n=21). This means that rebound to threshold took longer with decreasing numbers of individuals surviving the fumigation, and at Mill #1 mean trap capture after fumigation was lower following the change in management program compared to before. It is difficult to separate the effect of changing the time of year the fumigation is performed, which as described above impacts rebound rate, from the impact of the change in management program because only one fall fumigation occurred prior to the management change in Mill #1. However, we can analyze percentage change in mean beetle capture between sequential monitoring periods by both season and before and after change in IPM program. The overall GLM model was marginally significant for change in mean trap capture (*F*=2.70; d.f.=1,146; *P*=0.05) and season was a significant factor (*P*=0.02), but change in management and the interaction between season and change in management tactics were not significant (*P*>0.05). Sorting data by season, the change in mean trap capture between monitoring periods was 23.0±9.0% (n=79) in the cool season and 66.1±15.8% (n=71) in the warm season monitoring periods. For the proportion of traps with captures, the overall GLM model was not significant (*F*=0.81; d.f.=1146; *P*=0.49). This analysis suggests that while improving IPM program likely has important consequences on pest populations, changing the season fumigations are performed may have the largest single impact on rebound rate. Further evaluation using population models and before and after comparisons from other locations may provide more insight into the relative importance of the IPM program changes.

3.5. Development of risk thresholds

Theoretically, unchecked *T. castaneum* populations will grow exponentially and rebound in beetle capture data was frequently consistent with this pattern, although with a considerable amount of variation and with increases frequently truncated due to fumigations. There are currently no standardized pest management action thresholds for food facilities, but if a pest management program can keep *T. castaneum* trap captures in the relatively flat portion of the exponential curve there should be reduced risk of large increases in beetle captures in subsequent monitoring periods.

We first tested for a correlation between mean beetle capture and the increase in trap captures in the next monitoring period, but this was not significantly correlated (Pearson Correlation: r=0.08, *P*=0.15, n=292). This is likely because as mean trap capture increases it is more likely that interventions will increase – e.g., fumigation, insecticide applications, sanitation – resulting in slower rates of increase or decreases. Next, we tested for a correlation between change from the previous monitoring period compared to the current mean trap capture, which did have a significant positive correlation (Pearson Correlation: r=0.69, *P*<0.01, n=290). Finally, we used the rebound threshold values developed earlier and calculated the changes in trap captures above and below these thresholds. Below the mean trap capture of 2.5 beetles/trap/period, the increase in mean trap capture in the next monitoring period was 0.34±0.08 (n=202), but above this threshold the increase in mean trap capture in the next monitoring period was five times this amount (1.76±0.85 (n=90)), although the difference was not significantly different (Mann-Whitney Rank Sum Test: *U*=8746.5, *P*=0.61). Focusing just on intervals where beetle captures increased, the degree of increase was significantly greater above the 2.5 beetles/trap/period threshold (5.4±1.2, n=51) than below (0.9±0.2, n=119) (Mann-Whitney Rank Sum Test, U=1185.0, P<0.01). Similar results were obtained when analyzed using the probability of capture threshold. Preliminary analysis of a pooled dataset collected from a total of 12 wheat or rice mills gave similar results, which lends support to this approach, although further evaluation is still needed.

The rate of population increase is something that can be managed in food facilities such as flour mills: e.g., reduce population growth rate through increased mortality (sanitation and insecticide use), reduction in availability of food patches (sanitation and structural modification), reduced ability to colonize (exclusion and insecticide use), and reduced indoor temperatures (slower development rates). Pheromone based monitoring data can be used to evaluate the effectiveness of a management program, but it has been unclear how to use this data effectively. The mean trap capture and probability of capture thresholds appear to provide a good starting point for a risk threshold for mills. Given the limits of modern milling operations, flexible management targets based on risk thresholds may be more useful than traditional action thresholds based on responding when pest levels reach a certain point, as developed for other systems. In Mill #1 the enhanced pest management program and the shift in time of year that fumigations were performed resulted in trap captures exceeding the risk thresholds less frequently. The end result was less frequent fumigations and the suggestion that they might be reduced even further in frequency. This approach holds potential for improving management programs, since it is relatively simple to calculate, can be used to evaluate success of current program, and can be easily adapted to a given facility type and its management goals by adjusting the target threshold up or down based on criteria developed by management.

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Methyl iodide: a potential fumigant for post-harvest and quarantine disinfestation

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Abstract

Since the phase out of methyl bromide for post-harvest treatments, there is a lack of alternatives for many uses. Moreover, in the European Union methyl bromide is also banned for quarantine treatments since the 18th March 2010. That is why it is urgent to find and develop new fumigants because physical methods and/or contact insecticides cannot always replace all the capabilities of a fumigant (effectiveness, diffusion through the commodity, quick treatment...). A new fumigant that could replace methyl bromide for several uses is methyl iodide. This fumigant is already registered in the USA for soil disinfection and Japan for soil, timber and chestnut treatments.

Methyl iodide was studied in LNDS on some fields: grain, alfalfa seeds and timber disinfestations. For grain disinfestations, the study highlights the good results of methyl iodide after fumigations of infested wheat against the rice weevil: *Sitophilus oryzae* (L.). A CTP as low as 100 g.h/m^3 is sufficient to kill all the stages (eggs, larvae, pupae and adults) of this pest at 10°C. This fumigant could replace methyl bromide also for quarantine treatments to control the stem and bulb nematode *Ditylenchus dipsaci* (Kühn) in alfalfa seeds. After fumigation with an exposure time of 24 hours, and concentrations of 20, 30, 40, 50, 60 and 80 g/m^3, the results show that the efficacy of each fumigation at 15°C, is almost equivalent to that of methyl bromide. This fumigant could be also a potential fumigant for the pallet and wood packaging disinfestations under the ISPM n°15. Preliminary work shows that its diffusion through pine wood seems to be a good solution for the substitution of methyl bromide, with a time necessary to penetrate 8 cm of timber just a little bit longer than that for methyl bromide.

Keywords: Methyl iodide, *Sitophilus oryzae*, Alfalfa seeds, *Ditylenchus dipsaci*, Timber disinfestation

New prospects for ethyl formate as a fumigant for the date industry

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Abstract

Date infestation of nitidulid beetles poses a serious contamination problem for which methyl bromide (MB) provided a solution. However, because of the phase out of MB, alternatives were investigated. Thermal disinfestation method has been successfully applied to some dry date varieties except to Deglet-Noor, Zahidi, and Ameri which are handled in crates of 200 kg to 400 kg. Therefore, thermal disinfestation was not successful because of delayed heating due to the resistance of the dates to hot airflow. The fumigant formulation Vapormate™ was tested as alternative to MB for the disinfestation (proportion of insects found outside the feeding sites) and control of nitidulid beetles from artificial feeding sites at laboratory and for dates in crates at semi-commercial conditions. Vapormate™ contains 16.7% ethyl formate mixed with carbon dioxide. At laboratory conditions the effect of various dosages of Vapormate™ was tested at 30°C and at fixed exposure time of 12 h. Exposure of infested artificial feeding sites by larvae of *Carpophilus* spp. to the concentration of 280 g m^{-3} of Vapormate™ caused 69.3% disinfestation and 79.9% mortality, 350 g m^{-3} resulted in 72.7% disinfestation and 98.8% of mortality and the optimal results were obtained at 420 g m^{-3} that caused 69.6% disinfestations and 100% mortality.

Commercial pilot-plant tests were carried out by applying 420 g m^{-3} Vapormate™ for 12 h in a 9 m^3 flexible liner made of laminate composed of polypropylene/aluminum/polyethylene to cover crates containing infested dates. Disinfestation was tested on naturally infested dates that resulted in an average 100% disinfestation and 95% mortality, while with the artificially infested dates, disinfestation was 97% and mortality 96%. In a second series of tests, a commercial rigid fumigation chamber of 95.6 m^3 was used. After 12 h exposure, 100% mortality was recorded in all date samples. Following the promising results, Vapormate™ was registered in Israel for use by the date industry as an alternative to MB.

Keywords: Date, Nitidulid beetles, Ethyl formate, Vapormate™, Fumigation.

1. Introduction

Field infestations of nitidulid beetles pose a serious contamination problem of dates that requires their treatment immediately after harvest (Navarro, 2006). Until now, this problem has been addressed successfully using Methyl bromide (MB) because it causes a high proportion of larvae and adults to emigrate from the fruit before they succumb. This emigration phenomenon is associated with the disinfestation effect that leaves the fruit free from insect presence and more important than the toxic effect of the treatment. Since MB, under the terms of the Montreal Protocol, was phased out in 2005 the date industry needed to find alternative technologies to MB. Over the last two years the Israeli date industry has adopted thermal disinfestation to control nitidulid beetles infestation (Finkelman et al., 2006; Navarro et al., 2003; 2004; Navarro, 2006). A constant hot airflow can be achieved only when the dates are handled in trays of 3 kg or boxes containing 13 kg. However, date varieties Deglet-Noor, Zahidi, and Ameri are handled in two types of crates each containing 200 kg or 400 kg dates for which thermal disinfestations cannot be implemented. An option considered was the use of ethyl formate (EF) in its commercial form known as Vapormate™ in existing fumigation chambers. Ethyl formate occurs naturally in orange juice, honey, apples, pears and wine. It is used as a synthetic flavoring agent in the food industry and as fragrances; it is also a GRAS registered food additive. It decomposes slowly in water releasing formic acid and ethanol. Laboratory tests as a fumigant against insect pests of food commodities and field trials on bagged cereals, spices, pulses, dry fruits and oilcakes have been carried out (Muthu et al., 1984). Ethyl formate is currently registered as a fumigant in Australia as ERANOL® by Orica Chemnet for the elimination of insect pests in packed dried fruits like raisins. It is toxic to storage

insects including psocids (Annis et al., 2000). Vapormate™ is a low human risk fumigant formulated by BOC Australia, a member of the Linde Group, and contains 16.7 wt% EF in liquid carbon dioxide (CO_2) (Ryan and Bishop, 2003). The CO_2 in Vapormate™ has been added to eliminate the flammability of the EF and to enhance efficacy by its synergistic effect in reducing the time required to kill insects (Haritos et al., 2006). Vapormate™ is now fully registered for use in grain and horticultural products in Australia, in New Zealand for use in grain and for quarantine treatment of bananas (Krishna et al., 2002) and in Israel for dates and stored grains.

2. Materials and methods

2.1. Test insects

Carpophilus hemipterus (L.) (Coleoptera; Nitudilidae) and *C. maculatus* (Murray) were reared on media described by Donahaye and Navarro (1989) in an incubator at 30±1°C. To test infested dates, mixed populations of larvae from both species obtained from the field were incubated at 30°C for about 1 month.

2.2. Laboratory experiments

2.2.1. Evaluation of emigration from artificial feeding sites

The effectiveness of Vapormate™ in causing emigration of *Carpophilus spp*. larvae from artificial feeding sites was tested (Donahaye et al., 1992). The proportion of insects found outside the feeding sites was used to measure response and termed as percent of disinfestation. The artificial feeding sites consisted of cardboard rectangles placed on media in Petri dishes. Circles (9.5-cm diameter) were cut from polyethylene film (0.1-mm thickness) and these were used to line the lids of 9-cm-diameter plastic Petri dishes. The food medium was reheated and diluted as required to obtain a consistency at which it could be poured over the polyethylene to form a layer 5-mm deep. Cardboard rectangles (2 by 4 cm) were placed on top of the food medium, four rectangles to each Petri dish. After the media was solidified, the Petri dishes were opened and 30 larvae (4-5 d old) were introduced, so that each rectangle contained food medium bounded on its upper surface by cardboard and on its lower surface by polyethylene film. The proportion of larvae in feeding sites beneath the cardboard averaged 46% of the number placed in the Petri dishes 24 h earlier. Each feeding site generally held two to four larvae.

Humidity of the microenvironment within the desiccators ranged from 70 to 80% r.h. as determined by a humidity sensor (Hydrolog-D, Rotronic Instrument Ltd., Crawley, West Sussex, UK). Larvae in feeding sites were exposed to different treatments in 2.54-L desiccators. Each treatment was exposed at 30±1°C for 12 h to a series of doses of 280, 350, and 420 mg L^{-1} of Vapormate™ supplied from a pressurized cylinder (83% CO_2 and 17% EF w/w). In addition, the effect of 420 g m^{-3} and 12 h exposure to Vapormate™ on larvae emigration and mortality was studied at: 16ºC, 18ºC, 24ºC, 26ºC and 28ºC. Dosage calculations were converted to the gaseous phase and the required volume of Vapormate™ was obtained by evacuating the desiccator to the desired pressure, followed by restoration of atmospheric pressure using Vapormate™ supplied from a pressurized cylinder. The desired pressure was first calculated by converting the dose into a percentage of the desiccator volume to be treated, then desiccator was evacuated to the desired absolute pressure using a laboratory vacuum pump and the pressure measured using a portable transducer manometer (SE-2000, Celesco, Chatsworth, CA, USA), and then the equivalent to the partial pressure of in air was supplied by restoration of atmospheric pressure using the Vapormate™ supplied from the pressurized cylinder. The same process of evacuating to the desired pressure was carried out in the control desiccator but instead of the gas mixture, the pressure was restored using ambient air at atmospheric pressure. Before treatment, the desiccators were cleaned of any external infestation and the feeding sites were placed separately on the wire mesh floor. Each desiccator was loaded with ten feeding sites taken at random from the supply of feeding sites and during exposure were held in the dark 30±1°C. Upon completion of the exposure period they were removed from the desiccators and the larvae (dead or alive) present on the surface of the feeding sites and at the base of the desiccators were counted. Each feeding site was then opened length-wise using a scalpel and the adults and larvae (dead or alive) still present in each feeding site were counted. Each treatment was replicated four times and for each set of experiments, a control desiccator was exposed to the normal atmosphere for the same time period.

2.2.2. Egg mortality tests

Eggs were obtained by allowing 30 adult *C. hemipterus* to lay eggs over 24 h in the crevice between a microscope cover glass and glass slide formed by gluing them with a paper between both slides (Fig. 1). Eggs were exposed to 420 mg L^{-1} of Vapormate™ in 2.54-L desiccators for 12 h as described above. Mortality counts were made after 3 d and compared with the control group kept under the same temperature and humidity.

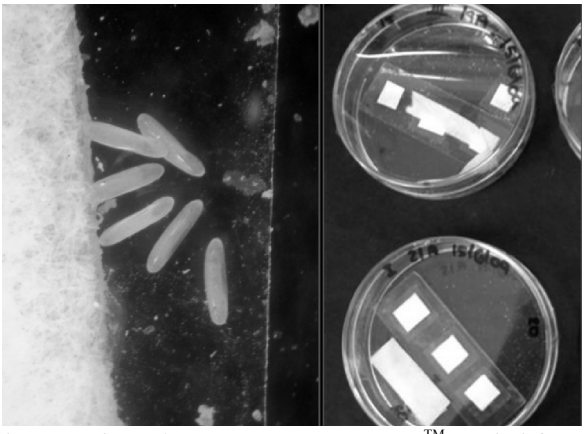

Figure 1 Experimental test exposing *C. hemipterus* eggs to Vapormate™: On the left: eggs under a cover slide. On the right: Slides with eggs placed in Petri dishes ready for exposure to the fumigant.

2.3. Commercial scale pilot trials on date fumigation

2.3.1. Date fumigation in portable flexible chamber

Four pallets containing Halawy variety dates were used for fumigation. Each pallet contained 20 rows and five columns of trays 60 x 40 x10 cm high. Each tray contained about 3 kg dates. Laboratory infested dates (50 fruits in each tray) were placed in pallets 1 and 4 at three layers (top, middle and bottom). Similarly field infested trays were marked in pallets 2 and 3.

The proportion of insects found after fumigation outside the dates and at the trays located below the test trays containing 50 laboratory-infested dates was used to measure percent of disinfestation. Mortality was calculated on the total number of insects found inside and outside the dates in the test trays and below the test trays.

The total volume to be fumigated was calculated as 9.5 m^3. Temperature of the dates was 24°C and ambient was 26°C during the trial. Vapormate™ supplied from the pressurized cylinder was mounted on a scale while the pressure tube was hold inside the sealed liner and secured to prevent movement of the injection tube due to back pressure of the gas. During the injection the opposite top of the chamber was kept open to release excessive pressure and to prevent sudden ballooning of the fumigation chamber (Fig. 2).

Figure 2 The portable disposable fumigation chamber made of flexible liner laminate on the right connected to a Vapormate™ cylinder on the left.

2.3.2. Date fumigation in commercial rigid fumigation chamber

A commercial rigid fumigation chamber of 95.6 m^3 located in Tzemach packing house was connected with a Vapormate™ pressurized cylinder directly to the existing system that was used for fumigation with MB (Fig. 3). Each of the 51 crates contained 400 kg Zahidi variety dates and 12 crates of 200-kg capacity containing Ameri variety dates were used for fumigation.

Figure 3 The commercial rigid fumigation chamber of 95.6 m^3 using Vapormate™ for dates stored in large crates each containing 200 kg or 400 kg dates.

Bioassays consisted of four small glass vials containing 30 laboratory-reared nitidulid larvae and 1 g of date (food) placed in four locations in the fumigation chamber. Two locations were in the top and bottom of crates near the door and two in the top and bottom crates at the far side of the chamber. Each glass vial was inserted 20-cm deep into the date pile. A gas-sampling opening in the chamber was used to measure gas concentration. An initial dosage of 420 g m^{-3} was used and concentrations were measured immediately after the gas release using a CO_2 gas analyzer.

3. Results

3.1 Laboratory experiments

Exposure to 280 mg L^{-1} for 12 h provided the lowest dosage with mortality values (79.9%) and disinfestation of (69.3%). Although mortality values at 350 mg L^{-1} averaged 98.8% and disinfestation was 72.7%, a higher dosage was also tested for complete control. Mortality at exposure to 420 mg L^{-1} resulted in complete kill with disinfestation value of 69.6% (Table 1). Table 2 shows the effect of 420 g m^{-3} and 12 h exposure to Vapormate™ on larvae emigration and mortality at 16ºC, 18ºC, 24ºC, 26ºC and 28ºC. Total larvae mortality was achieved at all temperatures above 18ºC, with the greatest larvae emigrations at 26ºC and 28ºC (Table 2). Table 3 shows the effect of 420 g m^{-3} and 12 h exposure to Vapormate™ on egg mortality at 24ºC and 30ºC. Total egg mortality was achieved at both temperatures.

Table 1 Effect of various dosage (mg L^{-1}) treatments of Vapormate™ on emigration of nitidulid larvae from artificial feeding sites simulating infested dates expressed in percent disinfestation, mortality and survival in control after 12 h at 30ºC (4 replicates for each dose).

Dose (mg/L)	Mortality (%)	SD	Disinfestation (%)	SD	Survival in control (%)	SD
280	79.9	16.26	68.3	15.34	66.7	24.58
350	98.8	1.39	72.7	9.81	76.0	26.82
420	100.0	0.0	69.6	12.85	74.9	21.31

Table 2 Larvae mortality and larvae emigration after 12 h exposure with 420 g m^{-3} of Vapormate™ at 16, 18, 24, 26 and 28ºC (4 replicates for each treatment).

	Mortality (%)					
Temperature (ºC)	Inside dates	SD	Outside dates	SD	Emigration (%)	SD
16	100	0.0	90.4	7.5	74.3	22.7
18	100	0.0	100	0.0	26.9	9.4
24	100	0.0	100	0.0	76.7	8.7
26	100	0.0	100	0.0	88.7	5.1
28	100	0.0	100	0.0	88.2	9.7

Table 3 Egg mortality after 12 h exposure with 420 g m^{-3} of Vapormate™ at 30ºC and 24ºC (4 replicates for each treatment).

	Control			Treatment		
Temperature (ºC)	Average number of eggs	Mortality (%)	SD	Average number of eggs	Mortality (%)	SD
24	21	65.9	1.2	113	100	0.0
30	33	53.0	4.2	123	100	0.0

3.2. Commercial scale pilot trials on date fumigation

Table 4 shows results using the flexible chamber on laboratory-infested dates after 12 h exposure with 420g m^{-3} of Vapormate™ ; there was with 97% disinfestation and 96% mortality calculated on total number of insects found inside and outside the dates. Table 5 shows results using the flexible chamber on naturally infested Halawy dates after treatment. All dates were clean from pests with a value of 100% disinfestation while mortality value of the beetles outside dates was 95%. The second type of fumigation chamber was a commercial rigid-fumigation chamber. All the bioassay test larvae in all four locations were killed and the dates collected from the same locations were free from insect contamination.

Table 4 Actual number of nitidulid beetles found outside and inside Laboratory infested dates after 12 h exposure with 420 g m^{-3} of Vapormate™ inside the flexible fumigation chamber. Calculated average disinfestation 97% and mortality 96%.

	Average number of insects outside date				Average number of insects inside date			
Layer of exposure in	Larvae		Adults		Larvae		Adults	
the experimental stack	Live	Dead	Live	Dead	Live	Dead	Live	Dead
Top	1	143	0	1	0	6	0	0
Middle	22	387	3	60	0	6	0	0
Bottom	10	297	6	39	0	13	0	0

Table 5 Actual number of nitidulid beetles found outside and inside naturally infested Halawy dates after 12 h exposure with 420 g m^{-3} of Vapormate™ inside the flexible fumigation chamber. Calculated average disinfestation 100% and mortality 95%.

	Average number of insects outside date				Average number of insects inside date			
Layer of exposure in	Larvae		Adults		Larvae		Adults	
the experimental stack	Live	Dead	Live	Dead	Live	Dead	Live	Dead
Top	0	0	0	0	0	0	0	0
Middle	21	456	0	76	0	0	0	0
Bottom	12	111	0	15	0	0	0	0

4. Discussion

The use of Vapormate™ as a fumigant to control nitidulid beetles has never been implemented before in the date industry. In order to comply with the registration demands in Israel there was a need to perform studies in the laboratory and to perform commercial scale trials. Under controlled laboratory conditions exposure to 420 g m^{-3} of Vapormate™ at 30ºC and 12 h exposure time resulted in 100% mortality of the nitidulid beetles and 69.6% disinfestations of the dates. Tests on larvae emigration and mortality at four temperatures resulted in total mortality at all temperatures (18º to 28ºC) with greatest larvae emigration at 26ºC and 28ºC. Vapormate™ was found to be effective fumigant to control nitidulid beetles including eggs, larvae and adults at exposure of 420 g m^{-3} of Vapormate™ for 12 h at temperatures above 24ºC.

The commercial trials using the flexible 9.5 m^3 chamber and commercial rigid fumigation chamber of 95.6 m^3 resulted in 100% mortality of nitidulid beetles and disinfestations greater than 95%. Vapormate™ was registered in Israel for the use by the date industry as an alternative to MB for the control nitidulid beetles at dosage of 420 g m^{-3} of Vapormate™ for 12 h at temperatures above 24ºC.

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Methyl bromide and sulfuryl fluoride effectiveness against red flour beetle life stages

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Abstract

The efficacy of methyl bromide (MB) and sulfuryl fluoride (SF) for managing all life stages of the red flour beetle, *Tribolium castaneum*, was investigated in the Hal Ross Flour Mill at Kansas State University. Eggs, young larvae, large larvae, pupae, and adults, confined in plastic compartments with dusting of flour and 2-cm deep flour, were exposed at 25 mill locations to two MB and two SF fumigations; in May and August 2009. MB and SF treatments were conducted by commercial fumigators, and each fumigation lasted 24 h. Gas monitoring lines were placed near the bioassay boxes to measure gas concentrations over time during fumigations. Both MB treatments killed 100% of all stages in the boxes except for large larvae in a few locations. In these locations, the mortality of large larvae ranged from 96-98%. SF treatments killed 100% of all stages except eggs. In the May treatment with SF, egg mortality ranged from 44-100% with only two boxes showing 100% mortality, because of under-dosing. Under-dosing occurred because the mill temperature was assumed to be greater than 27ºC when it was actually below 27ºC. In the second SF trial, only three boxes had egg mortalities that were less than 100%. However, data from the two replications showed that the mean mortalities of eggs and large larvae between MB and SF were not significantly different from each other.

Keywords**:** *Tribolium castaneum*, Methyl bromide, Sulfuryl fluoride, Ct product, Efficacy assessment.

1. Introduction

Methyl bromide (MB) is considered a Class I ozone depleting substance, and under the Montreal Protocol it was phased out in the United States in 2005 (UNEP 1998), except for certain critical uses under the critical use exemption category. Many MB alternatives for managing stored-product insects have been explored such as sulfuryl fluoride (SF), carbonyl sulphide, and heat treatment (Fields and White, 2002; Campbell and Arbogast, 2004; Boina et al., 2008). SF was registered by the United States Environmental Protection Agency under the trade name ProFume® by DowAgroSciences LLC, Indianapolis, Indiana, USA, in January 2004. SF was evaluated in flour mills and found to be effective, but the egg stage of the red flour beetle, *Tribolium castaneum* (Herbst), is reported to be relatively less susceptible when compared with postembryonic stages (Bell et al., 2003). Most comparisons of MB and SF treatments use different facilities for each of the different treatments or conduct treatments at different times of the year, and typically rely on pre- and post-trapping insect numbers to verify treatment effectiveness (Campbell and Arbogast, 2004; Small, 2007). The objective of the current study was to verify effectiveness of MB and SF fumigation in a pilot flour mill using insect bioassays under nearly identical environmental and sealing conditions.

2. Materials and methods

Bioassay boxes were constructed using large plastic craft boxes that contained twelve smaller compartments, each with individual lids (Fig. 1). Holes were cut in all four sides of the smaller boxes and the lids. These holes were covered with a metal mesh screen (90 µm opening) for ventilation and gas diffusion. Corresponding holes were also cut in the large outer box. In the top six small compartments, 2 cm of flour was added to simulate unsanitary mill conditions. In the remaining six compartments, a dusting of flour was added to simulate sanitary conditions. Eggs, small larvae (first instars), large larvae (late instars), pupae, and adults of *T. castaneum* were introduced into the separate compartments within each large box. Each compartment held 50 individuals of a life stage. Small data loggers to measure

temperatures (SmartButton, ACR Systems Inc., British Columbia, Canada) were added to the remaining two uninfested compartments. Data loggers were set to record temperature every two minutes. The boxes with insects were placed in 25 preselected locations across all five floors in the Hal Ross Flour Mill, a state-of-the-art pilot mill at Kansas State University. One box, placed in the laboratory growth chamber at 28ºC and 65% r.h., served as the control treatment.

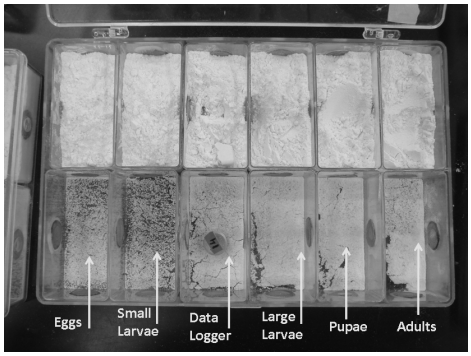

Figure 1 Rectangular plastic box with 12 compartments used in insect bioassays in the Hal Ross Flour Mill.

Some boxes were positioned directly on the floor, while others were placed within pieces of equipment. Gas monitoring lines were attached to the center of each box and during treatments, gas concentrations were continuously monitored. Treatments typically started in the late afternoon and each treatment lasted 24 h. Once the treatment was completed, the mill was opened for the gas to dissipate overnight and the boxes were retrieved the next morning when it was safe to reenter the mill. In the laboratory, boxes were placed in incubators set at 28ºC, and after 24 h adult mortality was determined by counting live and dead insects. For immature stages, insects and flour were removed from compartments and placed in 150 mL round plastic containers and reared to adulthood. Pupae were counted after 11 d, large larvae after 18 d, and small larvae and egg stages were counted after 45 d.

The first set of MB and SF treatments were performed during May 6-7 and May 27-28, 2009, respectively. The second set of treatments with MB and SF was conducted during August 11-12 and August 19-20, 2009, respectively. The final set of treatments will be conducted in May 2010. Data from four control treatments were averaged to obtain mean and associated standard errors for mortality of each of the five stages. Mortality data for each stage from the two MB and two SF replications were averaged to show the mean mortality and associated standard errors (SE). The effectiveness of MB and SF against each stage was compared by subjecting mortality data to two-way analysis of variance (ANOVA) using the GLM procedure (SAS Institute, 2002). Differences in mortality were considered to be significant at the $\alpha = 0.05$ level. Egg mortality was less than 100% in treatments with SF. Therefore, variation in egg mortality observed during May and August treatments were separately plotted against SF concentration x time (Ct) product at each of the 25 box locations.

3. Results and discussion

The fumigation starting time, mill temperature, amount of gas used, and Ct products for MB and SF are shown in Table 1. By using the same facility during the same month, the environmental conditions and experimental protocols followed were essentially similar, allowing us to do a true side-to-side comparison of MB and SF effectiveness against *T. castaneum* life stages.

Table 1 Starting time, amount of fumigant used, and range of mill temperatures and Ct products for May and August fumigations.

	May treatments		August treatments	
Variable	**MB**	**SF**	**MB**	**SF**
Starting time	6:40 p.m.	6:00 p.m.	2:50 p.m.	2:45 p.m.
kg of gas used	181.4	567.0	159	511
Mill temp. (°C)	22 - 23	23 - 26	27 - 31	28 - 32
Ct product (g-h/m^3)	283 - 327	923 - 1191	268 - 318	663 - 1003

The average control mortality across all life stages is shown in Figure 2. Mortality for both small larvae and eggs in compartments with flour dust was higher than expected (>40%). The higher mortality with these stages could be attributed to lack of sufficient food and possibly cannibalism. The achieved Ct product for MB in May and August resulted in 100% kill of all life stages, except for large larvae in a few locations (Table 2). However, the mean mortality for large larvae in compartments with flour dusting and in 2 cm deep flour was still close to 99.7%.

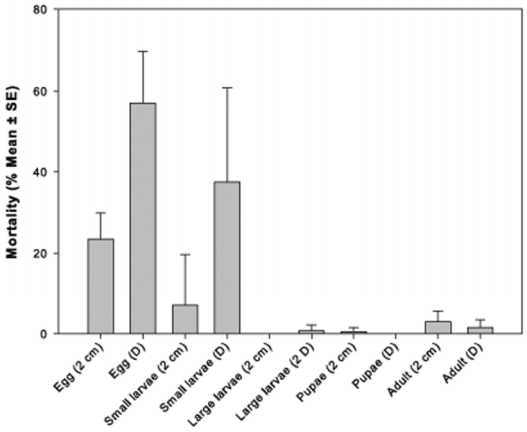

Figure 2 Mortality of life stages of *T. castaneum* in the control treatment (Each mean is based on four 4 replications).

Table 2 Average mortality of life stages of *T. castaneum* in bioassay boxes during the two MB and SF fumigations.

Treatment	Insect Stage	Flour Depth	Mean±SE Mortality (%)[a]
MB	Eggs	2 cm	100.00
	Eggs	Dusting	100.00
	Small larvae	2 cm	100.00
	Small larvae	Dusting	100.00
	Large larvae	2 cm	99.70 ± 0.16
	Large larvae	Dusting	99.96 ± 0.08
	Pupae	2 cm	100.00
	Pupae	Dusting	100.00
	Adults	2 cm	100.00
	Adults	Dusting	100.00
SF	Eggs	2 cm	91.00 ± 9.00
	Eggs	Dusting	92.55 ± 7.05
	Small larvae	2 cm	100.00
	Small larvae	Dusting	100.00
	Large larvae	2 cm	99.96 ± 0.04
	Large larvae	Dusting	100.00
	Pupae	2 cm	100.00
	Pupae	Dusting	100.00
	Adults	2 cm	100.00
	Adults	Dusting	100.00

[a]Each mean is based on *n*=2 replications.

The SF treatments were effective against all postembryonic stages. Interestingly, the effectiveness on eggs during the May SF treatment ranged from a low of 44 to a high of 100% (Fig. 3 and 4). During the August treatment egg mortality with SF only in three locations ranged from 94-98% (Fig. 5**).** The poor efficacy against eggs in May is due to using 14% less SF than that required to kill all of the eggs.

This occurred because a temperature of 26.7°C was planned and used for dosage calculation, but the actual mill temperatures ranged from 22.2-25.0°C.

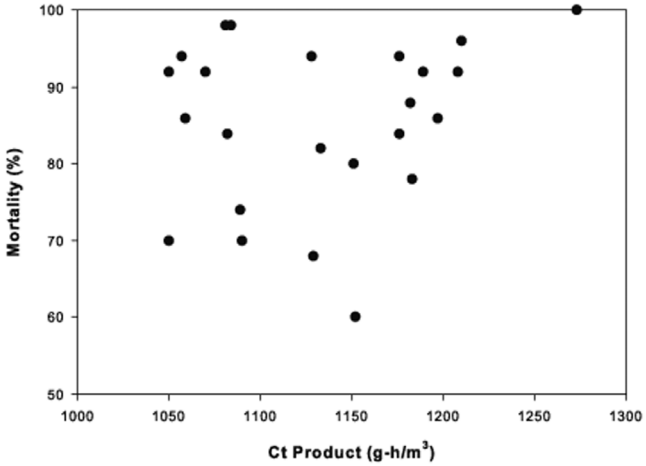

Figure 3 Scatter plot showing variation in egg mortality in compartments with flour dusting as a function of the concentration x time (Ct) product during May 6-7, 2009 fumigation with sulfuryl fluoride.

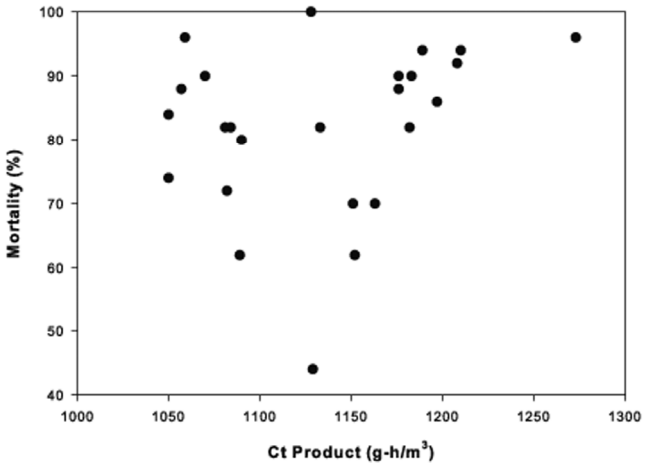

Figure 4 Scatter plot showing variation in egg mortality in compartments with 2 cm deep flour as a function of the concentration x time (Ct) product during May 6-7, 2009 fumigation with sulfuryl fluoride.

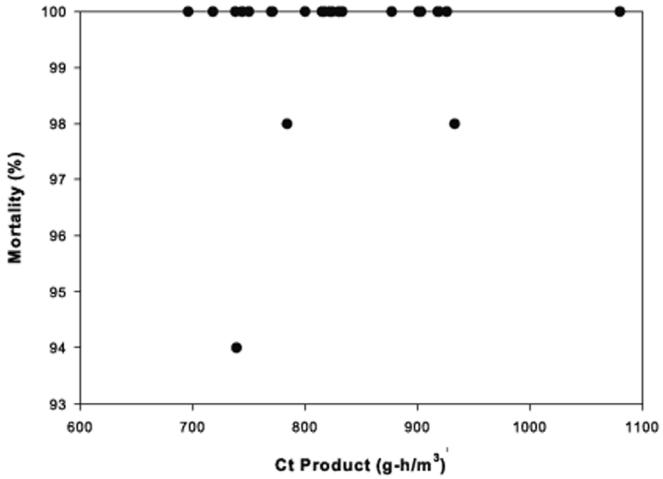

Figure 5 Scatter plot showing variation in egg mortality in compartments with flour dusting as a function of the concentration x time (Ct) product during August 19-20, 2009 fumigation with sulfuryl fluoride.

The only two stages that survived included the egg stage (with SF) and large larvae (with MB and SF). All other stages were completely controlled by MB and SF. Therefore, differences in the effectiveness of MB and SF on eggs or large larvae were compared. The comparisons for each stage show that the mortality of eggs or large larvae was not significant ($P > 0.05$) between MB and SF treatments (Table 3). Similarly, flour depth did not influence mortality of eggs or large larvae. The interaction term was also not significant, indicating that the mortality of eggs and large larvae were consistent between the two treatments (MB and SF) and at the two flour depths. The results from the two replications showed that under nearly identical environmental and sealing conditions, the effectiveness of MB and SF on the mortality of five life stages of *T. castaneum* was similar, despite differences in the survival of eggs with SF and large larvae with MB and SF.

Table 3 Two-way analysis of variance statistics comparing mortality of eggs and large larvae between MB and SF treatments in compartments with flour dusting and 2 cm deep flour.

Stage	Source	df	Mean square	*F*-value	*P*-value*
Egg	Treatment[a]	1	135.30	2.07	0.22
	Depth[b]	1	1.20	0.02	0.90
	Treatment x Depth	1	1.20	0.02	0.90
	Error	4	65.35	-	-
Large larvae	Treatment[a]	1	0.09	5.14	0.09
	Depth[b]	1	0.02	1.08	0.36
	Treatment x Depth	1	0.01	0.40	0.56
	Error	4	0.02	-	-

[a]Treatments are MB and SF. [b]Depths represent flour dusting and 2 cm deep flour in bioassay compartments.
*None of the *P*-values is significant at $P = 0.05$.

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❖ Mention of a proprietary product name does not constitute an endorsement by Kansas State University, Kasetsart University, University of Milan, or USDA.

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Fumigant toxicity of garlic essential oil in combination with carbon dioxide (CO_2) against stored-product insects

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Abstract

Fumigant toxicity of garlic essential oil alone and in combination with carbon dioxide (92% CO_2) to adults and pupae of *Tribolium confusum* and *Ephestia kuehniella* at 24 h of exposure time was studied. Results indicated that adults of *E. kuehniella* were the most sensitive, since garlic essential oil alone resulted in their complete mortality at each concentration, whereas adults of *T. confusum* were the most tolerant with LC_{90} value of 30 µL L^{-1}. In contrast to the results for the adults, pupae of *T. confusum* were more sensitive with LC_{90} value of 11.1 µL L^{-1} than those of *E. kuehniella* with LC_{90} value of 27.4 µL L^{-1}. When 92% CO_2 was combined with garlic essential oil, the LC_{50} and LC_{90} values for adult of *T. confusum* and pupa of *T. confusum* and *E. kuehniella* were significantly reduced. Combination of garlic essential oil with 92% CO_2 produced decrements in LC_{90} value of *E. kuehniella* pupa from 27.4 to 0.95 µL L^{-1}, while it resulted in reduction in LC_{90} value of *T. confusum* pupa from 11.1 to 1.0 µL L^{-1}. Combination of garlic essential oil with 92% CO_2 also produced significant decrements in LC_{90} value for adults of *T. confusum* (4.9-fold) compared with those exposed to garlic essential oil alone. These results indicated that 92% CO_2 had a synergistic effect on toxicity of garlic essential oil to *T. confusum* and *E. kuehniella*. In conclusion, present study revealed that the combination of garlic essential oil with CO_2 enhanced its fumigant toxicity to stored product insects.

Keywords: Garlic essential oil, Carbon dioxide, *Tribolium confusum*, *Ephestia kuehniella*, Fumigant toxicity, Synergistic effect

1. Introduction

Control of insect-pest infestation in storage may cause special problems on stored products. In many storage systems, methyl bromide and phosphine are the most economical fumigants for management of stored-grain insect pests. EPA (2001) has proposed elimination of the production of methyl bromide by 2005 because of its ozone depletion potential. Additionally, some stored-product insects are found to have developed resistance to methyl bromide and phosphine (Subramanyam and Hagstrum, 1995; Champ and Dyte, 1977). These problems have highlighted the need for the development of natural products derived from plants as an alternative to conventional insecticides. Many types of spices and herbs are known to possess insecticidal activities (Tripathi et al., 1999), especially in the form of essential oils (Shaaya et al., 1991). They do not leave residues toxic to the environment and have medicinal properties for humans with lower toxicity to mammals (Duke, 1985).

Essential oils are potential sources of alternative compounds to currently used fumigants. Various studies have demonstrated fumigant activity of various essential oils against various stored product insects (Shaaya et al., 1991; Shaaya et al., 1997; Tunç et al., 2000; Lee et al., 2003). Toxicity of various essential oils and their volatile constitutes against all life stages of the flour beetle, *Tribolium confusum* Jacguelin du Val (Coleoptera: Tenebrionidae) indicated that two essential oils, garlic and onion had potent fumigant activities (Karcı, 2006). Gözek (2007) reported that the adults and larvae of *T. confusum* were the most tolerant stages and the eggs and pupae were the most susceptible stages to treatments of garlic essential oil, their active compounds and mixture. In the same study, garlic essential oils, their active compounds and mixture required less than 1 µL L^{-1} to kill 90% of the eggs whilst garlic essential oil and its active compounds required the dosages ranging from 6.4 and 23.3 µL L^{-1} to kill 90% of the larvae.

The use of CO_2 together with conventional fumigants has also been studied. Carbon dioxide, a respiratory stimulant, is a known adjuvant for fumigants including phosphine and methyl bromide. The advantages of using CO_2 in the mixture are to increase the toxicity of the fumigant, improve the

distribution pattern, limit the levels of harmful residues in the treated commodity, and also eliminate the flammable hazard of some fumigants. Several general studies on fumigant/CO_2 mixtures have been made in the past (Jones, 1938), and these were followed by investigations which showed that the addition of CO_2 to methyl bromide (MB) resulted in an increase in the susceptibilities of some stored-product insects (Calderon and Leesch, 1983; Willliams, 1985). Laboratory tests with essential oils have shown a similar joint action with CO_2 atmospheres. Shaaya et al. (1997) demonstrated enhanced toxicity of essential oil, SEM76 (from a Lamiaceae plant), in the presence CO_2 to *T. castaneum* (larvae, pupae and adults), *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) (larvae and pupae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (adults). However, toxicity studies on mixture of essential oils with CO_2 against stored-product insects to demonstrate additive, synergistic or antagonistic effects are rare.

The present study was conducted to investigate fumigant toxicity of garlic essential oil alone and in combination with high concentration of carbon dioxide (92% CO_2) to adults and pupae of *T. confusum* and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae).

2. Materials and methods

2.1. Test insects

Tests were carried out on pupae and adults of *E. kuehniella* and *T. confusum.* Adults and pupae of *T. confusum* were obtained from cultures reared at 25 ± 1°C and 65 ± 5% relative humidity (r.h.) on a diet of wheat flour mixed with yeast (17:1, w/w) using standard culture techniques (Donahaye, 1990). Larvae of *E. kuehniella* were reared on a 10:2:1 mixture of wheat flour, wheat germ and dried brewer's yeast at the same environmental conditions as *T. confusum*. Pupae were obtained by daily separation from culture jars and were exposed to the treatments at the age of 2 d. Seven- to 10-d-old and newly-emerged (0-1 d) adults of *T. confusum* and *E. kuehniella*, respectively, were exposed to the treatments in empty exposure jars.

2.2. Fumigation chambers

Fumigation chambers consisted of 3-L glass jar, each capped with a ground-glass stopper equipped with entry and exit tubing. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps.

2.3. Garlic Essential oil

Essential oil from bulbs of garlic, *Allium sativum* L. (Alliaceae) was tested against all life stages of *T. confusum* and *E. kuehniella*. Garlic essential oil extracted by stem distillation method was provided commercially (Liberty Natural Products, Inc., Oregan City, OR, USA). After purchase, garlic essential oil collected in sealed glass containers was refrigerated in the dark at 4ºC until their use. Carbon dioxide was supplied from a cylinder and was 99% pur*e.*

2.4. Bioassay and Experimental Procedures

Garlic essential oil was introduced as a liquid into the desiccators using 10- or 50-µL gas-tight syringes. Carbon dioxide was transferred from the supply cylinder through a pipe equipped with a regulator valve. Concentrations of CO_2 inside the glass jars were checked by using hand-operated O_2/CO_2 analyzer (PBI Dansensor, Ringsted, Denmark). Relative humidity during fumigations was also measured by placing small hygrometers within the fumigation chamber. Prior to each test, 20 larvae and adults of *T. confusum* and *E. kuehniella* were confined, separately, inside 2.5-cm diameter by 5-cm long glass vials.

Firstly, preliminary bioassay tests on fumigation activity of garlic essential oil alone and garlic essential oil combination with 92% CO_2 were carried out to determine the effective concentrations of each treatment against adults and pupae of *T. confusum* and *E. kuehniella*. For garlic essential oil alone treatment, adults and pupae of each tested species were exposed to a concentration of 10 µL L^{-1} of garlic essential oil for 24 h. Garlic essential oils were applied on filter paper (2 x 8 cm) attached to lower side of the lids of fumigation chamber by using a 50-µL syringe. After adults and pupae of *T. confusum* and *E. kuehniella* kept in the glass vials were transferred separately into fumigation chamber, fumigation chambers were closed by screwed lids, which were made air-tight. Each treatment and control was

replicated three times. For the treatments with garlic essential oil in a CO_2 atmosphere, the insects were first placed in the fumigation chambers. Then, prior to the introduction of 10 µL L^{-1} of garlic essential oil concentration, the fumigation chambers were briefly evacuated to 60.8 mm Hg followed by flushing with CO_2 until restoration of atmospheric pressure so as to obtain a uniform concentration of 92±2% CO_2. The 24-h exposure was used throughout all the experiments. In addition to these treatments, separate exposure to 92% CO_2 alone was made and untreated control insects were exposed to atmospheric conditions. For all fumigations, conditions were maintained at 65±5% r.h. at atmospheric pressure and 25±1°C respectively.

Separate bioassay tests were carried out to determine LC_{50} and LC_{90} values of garlic essential oil alone and in combination with 92% CO_2 for adults of *T. confusum* and pupae of *T. confusum* and *E. kuehniella*. Each stage of tested species were exposed to different concentrations of garlic essential oil for 24 h. With garlic essential oil alone a range of 5 concentration levels from 10 to 40 µL L^{-1} for *T. confusum* adult, from 1 to 15 µL L^{-1} for *T. confusum* pupae and from 5 to 35 µL L^{-1} for *E. kuehniella* pupae was used. With garlic essential oil in combination with 92% CO_2 ranges consisted of 5 concentrations, from 0.5 to 8 µL L^{-1} for *T. confusum* adults, from 0.25 to 2.5 µL L^{-1} for *T. confusum* pupae and from 0.25 to 2 µL L^{-1} for *E. kuehniella* pupae. Concentrations were selected for the eggs of insect species on basis of preliminary bioassay tests. Three replicates were set up for each concentration and control. Fumigation procedures were the same as in above mentioned bioassay tests.

2.5. Data processing and analysis

After each treatment, adults and pupae of *T. confusum* and *E. kuehniella* were transferred to 250-mL jars containing standard diets and were held at 26±1°C and 65±5% r.h. until examined for mortality. Mortality counts for adults were made 1-2 d after exposure and pupal mortality was based on those pupae that failed to produce adults 9 d after exposure. Mortality data were subjected to arcsine transformation and then analyzed using one-way analysis of variance (ANOVA). The means were separated using the LSD (Least Significant Difference) method at the 1% level (SAS Institute, 1985). Data obtained from each zero dose control and concentration-mortality responses were subjected to probit analysis by using maximum likelihood program software (POLO-PC) (LeOra Software, 1989) to determine LC_{50}s (Lethal Concentration$_{50}$), LC_{90}s (Lethal Concentration$_{90}$) and their respective 95% confidence intervals.

3. Results

Percentage mortalities (%) of adults and pupae of *T. confusum* and *E. kuehniella* exposed to a concentration of 10 µL L^{-1} of garlic essential oil alone, 10 µL L^{-1} of garlic essential oil in combination with 92% CO_2 and 92% CO_2 alone for 24-h exposure time are given in Table 1. Preliminary bioassay tests indicated that all treatments except control resulted in almost 100% mortality for adults of *E. kuehniella*. However, only garlic essential oil in combination with 92% CO_2 achieved almost mortality of 100% against adult of *T. confusum,* which was significant higher mortality than those of garlic essential oil alone, 92% CO_2 alone and control treatment (P<0.0001). Similarly, garlic essential oil in combination with 92% CO_2 resulted in 100% mortality of pupae of *T. confusum* and *E. kuehniella*, which was significant higher mortality than those of garlic essential oil alone, 92% CO_2 alone and control treatment (P<0.0001). Preliminary bioassay tests indicated that garlic essential oil in combination with 92% CO_2 resulted in the increase of mortality of adults of *T. confusum* and pupae of *T. confusum* and *E. kuehniella*. Exposure to 92% CO_2 alone produced a very low mortality of *T. confusum* adults, but it resulted in relatively high mortality of *T. confusum* and *E. kuehniella* pupae (27% to 31%).

Table 1 Percentage mortalities (%) of adults and pupae of *T. confusum* and *E. kuehniella* exposed to a concentration of 10 µL/L of garlic essential oil alone, 10 µL/L of garlic essential oil in combination with 92% CO_2 and 92% CO_2 alone for 24-h exposure time.

	Mortality (%)±SE			
	T. confusum		*E. kuehniella*	
Treatments	**Adult**	**Pupa**	**Adult**	**Pupa**
Garlic oil alone	17.6±2.22 b	34.4±2.94 b	97.8±2.22 a	34.4±2.94 b
Garlic oil + 92% CO_2	98.3±1.67 a	100±0 a	100±0 a	100±0 a
92% CO_2 alone	23.3±3.85 b	31.1±5.88 b	100±0 a	26.7±3.85 b
Control	2.2±2.22 c	17.2±2.22 b	6.7±3.33 b	16.2±2.22 b

Means within a column with the same letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied for data.

Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for pupae of *T. confusum* and *E. kuehniella* resulting from 24-h laboratory fumigations are given Tables 2 and 3, respectively. The tables show that garlic essential oil in combination with 92% CO_2 reduced LC_{50} and LC_{90} values of pupae of *T. confusum* and *E. kuehniella*. Garlic essential oil in combination with 92% CO_2 produced a significant decrease in LC_{90} values from 11.1 to 1.0 µL L^{-1} and from 27.4 to 0.95 µL L^{-1} for pupae of *T. confusum* and *E. kuehniella* respectively. Thus, garlic essential oil in combination with 92% CO_2 had 11.1- and 28.9-fold reduction in LC_{90} values for pupae of *T. confusum* and *E. kuehniella*, respectively, compared with garlic essential oil alone (Tables 2 and 3). The decrements in LC_{50} values were higher than those in LC_{90} values, the LC_{50} values obtained from garlic essential oil in combination with 92% CO_2 were still reduced by 14.3- and 58.3-fold for pupae of *T. confusum* and *E. kuehniella*, respectively, compared with garlic essential oil alone.

Table 2 Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for pupae of *Tribolium confusum* resulting from 24-h laboratory fumigations.

Treatment	**n[a]**	**Slope±SE**	**LC_{50} (µL/L) (Fiducial limit)[b]**	**LC_{90} (µL/L) (Fiducial limit)[b]**	**λ^{2c}**
Garlic oil alone	315	4.15±0.64	5.45 (4.34-6.52)	11.09 (9.11-14.81)	4.712
Garlic oil + 92% CO_2	315	3.02±0.51	0.38 (0.22-0.42)	1.00 (0.78-1.46)	5.191
Ratio			14.34	11.09	

[a]: Number treated, excluding controls. [b]: Numbers in brackets give the 95% confidence range. [c]: Chi-square (chi-square is significant, $P < 0.05$).

Table 3 Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for pupae of *Ephestia kuehniella* resulting from 24-h laboratory fumigations.

Treatment	**n[a]**	**Slope±SE**	**LC_{50} (µL/L) (Fiducial limit)[b]**	**LC_{90} (µL/L) (Fiducial limit)[b]**	**λ^{2c}**
Garlic oil alone	315	10.99±2.56	20.98 (18.22-22.73)	27.44 (25.23-32.33)	9.897
Garlic oil + 92% CO_2	315	3.03±0.52	0.36 (0.31-0.40)	0.95 (0.74-1.39)	4.184
Ratio			58.28	28.89	

[a]: Number treated, excluding controls. [b]: Numbers in brackets give the 95% confidence range. [c]: Chi-square (chi-square is significant, $P < 0.05$).

Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for adults of *T. confusum* resulting from 24-h laboratory fumigations are given Tables 4. Garlic essential oil in combination with 92% CO_2 reduced LC_{50} and LC_{90} value of adults of *T. confusum*. Garlic essential oil in combination with 92% CO_2 produced a significant decrease in LC_{90} values from 30.1 to 6.1 µL L^{-1} for adults of *T. confusum*. Thus, garlic essential oil in combination with 92% CO_2 had 4.9-fold reduction in LC_{90} values for adults of *T. confusum* compared with garlic essential oil alone (Table 4). The decrements in LC_{50} values were higher than those in LC_{90} values, the LC_{50} values obtained from garlic essential oil in combination with 92% CO_2 were still reduced by 9-fold for adults of *T. confusum* compared with garlic essential oil alone.

Table 4 Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for adults of *Tribolium confusum* resulting from 24-h laboratory fumigations.

Treatment	n[a]	Slope ± SE	LC_{50} (µL L^1) (Fiducial limit)[b]	LC_{90} (µL L^1) (Fiducial limit)[b]	λ^{2c}
Garlic oil alone	420	5.38±0.54	17.38 (15.74-19.88)	30.07 (27.37-33.99)	9.099
Garlic oil + 92% CO_2	420	2.55±0.27	1.93 (1.55-2.29)	6.12 (4.98-.807)	6.727
Ratio			9.01	4.91	

[a]: Number treated, excluding controls. [b] : Numbers in brackets give the 95% confidence range. [c] : Chi-square (chi-square is significant, $P < 0.05$).

4. Discussion

From this study, it can be seen that the use of 92% CO_2 with garlic essential oil clearly resulted in significant reductions of LC_{50} and LC_{90} values for adults and pupae of *T. confusum* and *E. kuehniella*. This was particularly effective for the most tolerant pupal stage where combining garlic essential oil with 92% CO_2 decreased the LC_{90} value from 27.4 to 0.95 µL L^{-1}. It might be argued that low O_2 concentrations could influence the potentiating effect of CO_2 for pupae of *T. confusum* and *E. kuehniella*. However, data without garlic essential oil indicated that there was only limited mortality of the adults and pupae on exposure to 92% CO_2 alone for 24 h. Therefore, the results suggest that CO_2 has a synergistic effect on the test insects when exposed together with garlic essential oil.

Other studies have shown that the admixture of CO_2 could increase the toxicity of fumigants, mainly MB and phosphine (Monro et al., 1966; Dumas et al., 1969; Calderon and Leesch, 1983; Williams, 1985; Donahaye and Navarro, 1989). In all these studies the susceptibilities of test insects to fumigants combined with CO_2 were found to increase by only a factor of one to three. Laboratory tests with essential oils have shown a similar joint action with CO_2 atmospheres. The peel oils of *Citrus* spp. and *Eucalyptus citriodora* Hook at 10 and 20 µL L^{-1} doses were more toxic in presence of two different controlled atmospheres (15% CO_2 + 1% O_2 + 84% N_2 and 12% CO_2 + 5% O_2 + 83% N_2) to the psocid, *Liposcelis bostrychophilus* Badonnel (Psocoptera: Liposcelidae) (Wang et al., 2001). However, the results obtained from our studies reveal that reductions in LC_{50} and LC_{90} caused by garlic essential oil in combination CO_2 are much higher than those reported by the above authors.

In conclusion, the use of high concentration of CO_2 appears to have a synergistic effect on these species as evidenced by significant decrements in LC_{50} and LC_{90} values for the adults and pupae. These results indicate that combination of garlic essential oil with CO_2 can be potential as an alternative application to the most commonly used commercial fumigants, methyl bromide and phosphine.

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Improvement of phosphine fumigation by the use of Speedbox

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Abstract

Today, phosphine is turning to be a major fumigant for controlling insects in stored products. However, few limitations, such as low temperatures and relatively long exposure time, limit the phosphine use. In order to improve phosphine application, a special devise, containing a heater and a ventilator, called "Speedbox" has been developed by Detia Degesch GmbH Germany. For studying the effectiveness of phosphine fumigation using Speedbox, we have conducted two kinds of experiments: one in a fumigation room (Pilot) and other in commercial warehouse. For pilot fumigation, adults, pupae and late larvae of *Sitophilus oryzae,*, *Rhyzopertha dominica, Oryzaephilus surinamensis*, *Trogoderma granarium* and *Callosobruchus maculatus,* and all stages of *Tribolium castaneum* Herbst, *Plodia interpunctella* and *Ephestia cautella* were used as test insects. One to three Degesch Plates (about 2-6 g of phosphine gas per m^3) were used. Exposure time was 1 to 3 days. The phosphine concentration was monitored by Bedfont device model 415. At 4 g/m^3 for 48 h a maximum of phosphine concentration of 1460 ppm was reached. The total mortality of all tested insects and stages was recorded, except the eggs of *E. cautella* (98%). The commercial stack fumigation was done at the dosages of 2-4 g/m^3, exposure time of 2-4 days and commodity temperatures of 6-17ºC. At a target concentration of 4 g/m^3, 2 hours after beginning of the treatment, the concentration of the gas has reached 414 ppm, with a maximum of 1480 ppm. The total mortality of tested insects at adult, late larvae and pupae stages was recorded. The use of Speedbox allows one-day decrease in the plates degassing time, recirculation of the gas and its event distribution in the treated space and controlling major stored product insects for shorter exposure time at low temperatures.

Keywords: Fumigation; Posphine; Speedbox; Stored-product insects

1. Introduction

In the post methyl bromide era, phosphine is turning to be a major fumigant for controlling insects in stored products. However, few limitations, such as low temperatures and relatively longer exposure times in comparison to methyl bromide, limit the use of phosphine. Some techniques for direct application of gaseous phosphine from cylinder (ECO_2FUME, $VAPORPH_3OSTM$ Phosphine) and on-site generator sources (Horn generator, Chinese generator, QuickPHlo-R phosphine generator, QuickPHlo-C technology and the Degesch Phosphine Generator) and others have been developed to solve these problems (Williams et al., 2000; Mathews and Luzaich, 2003; Waterford and Asher, 2003; Waterford, 2004; Horn and Horn, 2006; Steuerwald et al., 2006; Rajendran and Sriranjini, 2007; Ryan et al., 2010). Each of them aimed for different purposes and has advantages and disadvantages. In order to improve phosphine application, especially for low temperatures and for shorter treatment time, a special devise, called "Speedbox" has been developed by Detia Degesch GmbH Germany (Jakob et al., 2006). The Speedbox is a waterproof aluminium box containing a heater and a ventilator. The Speedbox has been designed to be used exclusively with Degesch Plates®. For studying the effectiveness of phosphine fumigation using Speedbox, we have conducted two kinds of experiments: one in a fumigation room (pilot) and other in commercial warehouse.

2. Materials and methods

2.1. Experiments in the fumigation room

The Speedbox was connected to the fumigation room of 15-m^3 volume filled with wheat grain bags by 30% of the space. One to three Degesch Plates were used. One Degesch Plate contains 56% of magnesium phosphide, weighs 117 g and evolves 33 g of phosphine gas (about 2 g of phosphine gas per m^3). The target concentration was 2 to 6 g of phosphine gas per m^3. The plates were heated to 36ºC into

the Speedbox. The produced hydrogen phosphide was blown in the fumigation room and was pumped out from the room to the Speedbox for recirculation. The range of exposure time was 1 to 3 days. The phosphine concentration was monitored during all the treatment by Bedfont device model 415. Six phosphine gas-sampling points were located at the top, middle and bottom of the fumigation room space, at the point of entry of phosphine gas into the room and at two places between the bags containing wheat. The temperatures of the inter-granular air and of the room space were also recorded. Adults, pupae and late larvae of rice weevil *Sitophilus oryzae* (L.), lesser grain borer *Rhyzopertha dominica* (F.), cowpea weevil *Callosobruchus maculatus* (F.) (both pupae and late larvae inside the grain), saw-toothed grain beetle *Oryzaephilus surinamensis* (L), khapra beetle *Trogoderma granarium* Everts and all stages of Indian meal moth *Plodia interpunctella* (Hübner), tropical warehouse moth *Ephestia cautella* Walker and red flour beetle *Tribolium castaneum* (Herbst) were used as test insects. The test insects were placed between the bags in three replicates. The control insects were kept outside of the fumigation room under the same temperature conditions. The mortality of external stages was counted 24 h, a week and a month after treatment. The mortality of internal stages was counted a week, 2 weeks and a month after treatment. The egg hatching was counted 3 and 7 days after treatment. The progeny of tested insects at all stages were counted 6 weeks after treatment. All tested insects were reared and maintained after treatment under laboratory conditions at 28±0.5ºC and r.h. of 65±5%.

2.2. Experiments in commercial warehouses

The fumigation was carried out in commercial warehouses in the South and in the Center of Israel. Bags of wheat grain, rice, sunflowers, beans, peanuts, pistachio and nuts, each one of 40-50 kg weight, were hermetically sealed using plastic sheets. The range of stack volume was 15 to 60 m^3. The range of outdoor temperature was 15º to 23ºC at the beginning of the experiments and 8º to 25ºC during the experiments. The temperature of the above-mentioned treated products was 6º to 17ºC at the beginning of the experiments and 6º to 19ºC during the experiments. The Degesch Plates were used. The Speedbox was connected to the stack. The number of the used plates was counted according to the target concentration of phosphine gas. The plates were put into the Speedbox and were heated to 36ºC. The produced hydrogen phosphide was blown into the stack and was pump out from the stack to the Speedbox for recirculation. The dosage of the phosphine gas was 2-4 g/m^3. During the experiment, the concentration of phosphine was monitored by Bedfont device model 415. The exposure time was 2 to 4 days. The test insects were *S. oryzae*, *R. dominica*, *O. surinamensis*, *T. castaneum*, *E. cautella*, *P. interpunctella*, *T. granarium* and *C. maculatus*. All the insects were tested at the stages of adults, late larvae and pupae (while the insects were inside the kernels for relevant species). The test insects were inserted into the stack in three places. The control insects were kept outside of the stack in the same warehouse. The mortality of external stages was counted 24 hours, a week and a month after treatment. The mortality of internal stages was counted a week, 2 weeks and a month after treatment.

3. Results

3.1. Experiments in the fumigation room

The results showed, that at a target concentration of 4 g/m^3 and exposure time of 48 hours, the effective concentration of phosphine was reached in a short period. Half hour after beginning of the fumigation 95 -115 ppm of phosphine was recorded. After 15-17 hours, the maximum concentration of the gas has reached 1460 ppm. The gas concentrations in 6 sample points in the space and in the commodity were very similar. The total mortality of adults, larvae (late) and pupae of *S. oryzae*, *R. dominica*, *O. surinamensis T. castaneum, T. granarium, C. maculatus, P. interpunctella and E. cautella* was recorded. For *T. castaneum, P. interpunctella and E. cautella* 100, 100 and 98% of egg mortality was achieved, respectively.

3.2 Experiments in the commercial warehouses

In the field experiment (the 60-m^3 stack of wheat grain bags, the target concentration of 4 g/m^3, the exposure time of 2 days and commodity temperature of 17- 20ºC) the concentration of phosphine has reached 414 and 1480 ppm after 2 and 24 hours respectively. The total mortality of adults of *S. oryzae*, *R. dominica, O. surinamensis*, *T. castaneum, C. maculatus* and *T. granarium*, as well as larvae and pupae of *P. interpunctella*, *E. cautella* and above-mentioned coleopterans was recorded (Table 1). The same

results were obtained after fumigation of the 15-m^3 stack containing various grains, at the dosage of 2 g/m^3, exposure time of 4 days and commodity temperature of 6-8ºC.

Table 1 Effect of phosphine fumigation (4g/m^3 for 48h) using Speedbox against major stored- product insects.

Insect	Stage	Mortality (%)	
		Pilot	Field
Sitophilus oryzae	adults	100	100
	pupae	100	100
	larvae late	100	100
Rhyzopertha dominica	adults	100	100
	pupae	100	100
	larvae late	100	100
Oryzaephilus surinamensis	adults	100	100
	pupae	100	100
	larvae late	100	100
Tribolium castaneum	adults	100	100
	pupae	100	100
	larvae late	100	100
	eggs	100	-
Trogoderma granarium	adults	100	100
	pupae	100	100
	larvae late	100	100
Callosobruchus maculatus	adults	100	100
	pupae	100	100
	larvae late	100	100
	eggs	100	-
Plodia interpunctella	adults	100	100
	pupae	100	100
	larvae late	100	100
	eggs	100	-
Ephestia cautella	adults	100	100
	pupae	100	100
	larvae late	100	100
	eggs	98	-

Pilot: fumigation room of 15-m^3 volume filled with wheat grain bags by 30%. Field: stack of grain bags, 60-m^3 volume. The mortality of external stages was counted 24 hours, a week and a month after treatment. The mortality of internal stages was counted a week, 2 weeks and a month after treatment. The egg hatching was counted 3 and 7 days after treatment.

4. Discussion

The current results show that the Speedbox allows significantly decreasing the period of phosphine release from the Degesch Plates. The maximum of phosphine concentration in the treated area was achieved just after 12-17 hours from the beginning of the fumigation, depend on target concentration. As was established by Jacob et al. (2006), without the Speedbox the degassing rate of the Degesch Plates after 24 hours was only 60%. Therefore, the Speedbox allows getting the effective concentration of phosphine in a shorter period and thus to decrease the exposure time. The Speedbox allows also recirculation of the gas for its even distribution in the treated stack.

All developmental stages of the tested insect, except eggs, were totally killed at a target concentration of 4 g/m^3 for 48 hours both in pilot and field fumigations. The eggs were found to be the most tolerant developmental stage of the tested insects. Our data is consistent with the results obtained by Mills et al. (2003).

It is important to underline that in some field fumigations the temperatures of the outdoor and treated products were as low as 8-16ºC and 6-8ºC, respectively. Despite the strong gas sorption by the treated commodities, especially with high lipids content, the concentration of phosphine during the treatment

was high enough and total mortality of tested insects was achieved. The current results are consist with the findings from Jacob et al. (2006) studies, that with the Speedbox, only negligible degassing differences were observed between 10ºC and 20ºC.

To conclude, the Speedbox allows:

- To optimize the phosphine fumigation of stacked bags in controlling all developmental stages of the major stored product insects.
- To decrease significantly the period of phosphine release from the Degesch Plates and the exposure time.
- To enable effective phosphine fumigation at low temperatures.
- To achieve even distribution of the gas in the treated space.
- The advantages of the Speedbox open novel possibilities for phosphine fumigation also as quarantine treatment.

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Half-life time of ozone as a function of air conditions and movement

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Abstract

Stored grain products, such as corn, can harbor multiple microorganisms, including fungi such as *Aspergillus* species that produce toxins harmful to both humans and animals. In previous studies, we have demonstrated that ozone-treatment can significantly reduce the level of viable microorganisms on the surface of corn kernels. Ozone is a strong oxidizing agent, which is used in a growing number of industrial applications to control harmful microbes and volatiles. To achieve this goal, a better understanding of the properties of ozone is needed, especially with respect to the half-life of ozone and time/concentration criteria to reduce microbes on corn. The focus of this project was to determine the half-life time of ozone in air as a function of air speed (0 to 370 m^3/h), temperature (4 to 40°C) and relative humidity (0 to 80%) inside the cylinder. Half-Life Time (HLT) averaged ~1500 minutes in still air at room temperature (24°C) and zero humidity, which was substantially longer than previously published data (i.e., 30-40 minutes). As air speed, temperature and humidity increased, HLT decreased to ~40, 800 and 450 minutes, respectively. The results suggest that ozonation will be more effective in still air at low temperature and humidity (e.g., headspace ozonation of rail cars in the early spring) than at high flow rates of ozonated air at high temperature and humidity (e.g., grain storage silo in the middle of summer).

Keywords: Ozone, Ozone concentration, Half-life time, Treatment

1. Introduction

Ozone is a highly reactive gas that makes it useful in sterilizing surfaces, as it will react with many compounds. Due to the highly reactive nature of ozone, it is necessary to better understand key parameters about the gas before further investigation into its sterilizing abilities is undertaken. Methods for generating and analyzing ozone that will be used in this research must also be evaluated. Also, chemical reactions need to be explored as the ozone used will come in contact with various surfaces and compounds. These key parameters also involve evaluating ozone half-life at different airflow rates, temperatures and relative humidities. The half-life time (HLT) of ozone is the amount of time it takes to reduce the initial concentration by half. A lower HLT of ozone could result in less effective treatments during exposure. Once the HLT of ozone under varying conditions of airflow, temperature and relative humidity (r.h.) is determined, its effects on grain can be explored further. Ozone has been used for its sterilizing abilities in many types of containers. Grain bins as well as rail cars, which transport grains, have been treated with ozone before filling in order to sterilize container surfaces. At times these containers can be exposed to different air conditions and movement. Therefore, the objective of this study was to develop an equation that will quantify the HLT of ozone as a function of air conditions and movement. This equation can then be used to predict the HLT of ozone in containers that will be treated under varying air conditions and movement.

2. Materials and methods

2.1. Experimental set-up

Experiments were set up to determine the half-life time (HLT) of ozone produced by a lab-scale generator as a function of varying air movement, temperature and relative humidity inside a plexiglass cylinder. Air movement inside the cylinder was regulated with fans. Temperature was regulated by exposing the cylinder to different external environments. Relative humidity in the cylinder was

controlled with salt solutions. Ozone was pumped into a 40L cylinder (r_i=5.5", r_o=6", h=24") until it was filled to a pre-determined O_3 concentration. Two to three replications were performed for each test. To calculate HLT, the results from the tests were analyzed using SAS (version 9.3.1).

2.2 Ozone generation

The generator used in this work was provided by O_3Co Inc. (Idaho Falls, ID, USA) and utilized the corona discharge to produce ozone. The unit contains four electrodes where the oxygen-to-ozone transformation takes place. The system was placed in a fume hood and tap water flowed from the top of the system to the bottom, along the length of the electrode tubes, to keep the system cool. Ozone concentration was varied by adjusting the input voltage and air supply. The generator produces about 2.75 g/h of ozone at 115 V with dry air as the feed gas. The input voltage was varied from about 50 V to produce 0.5 g/h to 140 V to produce 3.5 g/h.

2.3. Ozone analyzer

The ozone analyzer used to monitor the ozone concentration from the generator was an IN2000LC unit from IN USA, Incorporated (Norwood,MA, USA). According to the analyzer's manual, the unit has a measuring range from 0 to 2000 ppm, and is calibrated according to US NIST traceable standards (+/- 1%). The ozonated air mixture is pumped into the analyzer at 1.0 L/min.

2.4. Data collection and analysis

Concentration measurements were taken through a valve at the top of the cylinder using a Kitagawa® Gas Sampling Tube. Over a period of time, samples were taken intermittently and recorded with a date and time stamp. Standard test conditions were all room temperature (about 24°C), ~0% humidity, and 0 m^3/h air movement. The other tests included an increased and decreased air temperature (40°C and 4°C), increased humidity (45% and 87%) and increased air movement (102 m^3/h, 187 m^3/h, 374 m^3/h).

3. Results

The results from the tests show that the HLT of ozone is affected by changes in temperature, air speed and humidity. For example, the addition of mixing fans into the cylinder allowed for the ozone inside of the cylinder to be moved around. As the fan speed increased, the HLT decreased exponentially. Incorporation of mixing fans showed a marked reduction of HLTs.

The average difference in HLT between the test with 0 m^3/h fan speed and 100 m^3/h fan speed was 1314 minutes. Table 1 shows that as temperature increased the half-life time of ozone decreased by about 38% from 4 to 24°C and 48% for 24 to 40°C. As humidity increased from 0 to 45%, HLT decreased by ~54%; and by ~35% from 45% to 87% r.h.

Table 1 Half Life Time results for tests on temperature, air speed, and humidity variations.

Temp (°C)	Fan Speed (m^3/h)	r.h. (%)	Reps	HLT (min)
24	0	0	3	1524
24	0	45	1	705
24	0	87	2	451
4	0	0	2	2439
40	0	0	2	796
24	100	0	1	210
24	185	0	2	49
24	370	0	2	39

3.1. Half-life time equation

By combining the results from the previous tests, stepwise regression could be performed in SAS. The SAS analysis yielded the following equation:

$$Y = 2274.4 + 0.483 * x_1 - 8.49 * x_2 - 51.64 * x_3 - 12.01 * x_4 \qquad \textbf{Eqn. 1}$$

Where y is the half-life time (min), x_1 is the initial ozone concentration (ppm), x_2 is the value for air flow rate (m^3/h), x_3 is the temperature (C), and x_4 is the relative humidity (%). Figures 1, 2 and 3 compare three variables at once using Eqn. 1. Figure 1 shows the relationship of air speed and temperature for HLT at ~24°C. For dry air at 0% r.h., the sterilization limit at which HLT = 0 is predicted to be an airflow rate of 290 m^3/h (170 CFM).

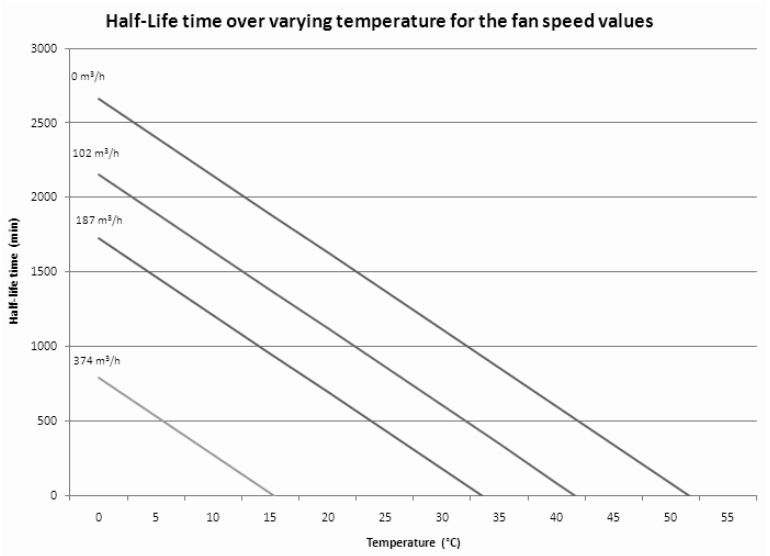

Figure 1 Comparison of HLT against temperature for four air flow values and 0% relative humidity air.

Figure 2 shows the relationship of relative humidity and temperature on HLT in still air. For the higher temperature (40°C) the HLT reaches zero at 50% r.h. This implies that a sterilization effect will unlikely be achieved in the treatment container above that level.

Interpolation was performed to find the temperature that would give an HLT of zero at 100% r.h., which was 28°C. This means that temperature lower than 28°C can sustain HLTs greater than zero at 100% r.h. An HLT of 1524 min was achieved at a temperature of 24°C and 0%r.h., and 2439 min was achieved at a temperature of 4°C. This implies that the equation does a good job of predicting the observed values.

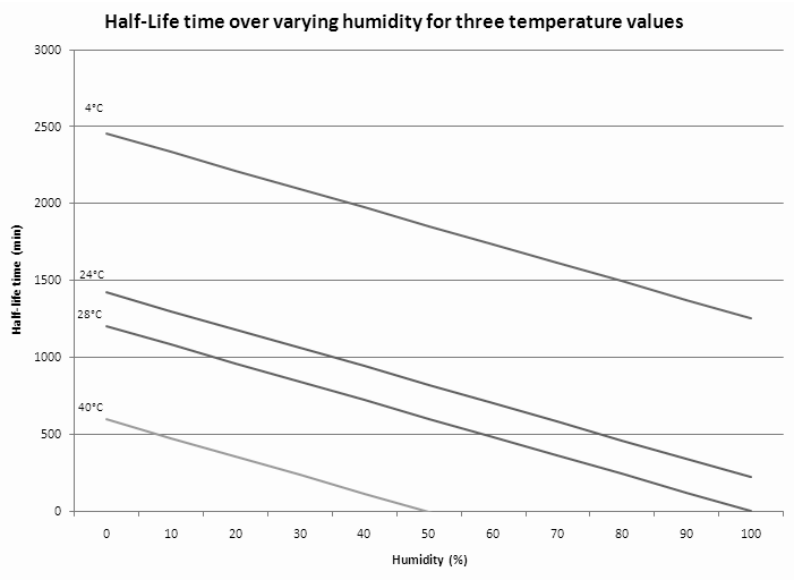

Figure 2 Comparison of HLT against humidity for four temperatures in still air.

Figure 3 shows the relationship of air speed and relative humidity for HLT at ~24°C. The equation both over and under predicts the true values. The 80% r.h. line predicts well, as the HLT for 80% r.h. is 451 minutes at 0 m^3/h.

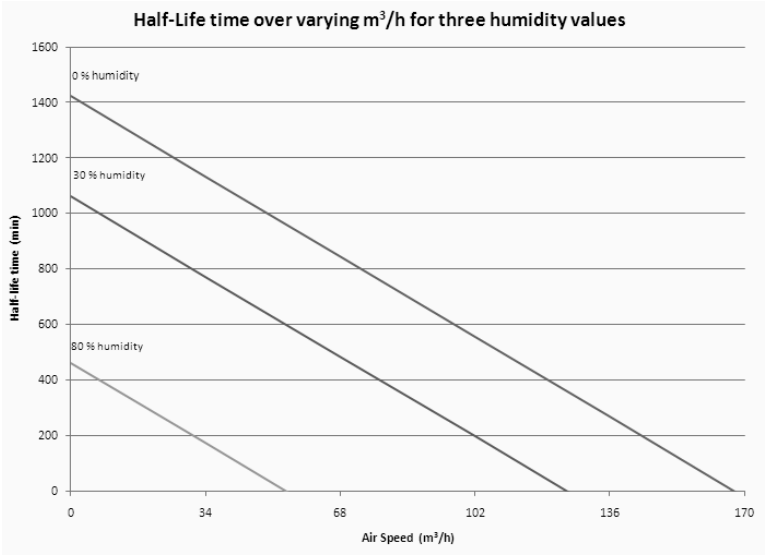

Figure 3 Comparison of HLT against air speed for three relative humidity value at ~24°C temperature.

4. Discussion

Ozone follows the common gas law. Therefore, as the air speed inside the cylinder increased the HLT of ozone decreased. The gas molecules inside the cylinder interact more rapidly when the air speed increases. This interaction of ozone molecules bouncing into other ozone molecules results in a breakdown of ozone to oxygen and, thus, a reduction in ozone concentration. Treating the plenum of grain bins before or after filling with grain has shown to sterilize the metal surfaces and kill mice when exposed to high enough ozone concentrations for sufficient time given the HLT in still air. Therefore, filling any container or chamber with ozone to a sufficiently high concentration and avoiding subsequent air agitation (mixing) for extended time periods has great potential as an alternative, non-chemical treatment. Strait (1998) showed that temperature affects ozone concentration. Gas half-life values ideally follow this linear trend concerning varying temperature. At lower temperatures gas molecules do not move quickly and therefore collide less frequently. At higher temperatures ozone molecules move faster and collide more frequently. The effect of relative humidity on ozone HLT was also tested. From Table 1 it can be noted that as humidity increased ozone HLT decreased. These results agree with Strait (1998) which stated that as humidity increases the HLT of ozone will decrease.

Applying these results to the headspace of a rail car and the plenum of a grain bin suggests that treatment with ozonated air would be more efficacious in drier western U.S. locations than more humid Eastern U.S. locations. Combined with the temperature results, these findings suggest that the efficacy of ozone treatment will be affected by the initial temperature and relative humidity of the air in the storage container or transport vehicle. Therefore, the initial ozone concentration will need to be adjusted upward under warmer and more humid weather conditions to achieve the same results as under cooler and drier weather conditions.

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Ozone technology in the post-harvest storage environment- a comparison of efficacy of high doses of ozone to insects treated under laboratory conditions and field conditions

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Abstract

Efficacy experiments were conducted to determine the ozone concentration (C) and treatment time (T) needed to effectively kill adult stages of red flour beetle (*Tribolium castaneum*) and maize weevil (*Sitophilus zeamais*). Under laboratory conditions, insects were exposed to concentrations of ozone ranging from 50 to 1800 ppm for 30 and 60 min. After treatment, insect mortality was scored, and if 100% mortality was not reached, more treatment-time was added in 30 min intervals at 1800 ppm. For both species, 100% mortality was reached after treatment at 1800 ppm for 120 min, which equates to a concentration*time (CT) product of 216,000 ppm.min. A similar CT product was attained under field conditions with a prototype auger designed to treat moving streams of grain with ozone during transfer. In field tests, 100% mortality was achieved for both insect species after a treatment of 47,000 ppm ozone for 6 min (CT = 282,000 ppm.min). The results indicate that CT values obtained in laboratory experiments correlated well with those from field experiments. Based on these results, we now can calculate ozone concentration and auger length needed to treat grain in a fast-moving stream.

Keywords: Ozone, Maize weevil, *Sitophilus zeamais*, Red flour beetle, *Tribolium castaneum*

1. Introduction

There is a need for an alternative treatment strategy for insect control in stored grains due to issues such as: loss of registered control strategies, interest in biologically safe alternatives to chemical control methods, lack of control options for organic producers, and even resistance to some traditional fumigants such as phosphine (Zettler et al. 1989; Zettler and Cuperusi 1990; Zettler 1991). Ozone technology is one alternative for treating stored grains to manage insect pests. Ozone is an excellent alternative to currently available products, especially for on-farm use and grain already in bulk storage. It is a strong oxidizer and very unstable, breaking down into atmospheric oxygen very quickly. There is no need to store or dispose of potentially hazardous chemicals. Ozone has the ability to sanitize, disinfect, and is "Generally Recognized As Safe" (GRAS) for food processing in the United States (Sopher et al., 2002). Ozone can be used to deodorize kill microbes, and is effective against insects (Kells et al., 2001). Grains can be treated with ozone for extended periods of time without affecting its quality. Mendez et al. (2003) treated grain for 30 d at 50 ppm and found no affect on grain quality.

Insects are a major problem in stored-grain ecosystems. The weevils, and in particular, maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), are internal feeders and are very destructive to grain. Adults bore holes into grain where eggs are laid and the entire life cycle takes place (Cotton, 1963). The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), an external feeder that feeds on cracked kernels and dust (Mason and Obermeyer, 2006). Both species have a worldwide distribution and a fast reproductive ability. The objective of this study was to compare the efficacy of higher dosages of ozone on *S. zeamais* and *T. castaneum* treated in the laboratory and in the field to determine the concentration-time parameters necessary in constructing a commercial grain-auger ozone-treatment system.

2. Materials and methods

2.1. Experimental setup-laboratory

The laboratory ozone generator creates ozone using a corona discharge (O3Co Aberdeen, ID). Concentrations of ozone were measured using an ozone analyzer (INUSA, L2-LC Model 040977) located at the terminal end of the flow through system, consisting of three identical sealed plastic sandwich containers (101.6 mm x 101.6 mm x 25.4 mm) connected in series via 12.7 mm x 2.38 mm (diameter) Tygon® tubing.

2.2. Experimental setup-field

A modified screw conveyer, made of stainless steel, (Lynntech, Inc., College Station, TX), was used for the continuous ozonation treatments of the grain. The conveyor had a length of 6.3 m and a diameter of 0.102 m. A hopper (22.7 kg capacity) was placed on one side to load the grain into the system. A manifold was also attached to the system to introduce ozone at different points of the conveyor. Ozone was produced by corona discharge using an Ozoblast system (O_3Co, Aberdeen, ID. USA).

2.3. Insects/Treatments

2.3.1. Laboratory experiment.

Adult red flour beetle (*T. castaneum*) and adult maize weevil (*S. zeamais*) were taken from colonies maintained in environmental chambers (Percival Scientific Inc., Perry, IA, USA) (30°C and 60% r.h.) at Purdue University, West Lafayette, IN, USA. Insects were placed into Petri dishes (10/dish) and treated with ozone. Insects were exposed to concentrations of ozone ranging from 50 to 1800 ppm for 30 and 60 min. After treatment, insect mortality was scored, and if 100% mortality was not achieved, more replications were included by adding treatment-time in 30 min intervals at 1800 ppm. Each concentration/time combination (CT) was repeated three times with a total of 30 insects per CT combination. After treatment, insects were held at colony maintenance conditions for 24 h and then scored as dead or alive. These data were compared to controls which were untreated and held in dishes for 24 h at colony maintenance conditions.

2.3.2. Field experiment

Bioassay bags were constructed with fine nylon mesh (104 x 104 openings/in^2; 94 µm thread diameter; 150 µm opening size) by folding a 3.81 cm x 4.45 cm piece in half and hot gluing the sides together, leaving the top open for insertion of insects, after which the top was sealed. The size of the completed bags was 3.81 cm x 2.22 cm. Different sized bags were tested before the final size was chosen. The criteria used to decide which bag was chosen were a low crush rate in the auger and the bag had to make it all the way through the auger intact. Insects (10 per bag, randomly chosen from colony jars) were placed in bioassay bags 24 h prior to the experiment start. On the day of the experiment the bioassay bags were placed in buckets of maize (each bucket held 15.876 kg maize; 5 bioassay bags per bucket). These buckets were then subsequently dumped into a hopper which opened into the auger. The maize and bioassay bags moved through the auger and then were deposited down a PVC pipe into another bucket. Random bioassay bags were chosen after each run through the auger and insects within the bags were examined for insect alertness. One run constituted one time through the auger, which equaled two minutes residence (treatment) time with ozone. Treatments included one, two and three-runs through the auger. Between each "run", the ozone concentration was allowed to build back up in the auger (~15 m). Each 'run' was replicated three times. The concentration of ozone for each treatment was 47,820 ppm. Controls were run the same way through the conveyor, but without ozone flowing.

2.4. Analysis of data

To determine differences between control and corresponding treatments, a two-tailed t-test was run. After differences were found, treatment mortality was normalized to account for control mortality using the correction for Abbott's formula determined by Rosenheim and Hoy (1989). Data for comparison of the treatments was analyzed for differences ($\alpha = 0.05$) using analysis of variance (ANOVA). A Fisher's least significant difference (LSD) test using general linear model statistics was also performed (PROC GLM) (SAS Institute, 2001).

3. Results and discussion

In the laboratory, for both *S. zeamais* and *T. castaneum*, controls exhibited no mortality. A t-test revealed that controls and corresponding treatments were significantly different ($P < 0.05$). For both species, 100% mortality was only reached after an ozone treatment of 1800 ppm for 120 min. This equates to a concentration*time (CT) product of 216,000 ppm.min. The 90 min treatment for both insect species was not significantly different than the 120 min treatment. Although short treatment times are desirable for moving grain, the dosage rate of 1800 ppm (currently the highest achievable dose in our laboratory) does not achieve the desired mortality rates. Thus, if 100% control is desired, then the 120 min treatment time must be considered and/or a target CT of 216,000 must be achieved. Similar CTs have been reported by Kells et al. (2001) who achieved 100% mortality of *T. castaneum* adults with a treatment of 3 d at 50 ppm (CT = 216,000 ppm.min).

A CT product similar to the laboratory was attained under field conditions. For both *S. zeamais* and *T. castaneum*, 100% mortality was achieved after a treatment of 47,820 ppm ozone for 6 min (three runs)(CT = 286,920 ppm.min). The four-minute treatment (two-runs) at 47,820 ppm was not a long enough treatment to achieve the desired 100% mortality (CT = 191,280 ppm.min). For undetermined reasons, the two-run treatment mortality of red flour beetles was lower than the one-and three-run treatment. This was not the case for *S. zeamais* where there was an increase in mortality as treatment time increased.

A residence time of 6 min at 47,820 ppm in the treatment auger, or a CT of 286,920 ppm.min, must be applied for both species to achieve 100% mortality. All other treatment combinations tested did not achieve 100% mortality. Based on the CT products, if a treatment time of 5 min could have been tested, to achieve 100% mortality CT of 239,100 ppm.min would be necessary. This was not feasible based on the design of the auger we were testing, but when designing future augers this could certainly be accommodated.

Laboratory determined CT products necessary for 100% mortality (216,000 ppm.min) were lower, but close to the CT products achieved in the field experiments (286,920 ppm.min). Based on these results, ozone concentration and auger length needed to treat grain in a fast moving stream can now be determined and used to engineer an effective commercial grain-auger ozone-treatment system.

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How is sulfuryl fluoride performing as a methyl bromide alterative?

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Abstract

This presentation will address the following areas for sulfuryl fluoride usage in post harvest disinfestations in the United States. Included is background experience, market growth, objective benefit, successful adjustments, determining effective dosage rate, and advancements.

We have worked with sulfuryl fluoride for 12 years. Starting with the first commercial post harvest fumigation in the US this activity has moved to treating grain, seed, mills and food processing and storages. Using a wide range of dosage rates and closely examining pre fumigation conditions, fumigation data, and post fumigation results successful treatments have been the result. This presentation will provide some of our findings during this period of discovery.

Sulfuryl fluoride was first commercially approved for post harvest use in the United Sates in May 2004. Since this release SF has been met with some resistance in certain markets and openly accepted in others. We will graphically show this annual progression within usage patterns.

For 5 years SF has defended its efficacy while largely ignoring strengths. The penetration capability of SF over that of methyl bromide has greatly improved balance of performance and cost. SF is not more difficult than the same methyl bromide application. The difference with SF is applicator awareness, observation, and recognition to detail is required to provide effectiveness and cost reduction.

Understanding strengths and weaknesses of SF developed efficacious and cost responsible dosage choices. Discussed will be methods to pinpoint a comparable SF dosage rate from field proven performance over 5 years.

Our industry has put SF under a microscope that never existed with methyl bromide. Benefits of this attention have provided unexpected results in forms of greatly improved gas monitoring technology and gas application instruments and equipment. This paper will provide dialog for how these advancements have improved safety, effectiveness, and a continued path for improvement.

A novel approach to the protection of cocoa beans by preventing free fatty acid formation under hermetic storage

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Abstract

Hermetic storage has provided a successful storage method for the protection of dry cocoa beans by replacing fumigants for insect control and for quality preservation. Hermetic storage is achieved in specially constructed flexible plastic structures and is based on the principle of generation of an oxygen-depleted, carbon dioxide-enriched interstitial atmosphere caused by the respiration of the living organisms in the ecological system of a sealed storage. An increase in free fatty acids (FFA) content in dry cocoa beans is a significant factor that determines its quality preservation. After fermentation of the beans, moisture content (m.c.) is usually high that poses a risk for the rise of FFA in the beans. Tests were carried out to study the effects of hermetic storage of dry cocoa beans under aerobic and hermetically sealed conditions on the development of FFA's in the beans at 7.0%, 7.5%, and 8.0% m.c. for periods of 90 and 160 d at 30°C. The beans under hermetic conditions responded by creating progressive depleted oxygen conditions that were accompanied by the increased carbon dioxide due to the respiration of the beans. The lowest oxygen concentration took place at 7.0% m.c. after 35 d, at 7.5% m.c. after 29 d, at 8.0% m.c. after 26 d of storage and thereafter, no significant increase in oxygen concentration was observed. The FFA content of cocoa beans at 7.0%, 7.5%, and 8.0% m.c. under hermetic conditions of 30°C remained below or close to 1.0% after 90 and 160 d of storage. This was more comparable to the results obtained when the beans were stored at 4°C rather than the controls. In comparison, the aerated control stored at 30°C showed marked increase in FFA levels of up to 1.48%.

Keywords: Hermetic storage, Modified Atmospheres, Cocoa beans, Quality preservation, Storage insect control, Flexible storage structures.

1. Introduction

The practice of producing cocoa beans is through a process of fermentation, which encourages yeast fermentation due to the partial anaerobic conditions (Schwan and Wheals, 2004). This process is necessary to moderate its initially bitter flavour and to develop the typical flavour of cocoa. After fermentation, infestation of cocoa beans starts from the drying mats and continues in storage. Climatic conditions in the tropics are characterized by high humidity levels of 70 to 90% r.h. and temperatures around 30°C which are ideal for storage insects and moulds to develop on cocoa beans.

Surface contamination is a major source of fungi in fermented and dried cocoa beans. According to Pitt and Hocking (1997), species of *Aspergillus* are the predominant spoilage fungi in tropical areas and *Penicillium* spp. occur in more temperate zones. Properties of fungal lipases and mycotoxin-producing abilities of fungi isolated from raw cocoa beans showed good evidence to support their potential toxicogenic abilities and the free fatty acids (FFA) content (Guehi et al., 2007). The cocoa butter is defined as the fat obtained from the cocoa beans which should not contain more then 1.75% FFA (expressed as oleic acid) (FAO/WHO, 1999).

Storage beetles are attracted to cocoa beans and cause damage by boring holes in the beans or feeding on the nib (Jonfia-Essien et al., 2007). A couple of methods are applied today to achieve a low FFA content of the cocoa beans: storing the beans at low temperature (4°C) or drying the beans. Hermetic storage has provided a successful storage method for the protection of dry cocoa beans by replacing fumigants for insect control and for quality preservation (Navarro et al., 2007). Hermetic storage is achieved in specially constructed flexible plastic structures and is based on the principle of generation of an oxygen-

depleted, carbon dioxide-enriched interstitial atmosphere caused by the respiration of the living organisms in the sealed storage.

The present paper is intended to study the effects of storage of cocoa beans with 7.0 to 8.0% m.c. under aerobic and hermetically sealed conditions on the development of FFA's in the beans.

2. Materials and methods

2.1 Moisture content

Cocoa beans imported from Ghana were used and their m.c. levels were checked at the outset of the storage and following 90 and 160 d of storage by electronic sensors that measures water activity (Rotronic, Instrument Ltd., Crawley, UK) converted to m.c. (wet basis). The tested cocoa beans had an initial equilibrium relative humidity (ERH) of 69% equivalent to a moisture content (m.c.) of 7.2%. To raise the moisture content, the total quantity of water needed to attain given m.c. was calculated and applied in successive small aliquots on the beans spread out in a single layer on a polyethylene liner. Time was allowed for the added water to be absorbed, and this procedure was repeated until the calculated amount of water had been added and absorbed. After moisture adjustment these samples were allowed to equilibrate at least 4 wks at 4°±1°C.

2.2. Storage conditions

The cocoa beans were tested at approximately 7.0%, 7.5% and 8.0% m.c. At each moisture content., about 0.56 kg of cocoa beans was placed in 0.9-L mason jars. Aerobic and hermetic storage at three moisture contents were replicated four times. Cocoa beans were stored at a controlled temperature of 30±1°C for 90 and 160 d. In addition to controls kept at 30°C, another group of jars were kept at 4°C in two replicates.

2.3. Testing methods

2.3.1. Respiration rate of cocoa beans

The respiration rate was determined based on the oxygen consumption and the carbon dioxide evolved from the cocoa beans using the oxygen monitor (Emproco Ltd., HGA11-PB, Israel) equipped with inlet and outlet gas ports that enabled gas circulation by a closed loop gas flow system using flexible tubes connected with the two copper tubes soldered to the jar lid. The top end of each copper tube was equipped with T-type two-way valves from outside the jars situated between each flexible and copper tube. Similar to oxygen concentration the carbon dioxide evolved from the beans was measured using a Gow-Mac carbon dioxide analyzer model 20-600 using a thermal conductivity detector.

2.3.2. FFA content

At the outset of storage as well as following 90 and 160 d of storage the FFA content of the cocoa beans was tested according to the method of the International Office of Cocoa, Chocolates and Sugar Confection (IOCCC 1996). In this method the percentage FFA was calculated as percentage of oleic acid.

3. Results

Figures 1, 2 and 3 show the average gas concentrations of the four replicates that reflect the changes in oxygen and carbon dioxide concentration of 7%, 7.5% and 8% m.c. of the cocoa beans stored under hermetic conditions for 160 d at 30°C, respectively. The progressive depletion in oxygen concentrations were accompanied by the increased carbon dioxide that evolved from the living organisms prevailing on the tested beans. The lowest oxygen concentration (<0.5%) was recorded at 7.0% m.c. after 35 d, at 7.5% m.c. after 29 d and at 8.0% m.c. after 26 d of storage and thereafter no significant increase in oxygen concentration was observed. The highest carbon dioxide concentration at 7.0%, 7.5% and 8% m.c. was 20.2, 25.3 and 25.8%, respectively.

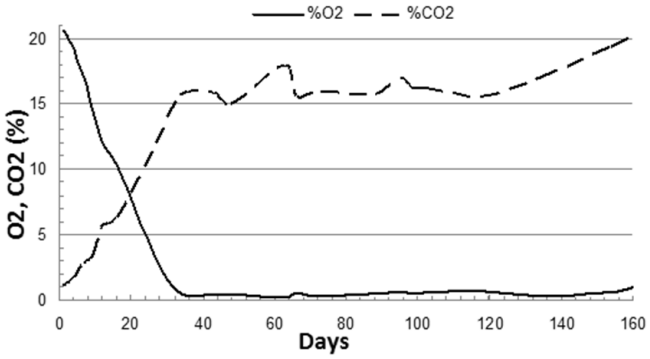

Figure 1 Changes in oxygen and carbon dioxide concentration of 7.0% moisture content cocoa beans stored under hermetic conditions for 160 d at 30°C. Results are averages of four replicates.

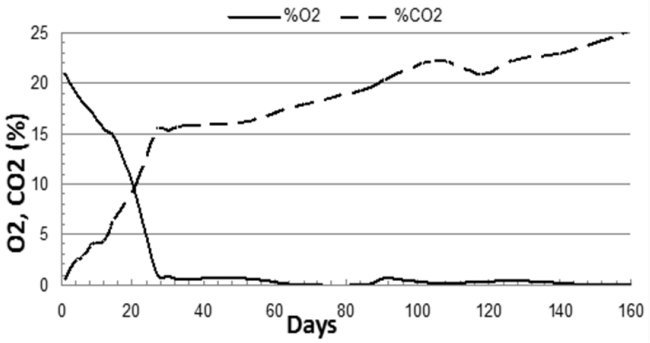

Figure 2 Changes in oxygen and carbon dioxide concentration of 7.5% moisture content cocoa beans stored under hermetic conditions for 160 d at 30°C. Results are averages of four replicates.

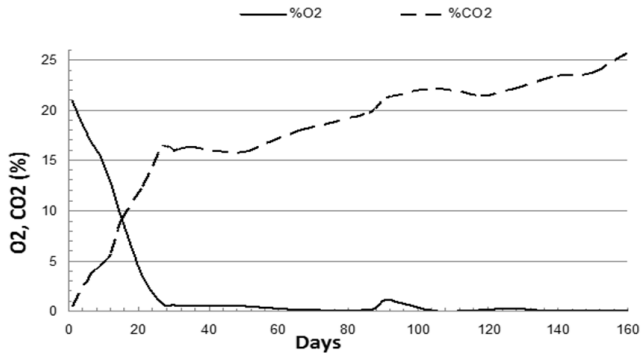

Figure 3 Changes in oxygen and carbon dioxide concentration of 8.0% moisture content cocoa beans stored under hermetic conditions for 160 d at 30°C. Results are averages of four replicates.

Figures 4, 5 and 6 show the average gas concentrations of the four replicates that reflect the changes in oxygen and carbon dioxide concentration of 7%, 7.5% and 8% m.c. of the cocoa beans stored under aerated conditions for 160 d at 30°C, respectively. There were slight changes in the oxygen and carbon dioxide concentration at 7.0%, 7.5% and 8.0% m.c. of the cocoa beans stored under aerated conditions for 160 d at 30°C. They maintained approximately the level of 20% oxygen as at start and with the highest percent of 2.8, 5.0 and 4.9 carbon dioxide respectively (Figures 4, 5 and 6).

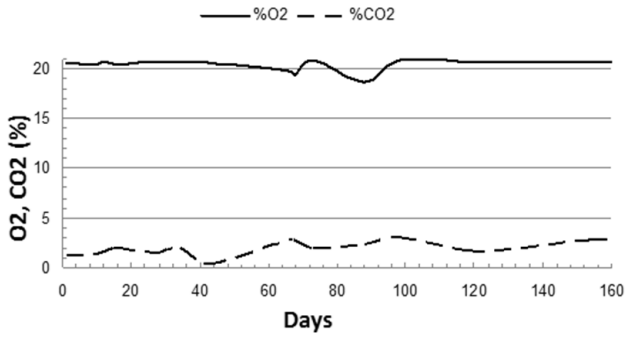

Figure 4 Changes in oxygen and carbon dioxide concentration of 7.0% moisture content cocoa beans stored under aereated conditions for 160 d at 30°C. Results are averages of four replicates.

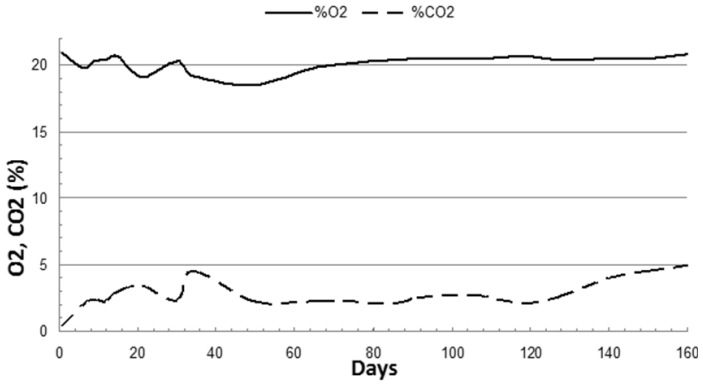

Figure 5 Changes in oxygen and carbon dioxide concentration of 7.5% moisture content cocoa beans stored under aerated conditions for 160 d at 30°C. Results are averages of four replicates.

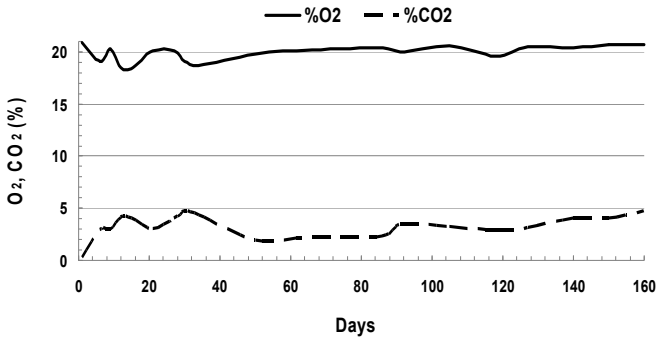

Figure 6 Changes in oxygen and carbon dioxide concentration of 8.0% moisture content cocoa beans stored under aerated conditions for 160 d at 30°C. Results are averages of four replicates.

Table 1 shows the FFA (% oleic acid) of cocoa beans at 7.0%, 7.5% and 8.0% m.c. stored under hermetic condition at 30ºC, in aerated conditions of 30°C and 4°C at 0 d, 90 d and 160 d of storage. Results were reported as average of four replicates and the calculated standard deviation for each set. Results in Table 1 clearly indicate that FFA levels under hermetic conditions of the 7.0% m.c. after 90 d storage are more comparable to the results obtained when the beans were at 4°C rather than the controls for which there was a significant and marked increase of FFA levels from 0.70% up to average of 0.95%.

Table 1 FFA (%) (± SD) level of 7.0, 7.5 and 8.0% moisture content cocoa beans stored under both hermetic and aerated conditions at 30°C and at 4°C at the start of storage, after 90 d and after 160 d of storage.

		After 90 d storage			After 160 d storage		
M.C. (%)	**Initial FFA (%)**	**Hermetic 30°C**	**Control 30°C**	**Control 4°C**	**Hermetic 30°C**	**Control 30°C**	**Control 4°C**
7.00	0.70±0.100	0.74±0.118	0.95±0.232	0.70±0.000	0.91±0.062	1.03±0.126	0.54±0.000
7.50	0.69±0.070	0.85±0.070	1.13±0.287	0.69±0.120	0.98±0.085	0.94±0.043	0.93±0.106
8.00	0.70±0.000	0.83±0.047	1.13±0.386	0.72±0.113	1.09±0.160	1.48±0.222	0.83±0.127

After 160 d of storage FFA results did not differ significantly compared to the control, but at 8.0% m.c. there was a marked increase of the FFA content in the control (1.48% FFA). Because of the moderate increase in FFA, and additional test for infestation and broken kernels revealed that the beans were sound and of good quality with percentage of broken kernels less than 1%.

4. Discussion

Among the potentially mycotoxigenic filamentous fungi on bean samples found by Rahmadi et al. (2008) the main species were *Aspergillus flavus* Link (Trichocomaceae), *A. niger* Tieghem, *A. wentii* Wehmer, *A. clavatus* Desmazières, *Penicillium citrinum* Thom (Trichocomaceae), and *P. spinulosum* Thom. *Aspergillus* spp. require higher temperature but lower water activity compared with *Penicillium* spp., and it grows more rapidly as well (Hocking, 2006). Although *Penicillium* spp. produce more chemical resistant spores (Hocking, 2006; Pitt, 2006), Guehi et al. (2007) found that FFA accumulation in raw cocoa beans could be attributed mainly to the presence and the action of *Rhizopus oryzae* Went & Prins. Geerl. (Mucoraceae) and *Absidia corymbifera* (Cohn) Sacc. & Trotter (Mucoraceae).

During storage, beetles and moths cause damage to dry cocoa beans by boring into the beans. The beans then become naked from their natural defence mechanism attracting moths' larva (Jonfia-Essien, 2004) and allowing fungi to penetrate the beans. Increased levels of broken beans and fragments in a consignment can significantly increase the average FFA content of the fat extracted. The greater the initial FFA content of the raw cocoa beans, or the lower the quality of the beans, the greater is the increase in FFA. Moulds could produce lipase (Wood and Lass, 1985) which in contact with cocoa butter of broken cocoa nibs releases FFA from triglycerides. Guehi et al. (2008) showed that on artificially broken cocoa beans FFA content increased substantially regardless of cocoa beans initial quality. Their low FFA content in whole healthy beans increased from 0.48 to 0.78% and did not exhibit an appreciable change during over 12 wks' storage. The present work was carried out using high quality cocoa beans imported from Ghana that contained very low fragmented or broken beans percent (<1%) and consequently the initial FFA was at 0.7%. According to cocoa bean grading and marketing rules (India Government, 1997), the percent of broken beans should not exceed 3% for grade A quality.

In previous work using biogenerated atmospheres of stored cocoa commodity for quality preservation and insect control, where the respiration of cocoa beans depleted oxygen concentration to <1% and increased carbon dioxide concentration up to 23%, no insects survived and the quality of the beans was preserved (Navarro et al., 2007, Jonfia-Essien et al., 2008 a, b).

In conclusion, storing cocoa beans in hermetically sealed structures inhibits activity of insect pests and mould development; as a consequence the FFA deriving from microflora development was inhibited. Most possible that the toxicogenic mycotoxins are also inhibited, though this requires further research for extended period of time in hermetically sealed structures with a higher percent of fragmented beans or a low quality cocoa beans

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Developing strategies to manage highly phosphine resistant populations of flat grain beetles in large bulk storages in Australia

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Abstract

Development of high level resistance to phosphine fumigant in flat grain beetles (*Cryptolestes ferrugineus*) in large bulk storages in Australia poses a serious threat to the biosecurity of Australian grain. The level of resistance in this species is the highest ever detected in any stored grain insect pest in Australia with a resistance factor of 875. Laboratory studies showed that at 0.5 mg/L and at 1 mg/L of phosphine 30 and 24 days are required, respectively, to attain population extinction. These doses are currently being tested in field trials for their validation. Moreover, we have developed an action plan in collaboration with project scientists and the major Australian bulk handling companies aimed at eradicating infestations of phosphine resistant flat grain beetles and preventing their spread. The key components of this plan include the use of grain protectants and sulfuryl fluoride to eliminate phosphine-resistant populations, adoption of an intensive hygiene program and monitoring of insect populations through inspection, sampling and resistance testing.

Keywords: Flat grain beetle, *Cryptolestes ferrugineus*, Phosphine, Resistance, Fumigation protocols

1. Introduction

Both domestic and international markets demand insect-free grain. Due to a range of advantages such as its universal acceptance as a residue-free treatment, cheap price, and versatility in application, phosphine is the fumigant of choice used by the Australian grain industry to maintain its grain free of insects and to mainatin market access. This situation is unlikely to change in the foreseeable future due to the lack of suitable alternatives to phosphine.

However, a major threat to the sustainability of phosphine, is the development of resistance in key stored grain pests. Australia has already witnessed the development of strong resistance in the lesser grain borer, Rh*yzopertha dominica* (F.), (Collins et al., 2005) and the psocid, *Liposcelis bostrychophila* Badonnel (Nayak and Collins, 2008). However, research has characterised these resistances and appropriate fumigation protocols have been developed to successfully manage them (Collins et al., 2005, Nayak and Collins, 2008). Among the range of storage pests that occur in both farms and bulk storage environments in Australia, flat grain beetle (FGB), *Cryptolestes ferrugineus* (Stephens), has always been considered to be a minor pest compared with the lesser grain borer, the rice weevil and the rust red flour beetle (Emery and Nayak, 2007). In 2007, however, this changed with the detection of a much higher level of resistance to phosphine in the FGB than that reported earlier for the lesser grain borer and psocids. Since then, there has been a steady increase in the incidence of strongly resistant populations in the bulk storage environment, and this poses a serious threat to the biosecurity of Australian grain.

Currently, as there is no practical alternative to phosphine, failure to control FGB with this fumigant will jeopadise market access for Australian grain. Our previous experience with the lesser grain borer and psocids has proven that manipulation of concentration and exposure periods can be utilised to manage strong resistant populations. Taking the same approach, we aim to develop appropriate phosphine fumigation doses to eradicate strongly resistant FGB populations.

2. Materials and methods

2.1. Characterisation of resistance in FGB

A population sample of strongly resistant FGB, which was originally collected from Edgeroi, New South Wales, was subjected to mass-selection in the laboratory to establish a purified strongly resistant strain. This process involved exposure of 1000 adult FGB to phosphine at 1 mg/L (720 ppm) for 7 d (168 hours) for six generations to maximise homozygosity of resistance genes. Adult insects were also exposed to phosphine at fixed periods of 48, 72 and 144 h to establish base-line response. Probit analysis was used to estimate the concentrations required to achieve different mortality levels including the LC 50 and LC 99.9 (Genstat, 2008). Mortality levels were compared with that of a laboratory susceptible strain for estimation of resistance factor. Once the purified strain was established, 50 adults were taken and mass cultured in 500 mL glass jars on a diet of 100 g of rolled oats and 5 g yeast powder at constant regimes of 30°C and 60% r.h. Several of these culture jars were organised to generate mixed-age populations (containing all life stages living in the culture medium) over a period of 6-8 wk for the following experiments.

2.2. Development of fumigation protocols

At the time of the initiation of the research, the industry prioritised 20°C as the temperature for the development of fumigation doses as this was regarded as the 'worst case' i. e., lowest temperature that infestation were likely to be detected. This was in view of anecdotal evidence that in bulk storages the FGB populations show a preference for cool grain. Fumigation doses were developed at two phosphine concentrations, 0.5 mg/L (360 ppm) and 1 mg/L (720 ppm) against the purified strongly resistant FGB at 20ºC and 60% r.h.

The experimental set-up was essentially similar to that previously described by Collins et al. (2005) for lesser grain borer. Batches of culture media containing mixed-age of strongly resistant FGB were exposed to constant phosphine concentrations in a continuous flow application of phosphine mixed with air aimed at establishing time to achieve population extinction. Briefly, the continuous flow apparatus which mimics a series of small air-tight silos consisted of six cylindrical stainless steel fumigation chambers that were connected in parallel via stainless steel tubing to cylinders of phosphine and compressed air (BOC, Brisbane Australia). Six cages with the mixed-age cultures (100 g) were inserted into the six fumigation chambers. These cages were sealed at both ends with stainless steel mesh to facilitate gas flow, and to prevent the escape of insects from the mixed-age culture. Controls were organised in the similar fashion, except that they were not fumigated with phosphine.

Fumigation was carried out with the coordination of mass flow controllers and a series of flow monitors, which force the flow of gas in one direction from the cylinders through the tubing and fumigation chambers and vents it to the atmosphere through a fume cupboard. During the fumigation, relative humidity was maintained at around 60% by passing the phosphine-air mixture through chilled water at 19°C inside a camping fridge. Phosphine concentration was monitored on a daily basis to ensure that the required concentration was maintained. Gas samples were taken from both ends of each fumigation chamber and measured using a pulsed-frame photometric detector mounted in a gas chromatograph. Test cages were removed from the fumigation chambers after pre-determined exposure periods and insects were sieved and the numbers of live and dead individuals recorded. After this, the counted insects were discarded and rest of the test material was kept at 30°C and 60% r.h. for 8 wk for an assessment of emergence of live insects. If there were no adults recovered, the grain was stored for a further 8 weeks for a final assessment of live adults. Time to population extinction was defined as the earliest exposure period (in whole days) from which there was no emergence of live insects. For both concentrations (0.5 and 1 mg/L), the experiments were replicated once.

2.3. Field trial

A field trial was undertaken in December 2008 to validate the first dose. A fumigation of 30 d at 0.5 mg/L (360 ppm) of phosphine was undertaken in a 3450 m^3 vertical silo at Clifton, Queensland containing 2750 tonnes of sorghum. Mixed-age populations of the strongly resistant FGB were placed in four test cages, two of which were buried a metre below the surface of the grain and other two in the bottom of the silo. There was provision for monitoring phosphine concentration at two points at the bottom of the silo and one point at the top. Three insect cages representing controls were buried inside sorghum in a bag kept inside an office near the silo. Data loggers (I-buttons, Maxim Integrated Products, Inc., CA, USA) were kept inside each of the test and control cages for hourly monitoring of temperature and humidity. At the end of the fumigation, the test and control cages were brought back to the laboratory where the numbers of live and dead adults were recorded immediately and again after the media had been incubated for 8 wk at 30°C and 60% r.h.

3. Results

3.1. Characterisation of resistance

During the purification process, a series of results were obtained on the response of FGB at fixed exposure periods to phosphine. To calculate resistance factor in the resistant FGB, we used the 50% mortality level (LC_{50}), as recommended in the FAO method (Anonymous, 1975). The LC_{50} for the susceptible strain exposed for 72 h was 0.007 mg/L (95% fiducial limits: (0.0052 -0.00863) compared with 6.12 mg/L (5.24 – 7.24) for the resistant strain (Table 1). Based on this comparison the resistance factor in the resistant FGB was approximately x 875.

Table 1 Probit analysis of results of exposure of flat grain beetle adults to phosphine for 72 h at 20ºC and 60% r.h..

Strain	Status	LC_{50} (mg/L) (95% fiducial limits)	$LC_{99.9\,9}$ (mg/L) (95% fiducial limits)
QCF31	Susceptible	0.007 (0.0052 -0.00863)	0.012 (0.00905-0.09124)
QNCR73	Resistant	6.12 (5.24 – 7.24)	26.24 (18.09-50.81)

3.2. Fumigation protocols

Both fumigation doses were developed at 20°C. At 0.5 mg/L (360 ppm), time to population extinction of the strongly resistant FGB required a 30 d fumigation at this concentration to achieve population extinction. At 1 mg/L (720 ppm) 24 d was required fumigation for population extinction of the strongly resistant FGB.

3.3. Validation of the first fumigation protocol through field trial

The first dose of 30 d at 0.5 mg/L (360 ppm) was trialled in a 3450 m^3 vertical silo at Clifton, Queensland. On the first day of fumigation, 6296 g of phosphine was applied from cylinders and a gas reading of >1000 ppm was recorded after 4 d (Fig. 1).

The concentration declined to 200 ppm on 23rd day of the fumigation. To compensate this loss of gas, 2528 g of phosphine was added on the 26th day, which resulted in a rapid increase in gas concentration inside the silo.

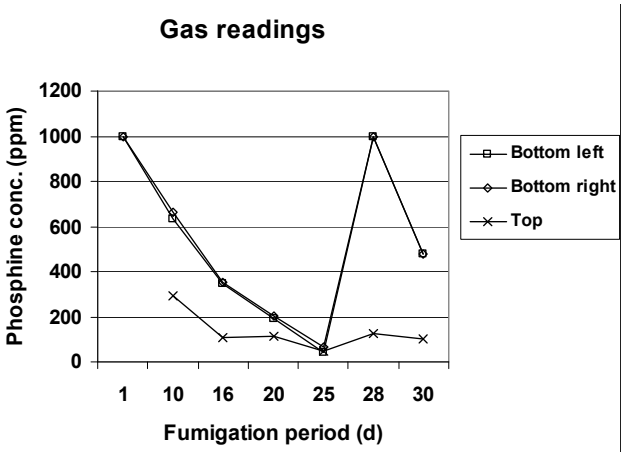

Figure 1 Phosphine concentrations in the silo under fumigation at Clifton for validation of the fumigation protocol of 360 ppm for 30 days.

A final concentration of 480 ppm was recorded at the time of ventilation after the day 30 of the fumigation (Fig. 1). Laboratory assessment of insect cages revealed no live FGB in the test cages compared with an average of 250 adult FGB in the control cages. Similarly, the assessment for progeny after 8 wk revealed no live progeny in the test cages compared with an average of 50 adult progeny in the controls. Data from the temperature-humidity loggers indicated that the temperature at the top of the silo ranged from 23.7°C to 43.2°C, with an average of 42.1°C. The temperature at the bottom of the silo varied between 23.2 to 26.2°C, with an average of 24.2°C. Temperatures inside the control cages ranged from 19.1 to 33.6°C with an average of 24.5°C.

4. Discussion

The main aim of the current research was to develop fumigation doses in the laboratory for control of strongly resistant FGB, which has recently been detected with the highest ever resistance to phosphine in any stored-grain pests in Australia, and to validate them through field trials. We have established protocols at two phosphine concentrations (360 ppm and 720 ppm) at 20°C and one of them (360 ppm for 30 days) has been successfully validated through a large scale field trial in a vertical silo with sorghum. Our results from this research suggest that to control strongly resistant FGB populations, we need much longer fumigation periods than that currently registered for phosphine to manage the previously established strongest resistant pests in Australia such as the lesser grain borer and psocids.

For example, at 20°C and a phosphine dose of 1 mg/L (720 ppm), we require a fumigation period of 24 d to achieve population extinction of strongly resistant populations of FGB compared with current recommended periods of 10 and 12 d for the control of strongly resistant populations of lesser grain borer and psocids, respectively (Fig. 2).

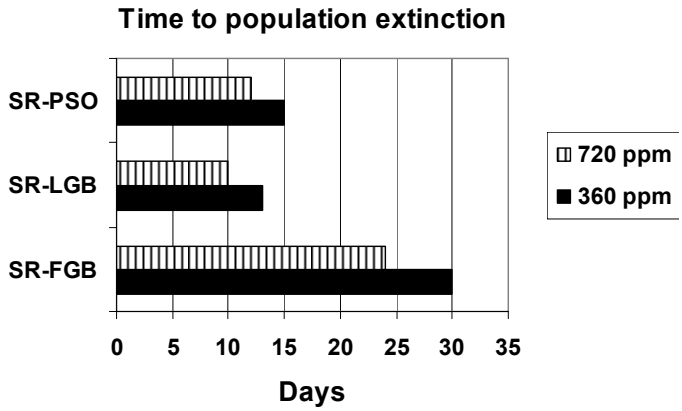

Figure 2 Time to population extinction of key phosphine resistant pests at two phosphine concentrations at 20°C. (SR-FGB: strong resistant flat grain beetle, SR-PSO: strong resistant psocid, source: Nayak and Collins (2008) and SR-LGB: strong resistant lesser grain borer, source: Collins et al., (2005).

Similarly, at 20°C and 0.5 mg/L (360 ppm), a fumigation period of 30 d will be required to control strongly resistant FGB compared with the current recommendations of 13 and 15 d for control of strongly resistant lesser grain borer and psocids, respectively (Fig. 2). This comparative analysis means that appropriate changes to the current label rates of phosphine may be required to accommodate the newly emerged strongly resistant FGB.

Strong resistance to phosphine in FGB was first reported in the 1980s in a population collected from Bangladesh (Mills, 1986). According to this report adults of the strong resistant FGB strain from Bangladesh were successfully controlled in 7 d at 0.66 mg/L (475 ppm) at 25°C, but a higher rate of 2.5 mg/L (1800 ppm) was needed at 15°C. From a more recent study in China, Wang et al. (2008) reported that to control phosphine resistant FGB populations in warehouses, the protocol should aim at achieving an initial concentration of 1 mg/L (720 ppm) and to maintain a concentration above 300-500 ppm for 16-25 d. From another study in China, Li and Yan (2008) recommended a dose of 200 ppm for more than 28 d to control strongly phosphine resistant FGB populations in warehouses. Both these studies concluded that once phosphine concentration drops below 200 ppm there was a possibility of control failure. Our recommendation of 360 ppm phosphine for 30 d falls in between the findings of these studies from China.

While research was in progress on development of new phosphine recommendations, other treatments were also considered for management of FGB. Laboratory evaluation of two currently registered grain protectants, chlorpyrifos-methyl (5 mg/kg) and fenitrothion (6 mg/kg) against 10 field collected FGB populations confirmed that these treatments can effectively control both adults and progeny. This information has been passed onto industry and grain at several bulk storages has been treated with either one of these chemicals. Personal communication from a major bulk storage operator suggests that this treatment has been successfully controlling the phosphine resistant FGB populations (Robin Reid, GrainCorp Operations Ltd., Queensland, Australia, personal communication). Moreover, several bulk storage operators have recently started using sulfuryl fluoride as an alternative fumigant to phosphine and claiming successful control of phosphine resistant FGB populations.

An action plan has been developed collaboratively by the bulk handling companies and the researchers aimed at eradicating infestations of phosphine resistant FGB and preventing their spread. The key components of this plan include use of an alternative fumigant such as sulfuryl fluoride, strategic application of grain protectants (chlorpyrifos-methyl, fenitrothion) and adoption of an intensive hygiene program and monitoring of insect populations through inspection, sampling and resistance testing.

Although the new fumigation rates and the eradication strategy developed through this research have practical application for the management of strongly phosphine resistant FGB populations, there are several research gaps that need to be addressed. These include identifying the causes for the development

of strong resistance and investigating whether the strong resistance is developing independently at different sites or resistant populations are spreading through transport.

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Integration of monoterpenoids with low pressure simulating vacuum for control of diapausing Indian meal moth larvae and red flour beetle adults

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Abstract

Monoterpenoids as well as low pressure simulating vacuum, when applied alone have been demonstrated to cause mortality of stored-product insect pests. The current report explored the possibility of integrating these two control methods in the management of stored-product insects. The insects used for this study were the adults of the red flour beetle, *Tribolium castaneum*, and diapausing larvae of the Indian meal moth, *Plodia interpunctella*. The monoterpenoids investigated were *E*-anethole, estragole, *S*-carvone, linalool, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor. Exposure of the insects to all the monoterpenoids alone, with the exception of camphor, at a concentration of 66.7µL/1L of volume required more than 24 h to generate 100% mortality at 28.0 ± 0.8°C and 70 ± 2.5 r.h. However, exposure of the insects to camphor alone generated 100% mortality with 3 h exposure in *T. castaneum*. Exposure of the insects to low pressure at 36.5 mm Hg generated 100% mortality in beetles with 24 h exposure and in the diapausing *P. interpunctella* larvae with 48 h exposure. However, combination of the monoterpenoids with low pressure reduced exposure periods (3-24 h) required to generate 100% mortality in both diapausing larvae of *P. interpunctella* and the adult beetles of *T. castaneum*. In all cases *T. castaneum* showed signs of weakness faster than diapausing *P. interpunctella* larvae.

Keywords: Low pressure, *DL*-camphor, Estragole, γ-terpinene, Carvone

1. Introduction

Physical methods, such as controlled atmospheres or extreme temperatures, are attractive for post-harvest commodities because they do not leave chemical residues on food, but many are expensive, damaging to the commodity, or impractical. Low-oxygen controlled atmospheres from the application of a vacuum to achieve low pressure have potential for effective postharvest insect control in some applications. Back and Cotton (1925) and Bare (1948) were among the first to study the use of low pressure for controlling stored-product insects. Insect mortality under low pressure is predominantly a result of the low oxygen concentration affecting key cell physiological processes such as glycolysis, and not to low pressure per se (i.e., physical pressure effects) or dehydration from lowered water concentrations under vacuum (Navarro and Calderon,1979; Friedlander and Navarro, 1983). Some studies considered the activity of vacuum alone applied to stored-product insects (Back and Cotton, 1925; Bare, 1948; Calderon et al., 1966; Calderon and Navarro, 1968), while other studies investigated the effects of temperature and pressure level on different life stages of post-harvest insects (Mbata and Phillips, 2001; Mbata et al., 2004). Other studies investigated the combination of low pressure with increases of other atmospheric gases or addition of fumigants (Calderon and Leesch,1983; Donahaye and Navarro, 1989; Locatelli and Daolio, 1993). Mbata et al. (2009) investigated the integration of low pressure with bruchid resistant cowpea varieties for the control of the cowpea weevil, *Callosobruchus maculatus* (F.). Controlled atmosphere has also been combined with essential oils for the control of *Liposcelis bostrychophila* Badonnel (Wang et al., 2001). Vacuum and fumigation have been used together in commercial practice for several decades (Bond, 1984), but we are unaware of any reports of procedures using combination of monoterpenoids with low pressures.

Monoterpenoids are 10-carbon, secondary plant chemicals that are major components of essential oils extracted from leaves or fruits of herbs such as *Eucalyptus*, *Ocimum* spp., *Carum carvii* L. (caraway), *Coriandrum sativum* L., and many others (Rice and Coats, 1994; López et al., 2008). The monoterpenoids are believed to aid plants in chemical defense against phytophagous insects and are now being exploited as insecticides. Monoterpenoids that have been investigated for insecticidal actions

include *E*-anethole, estragole, *S*-carvone, linalool, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor (Lee et al., 2002; Pascual-Villalobos and Ballesta-Acosta, 2003; Pascual-Villalobos et al., 2004; Lopez et al., 2008). Many of these monoterpenoids have been found effective against several post-harvest insects (López et al., 2008). It is hypothesized that combining low pressure with monoterpenoids will accelerate the mortality of exposed insects and also shorten the exposure period required to generate 100% mortality compared to when either low pressure or monoterpenoid is used alone.

The objective of the research reported herein was to investigate in the laboratory the effects of exposure of adults of *Tribolium castaneum* (Herbst.) and diapausing larvae of *Plodia interpunctella* (Hübner) to combinations of monoterpenoids and low pressure on their mortality. From these experiments we intended to determine monoterpenoids that can be applied simultaneously with low pressure to achieve 100% mortality of post-harvest insect pests in commodities within short exposure periods.

2. Materials and methods

2.1. Insects

Diapausing larvae of *Plodia interpunctella* (Lepidoptera: Pyralidae), and adults of *Tribolium castaneum* (Coleoptera: Tenebrionidae) were obtained from laboratory colonies. Laboratory colonies of insects were maintained at 28.0 ± 0.8°C and 70 ± 2.5 r.h. and reared using standard methods (Mbata,1985; Howe, 1991).

Diapausing *P. interpunctella* were generated by rearing larvae up to fourth instar (12 days following egg hatch) at 28.0 ± 0.8°C and 70 ± 2.5% r.h. and transferring them to a chamber maintained at 16ºC, 67% r.h. and a photoperiod of 8 h:16 h L:D (Mbata, 1987). Up to a third of the transferred larvae entered diapause within 4 weeks. Diapausing larvae were identified based on their extended larval developmental period, big sizes and yellowish color of larvae due to accumulated fat (Bell, 1977, Mbata 1987).

Adults of *T. castaneum* used for the study were sieved from the food as pupae and placed in a 500 mL jar for eclosion of adults. Following eclosion, adult red flour beetles were kept for 3 days before use in experiments. Both *T. castaneum* adults and the *P. interpunctella* diapausing larvae were separately placed in glass vials containing 2.0 g of rearing food, milled maize for the red flour beetle and moth rearing medium (Mbata and Osuji, 1983) for *P. interpunctella*. The insects were: five adult red flour beetles or five *P. interpunctella* diapausing larvae per vial and five vials were set up for each species.

2.2. Monoterpenoids

E-anethole (99%), Estragole (98%), *S*-carvone (98%), Linalool (97%), *L*-fenchone (98%), Geraniol (99%), γ-Terpinene (98%) and *DL*-Camphor (96%) were obtained from ACROS Organics BUBA/SPRL.

2.3. Experimental protocol

Three trials were carried out for each treatment and the treatments included exposing the insects to monoterpenoids alone, monoterpenoids and low pressure together, and low pressure alone. Exposure of test insects to low pressure was conducted by placing vials of test insects into 1L, thick-walled Erlenmeyer vacuum flasks with a side arm. Rubber stoppers were fitted with dial-type pressure gauges that had been previously calibrated to a mercury column manometer and placed in the flask opening. The side-arm outlet of the flask was connected to a vacuum pump (Budget Dyna-Pump; Fisher, Pittsburgh, PA) via a Tygon vacuum hose (4.76 mm i.d. and 1.59 mm wall thickness) equipped with a screw-type hose clamp. The air in the flasks was evacuated with the vacuum pump to an absolute pressure of 36.5 mmHg. Once the target pressure was attained the vacuum hose was clamped, the pump shut off, and for treatments requiring the addition of monoterpenoids, the chemical was injected with a 100 µL syringe through the rubber stopper. The concentration of the monoterpenoids used was 66.7µL/L. The syringe was removed by pulling from the needle, and the point of needle insertion through the rubber stopper was sealed with a glue to prevent the loss of pressure. The flasks were placed in an environmental chamber maintained at 28.0 ± 0.8°C and 70 ± 2.5% r.h. for given time periods needed for experiments. Untreated control flasks were set up with insects in the same way as treated flasks, but were vented so they were at ambient pressure and maintained in a chamber at 30°C. Approximately 30 vacuum flasks were available for use on any given day of experiments.

Vacuum flasks were set up with 5 vials for insect species, so there were 10 vials per flask for each trial. Treatment flasks were held at low pressure for five or more time periods that were 0.5, 1, 2, 3, 6, 12, 24, 48, 72, 96 h after evacuation. Flasks were removed from controlled temperature chambers at the end of the exposure period, vented to ambient pressure and placed in an environmental chamber at 28°C for an appropriate period of recovery. *T. castaneum* adults and larvae of *P. interpunctella* were observed for mortality after 2 d of recovery.

3. Results and discussion

Tables 1 and 2 summarize the results of our experiment. Less exposure time is required to kill *T. castaneum* (24 h) than *P. interpunctella* (48 h) at low pressure.

Table 1 Exposure time required to generate 100% mortality of *T. castaneum* adults exposed to low pressure, monoterpenoids, or their combinations[a].

Monoterpenoids	**Exposure period (h)**	
	Monoterpenoids[b]	**Monoterpenoids & low pressure[b]**
E-anethole	96	24
Estragole	24	6
S-carvone	48	12
Linalool	48	12
L-fenchole	72	12
Geraniol	48	24
γ-terpinene	24	3
DL-camphor	3	3
None	-	24

[a] complete mortality was achieved at >96 h of control (red flour beetles exposed to neither low pressure nor monoterpenoids); [b] The concentration of the monoterpenoids used was 66.7 µL/L.

Table 2 Exposure time required to generate 100% mortality of diapausing larvae of *P. interpunctella* exposed to low pressure, monoterpenoids, or their combinations[a].

Monoterpenoids	**Exposure period (h)**	
	Monoterpenoids[b]	**Monoterpenoids & low pressure[b]**
E-anethole	96	24
Estragole	24	12
S-carvone	>96	24
Linalool	72	24
L-fenchole	>96	24
Geraniol	>96	24
γ-terpinene	48	24
DL-camphor	24	6
None	-	48

[a] complete mortality was achieved at >96 h of control (diapausing larvae of *P. interpunctella* exposed to neither low pressure nor monoterpenoids); [b] The concentration of the monoterpenoids used was 66.7 µL/L.

The volatile toxic effects of monoterpenoids alone on both insects were faster (3-48 h) when *DL*-camphor, γ-terpinene or Estragole were used. The time required to kill 100% *T. castaneum adults* was at least 96 h when exposed to *E*-anethole, while exposure of diapausing *P. interpunctella* to Geraniol, *L*-fenchole, *S*-carvone and *E*-anethole required more than 96 h to achieve 100% mortality.

When monoterpenoids were used in combination with low pressure, the exposure periods required to generate 100% mortality were shortened. For instance, exposure periods were shortened from 24 h to 3 h with *DL*-camphor and γ-terpinene against *T.castaneum* or from 48 h to 6-12 h with *DL*-camphor and Estragole against diapausing *P. interpunctella*.

Rajendran and Sriranjini (2008) reviewing information on plant products as fumigants for stored-product insects documented that essential oils proved effective in mixture with carbon dioxide or ethyl formate.

CO_2 accelerate the penetration of fumigants by keeping the respiratory spiracles open. It is also probable that low pressure simulating vacuum will enhance the penetration of essential oils by keeping the spiracles open.

Some authors have focussed on enhancing the toxicity of gases, for example with a 95:5% v/v mixture of ethyl formate: carvone against *Sitophilus oryzae* (L.) (Waterford et al., 2004) or by combining carbon monoxide with carbon dioxide against *T.castaneum* (Wang et al., 2009). Ethyl formate efficacy against *Carpophilus hemipterus* (L.) can be increased (Rouzes et al., 2008) if applied at low pressure simulating vacuum.

Others have reported an enhancement of the toxicity of essential oils in the presence of controlled atmospheres (Wang et al., 2001) or of allyl acetate with carbon dioxide (Leelaja et al., 2007).

Besides enhancing the toxicity of monoterpenoids, their application at low pressure reduced considerably the long exposure times usually needed to kill the insects, either at exposure to low pressure alone or to monoterpenoids alone.

Our preliminary results indicate that *DL*-camphor, γ-terpinene and estragole at 66.7 µL/L have the potential to be applied simultaneously at low pressure (36.5 mmHg) to reduce the exposure periods required to achieve stored product pests mortality. But optimum combinations for each pest species have to be established.

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Evaluation of headspace Solid Phase Micro-extraction method for analysis of phosphine residues in wheat

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Abstract

This new method utilizes headspace-solid-phase micro extraction (HS-SPME) for pre-concentration of PH_3. Phosphine was determined with gas chromatography/pulsed flame photometric detector (PFPD). Spiked samples were used for calculation of phosphine residue in grain. Four types of fibres (100µm-PDMS, 85µm-CAR/PDMS, 75µm-CAR/PDMS and 65µm-PDMS/DVB) were tested. The bipolar fibres (CAR/PDMS and PDMS/DVB) can extract PH_3, but the non-polar fibre (PDMS) did not. Larger size fibres extracted PH_3 more efficiently than the smaller size fibres (e.g., 85 µm > 75 µm > 65 µm). The 85µm CAR/PDMS fibre was used to optimize the different parameters that affect the SPME extraction efficiency of PH_3. In the validation study, 50 grams of wheat in a 250 mL glass flask and capped with an open-top screw cap and PTFE/Silicon septa were spiked at 0.02 ng PH_3/g of wheat. The flask was then heated to 45°C in an oil bath for 45 min, after which time the 85 µm CAR/PDMS fibre was exposed for 20 min and then exposed in the heated injection port of a GC/PFPD and desorbed for 2 min. Under conditions of the validation study, the limit of detection (LOD) or level of quantification (LOQ) was in the range of 0.005–0.01 ng PH_3/g of wheat.

Keywords: Fumigant, Phosphine, Residue, SPME, HS-SPME

1. Introduction

The world grain industry relies heavily on chemicals for grain treatment. Currently, phosphine (PH_3) is the only fumigant available to treat bulk grains and oil seeds (more than 85% grains are treated/re-treated with PH_3) in each of the linkages from on-farm storage to central storage (Collins et al., 2000; Collins et al., 2002; Ren and Mahon, 2007). However, a restrictive Codex Maximum Residue Limit (MRL) of 0.1 mg/kg, and lower limits set by some purchasers, are challenging the use of PH_3 for treatment of grain. Phosphine residues can remain in the grain for several months. Multi-fumigation with PH_3 can also cause accumulation of residues in the grain exceeding the MRLs. It is also important to note that levels of PH_3 residues in grain, producing low aerial concentrations of PH_3, can also facilitate resistance. Therefore, it is necessary to understand PH_3 residues in bulk grain to establish better procedures for multi-fumigation. This will guide industry in the conduct of good PH_3 fumigation practice to minimise PH_3 residue in fumigated grain. These include application methods and choice of the right periods of exposure and airing.

Typical procedures for analysis of PH_3 residues include: 1) removal from the commodity matrix by either purge and trap techniques or by solvent extraction (Miyahara and Saito, 1994), and 2) and then analysis by gas chromatography (GC) or gas chromatography-mass spectra (GC-MS). However, purge and trap methods are not suitable for highly volatile fumigants such as PH_3 as they are unable to trap all fumigant (Ren and Desmarchelier, 1998). Solvent extraction has the problem of solvent interference and is time-consuming. None of the above methods are suitable for analysis of PH_3 residues in grains, particularly at very low levels. Microwave irradiation is being increasingly used in the digestion of samples (Ren, 2001; Desmarchelier et al., 1998), and, in recent work (Chemfate 2004), excellent recoveries and precision have been obtained from microwave extractions of PH_3 from wheat and hay (Ren and Mahon, 2007). However, care is required in selecting the appropriate power setting and the safety implications of heating sealed flasks in microwave ovens is of concern, and water vapour generated from microwave irradiation can significantly interfere with GC results (Ren and Mahon, 2007).

Solid-phase microextraction (SPME) in combination with head space (SH-SPME) analysis by gas chromatography is a convenient alternative method for volatiles. Solid-phase micro extraction is a simple, sensitive, and solvent-free technique that has become popular in a wide range of applications (Penalver et al., 2001). It has been used to study pollutant gases and volatile degradation products (Lattuai-Derieux, et al., 2004). For example residues of methyl isothiocyante (MITC) were analysed by HS-SPME with the 85 µm Polyacrylate (PA) fibre (Ren et al., 2008a). Using the HS-SPME method, MITC residues from wheat can be successfully analysed at levels below 0.1 ng/g, compared with purge and trap methods where the limit of detection is 10 ng/g (Ren et al., 2008a,b). This paper describes a new method for analysis of PH_3 residues in wheat which utilises headspace-solid-phase micro extraction (HS-SPME). It includes selection of the SPME fibre and factors that affect the extraction efficacy of PH_3.

2. Materials and methods

2.1. Reagents and apparatus

One litre Erlenmeyer flasks (Bibby Sterilin, Staffordshire, Cat. No. FE 1 L/3) were used for fumigation of wheat and preparation of standards. The measured volume of each Erlenmeyer flask and inlet system was calculated from the weight of water required to fill the container and was used for calculations. Bottles of 250 mL (Alltech Cat. No. 9535) were used for the microwave "extraction". Each bottle was fitted with a Mininert valve equipped with septa (Alltech Cat. No. 95326). A 100 µL air tight syringe with valve (SGE, Melbourne, Australia; Cat. No 005279) was used for GOW-MAC gas density balance injection and transfer of PH_3 from source to fumigation chambers and flasks.

The SPME fibres used were coated with 85 µm Carboxen/Polydimethylsiloxane (CAR/PDMS) fibre (Sigma-Aldrich Australia, Cat. 57334-U), 75 µm Carboxen/Polydimethylsiloxane (CAR/PDMS) fibre (Sigma-Aldrich Australia, Cat. 57344-U), 65µm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fibre (Sigma-Aldrich Australia, Cat. 57310-U) and 100 µm Polydimethylsiloxane (PDMS) fibre (Sigma-Aldrich Australia, Cat. 57301), respectively. All fibres were conditioned at 270°C for 1 h prior to use in accordance with manufacturers' recommendations.

Phosphine (85.0% PH_3 and 15.0% air and CO_2) was laboratory prepared by the FAO method (FAO, 1975). The purity of PH_3 was determined using a GOW-MAC gas density balance (GOW-MAC Instrument Co., Madison, N.J.) after separation of the gases on a 1 m × 5 mm i.d. Porapak Q 100/120 mesh (Alltech Associates, Cat. No. 2702) column at 105°C with a carrier flow (N_2) of 150 mL/min. The reference gas used was tetrafluoroethane (> 99.9%), which was supplied by ACTROL Ltd, Australia.

Phosphine (PH_3) was determined on a Varian CP-3800 (Varian Instruments, Sunnyvale, CA), equipped with a pulsed flame photometric detector (PFPD) with phosphorus filter. Separation was achieved on a 30 m × 0.53 mm ID, AT-Q column (Alltech Associates, Cat. No. 0810025, Part No. 13939) at 125°C and carrier flow (N_2) of 5.0 mL/min at 5.0 psi. Injector and detector temperatures were 200°C. Injection volumes of gases were 40 µL. A minimum interval of 5 min was kept between injections, in order to elute interfering chemicals.

2.2. Wheat sample and fumigation of wheat

Wheat used was Australian standard white wheat, 10.9% moisture content, w/w wet basis. The moisture content of the wheat was measured by oven drying at 105°C for 2 hours (ISO 712 International Standard 1998). In order to ensure the moisture result is precise, 500 kg wheat sample was quartered equally using a Boerner grain divider. One quarter sample (125 g) was quartered equally again and then one quarter sample of about 30 g was collected and ground with a laboratory sealed metal grinder for measurement of moisture content. Four replicates samples were dried at 105°C in mechanical convection oven (DK 62 American Scientific Products Columbus, OH, USA) and the loss of weight was used to calculate the moisture content of the sample.

Wheat samples (860 g) were fumigated in an Erlenmeyer flask (1 L) equipped with a lid fitted a septum injection system for 7 days at concentrations of 0.5 mg (PH_3)/L. After 7 days exposure, the flask was opened and aired for 7 days in a fume hood to obtain samples containing residual fumigant.

2.3. Preparation of diluted PH_3 gas and spiking standards

Diluted PH_3 gases were prepared by first removing the same volume of air as the known volume (0.5 mL) of concentrated PH_3 to be injected into an Erlenmeyer flask (1 L) containing six glass beads (2-3 mm OD). For validation study, the limit of detection (LOD) or level of quantification (LOQ) was investigated using spiked standards. Spiked wheat samples at and 0.02 and 0.5 ng PH_3/g of wheat were prepared by adding appropriate volumes of PH_3 into sealed flasks (250 mL) containing wheat (50 g) 3 min before oil bath heating. Triplicate samples were used and each sample was injected duplicates (*n=6*). The dosages and required volumes for PH_3 concentrations, calibrated to the current laboratory temperature and pressure, were calculated from Eq. 1

$$\mathbf{V_f} = (1-\frac{T}{273})\,(\frac{1.7\times10^4\times C\times V}{P\times M\times N}) \quad \text{Eq. 1}$$

Where: ***V*** is volume of fumigation container (L); ***P*** is pressure (mm Hg); ***T*** is temperature (°C); ***C*** is the intended concentration of methyl bromide (mg/L); $\mathbf{V_f}$ is dosage volume of fumigant (mL); ***M*** is molecule weight of fumigant; ***N*** is purity of gas (%).

2.4. Selection of fibre and HS-SPME extraction time

Four different types of fibres (100 µm-PDMS, 85 µm-CAR/PDMS, 75 µm-CAR/PDMS and 65 µm-PDMS/DVB) were tested at 20 min extraction time. Diluted PH_3 gas was injected into a 250 mL flask fitted with a sample port to obtain 0.3, 0.1, 0.05 and 0.01 ppm of PH_3, respectively. Headspace samplings were carried out on all four types of fibres. The needle was carefully inserted into the headspace of the flask and the fibre exposed into the headspace for 20 min. At the end of the defined extraction time, the fibre was withdrawn from the headspace into the needle. The fibre holder was removed from the extraction flask and inserted into the injection port. The fibre was extended into a GC-PFPD inlet where sample components were desorbed at 200°C for 5 min to clean it between extractions.

For evaluation of HS-SPME extraction time on the efficacy of PH_3 extraction, the SPME equilibration time was determined by exposing the fibres (85 µm-CAR/PDMS, 75 µm-CAR/PDMS *and* 65 µm-PDMS/DVB) to 0.3 ppm of PH_3 for 1, 5, 10, 15, 20 and 30 min, respectively. Finally the fibre was retracted and then exposed in the heated injection port (200°C) of a GC-PFPD and desorbed for 2 min. Results are the mean of duplicate samples and injections for each sample (*n=4*).

2.5. Evaluation of GC Injector temperature for desorption of PH_3 from fibre (85µm CAR/PDMS)

Efficient thermal desorption of the analysis in a GC injection port is dependent on the type of fibres and on the GC injector temperature. The 85 µm CAR/PDMS fibre was used to optimize the injector temperature. It was inserted into the headspace of the flask containing 0.1 ppm of PH_3 and exposed for 20 min. The fibre was carefully injected into the GC at different injector temperatures (100,150, 200 and 250°C) and desorbed for 5 min. Results are the mean of duplicate samples and injections for each sample (*n=4*).

2.6. Evaluation of wheat treatment and HS-SPME extraction temperature for extraction efficacy of PH_3

A fumigated wheat sample of 50 g in a 250 mL flask fitted with a sample port was immersed in a heated oil bath at 30°C for 30 min, after which time the fibre was inserted into the headspace of the flask and exposed for 20 min. Equivalent flasks were heated a 35, 40, 45 and 50°C. The fibre was injected (200°C) into a GC-PFPD and desorbed for 5 min. Spiked wheat samples were treated in exactly the same manner. Levels of PH_3 residue were determined against spiked standards. The peak areas were calibrated periodically using a fortified standard, and the data presented are the mean of duplicate samples and injections for each sample (*n=4*).

2.7. Statistical analysis

The GC readings of fumigant concentration were averaged respectively. The average variation of GC readings and PH_3 concentration and standard deviations (SD) between the duplicate treatments and injections were analysed with Microsoft Excel.

3. Result and discussion

3.1. Effect of fibre types and HS-SPME extraction time on extraction efficacy of PH_3

The amounts of PH_3 absorbed by each of four different SPME fibres are shown in Figure 1 for each of four PH_3 concentrations. These four fibres showed different efficiency and selectivity in extracting PH_3 in the headspace. The bipolar fibres (CAR/PDMS and PDMS/DVB) which dispersed solid adsorbents in polymer extracted PH_3, but the nonpolar fibres (PDMS) extracted no PH_3. The larger size of polymer fibre (e.g., 85 µm) extracted PH_3 more efficiently than the smaller size of polymer fibre (e.g., 75 µm and 65 µm).

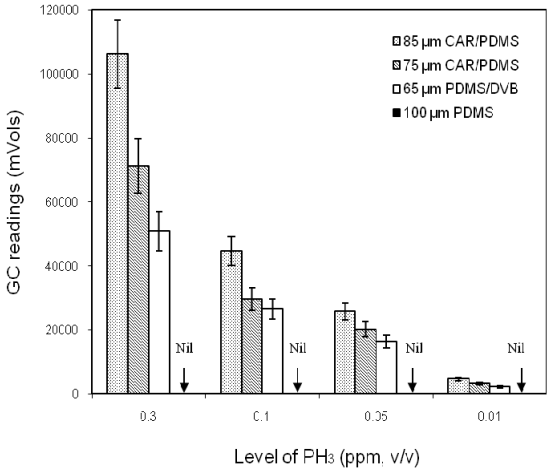

Figure1 Fibre types (100 µm PDMS, 85 µm CAR/PDMS, 75 µm CAR/PDMS and 65 µm PDMS/DVB) affect the extraction efficacy of PH_3 after 20 minutes extraction.

The effect of time of extraction of PH_3 is shown in Figure 2. The responses of GC peak areas progressively increased with increasing the extraction time, but no increase in the response occurred after 20 min. That is, the amount of PH_3 absorbed by the tested fibres increased over a period of 20 min and then attained equilibrium.

After 30 min extraction, the amount of absorption was 85 µm CAR/PDMS > 75 µm CAR/PDMS > 65 µm PDMS/DVB. The 85 µm CAR/PDMS fibre was used in this study to optimize the different parameters that affect the SPME extraction efficiency of PH_3.

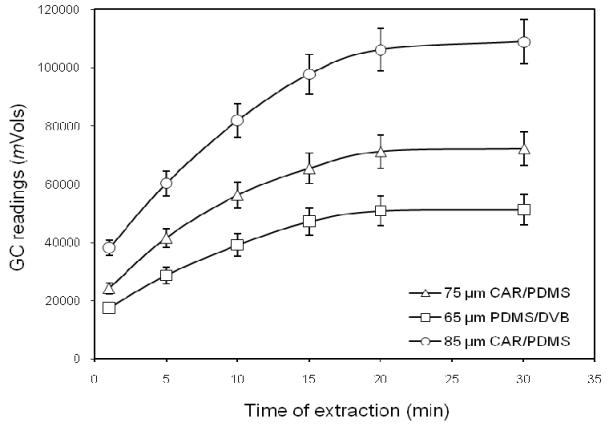

Figure 2 Time of extraction with different type of fibres (85 µm CAR/PDMS, 75 µm CAR/PDMS and 65 µm PDMS/DVB) affects the extraction efficacy of PH_3 (0.3 ppm) at 1, 5, 10, 15, 20 and 30 min.

3.2. Effect of GC Injector temperature for desorption of PH_3 from fibre (85 µm CAR/PDMS)

The effects of GC injector or desorption temperature were evaluated by varying the temperature from 100 to 250ºC. The injector temperature profile obtained is shown in Figure 3. The amount of desorbed PH_3 increased with increasing the injector temperature and then reached a maximum at 200ºC. The GC used had split/splitless capillary injectors. It was suitable for direct introduction of the fibre into the injection port. In this study, the CG injector operated at 200ºC of optimal desorption temperature and did not cause peak broadening and tailing (Fig. 4).

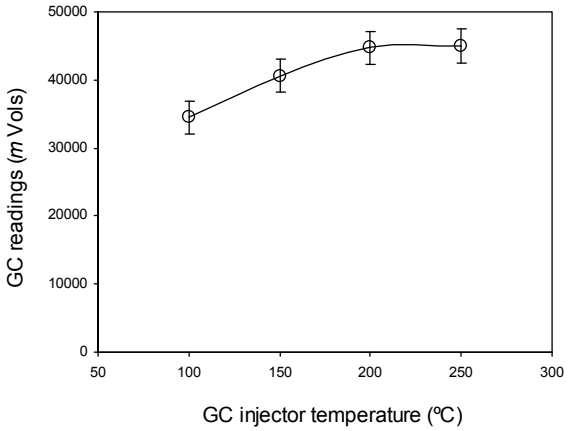

Figure 3 The GC injector temperature affects desorption of PH_3 from fibre (85 µm CAR/PDMS) at 0.1 ppm of PH_3 after 20 minutes extraction.

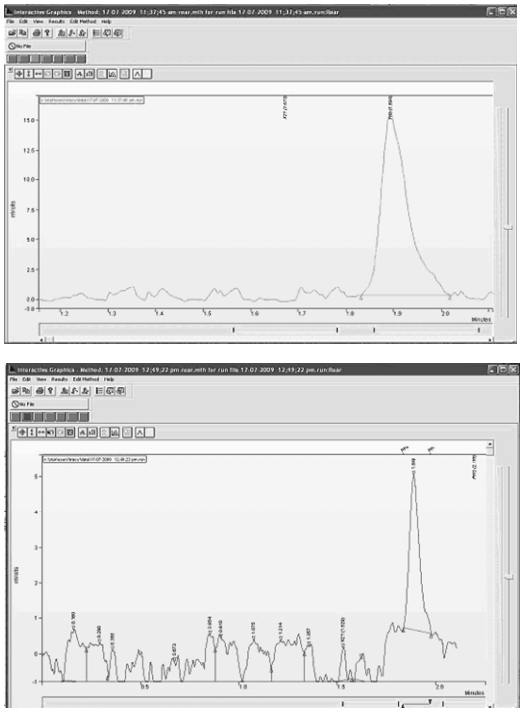

Figure 4 GC spectra with fibre (85 um CAR/PDMS) after 20 min extraction at levels of PH_3 at 0.5 ng/g (A) and 0.02 ng/g (B).

3.3. Effect of wheat treatment and HS-SPME extraction temperature on extraction efficacy of PH_3

The effects of temperature for treatment of wheat sample and extraction efficacy were evaluated by varying the temperature from 30 to 50ºC. The temperature profile for PH_3 released from fumigated wheat into the headspace and absorption of PH_3 on the fibre (85 µm CAR/PDMS) obtained is shown in Figure 5.

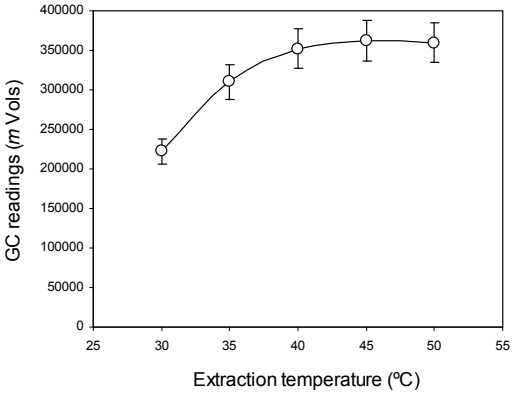

Figure 5 Temperature of extraction with fibre (85 µm CAR/PDMS) for 20 minutes extraction affects the extraction efficacy of PH_3.

The amount of absorbed PH_3 increased with increasing the injector temperature and then reached a maximum at 45ºC. As the temperature increases, the liquid and gas phases equilibrate faster and drive more PH_3 partitioning into the headspace, but increasing fibre temperature causes a competitive effect of

desorption from the fibre, ultimately limiting its analytical sensitivity of PH_3. This is consistent with the results of Vás and Vékey (2004). Therefore, we used the low temperature (45ºC) and long term (30 min) extraction for treatment of wheat samples. The limit of detection (LOD) or level of quantification (LOQ) was estimated based on a signal to noise ratio shown in Figure 5 (B) in the range of 0.005–0.01 ng/g.

Although the determination of PH_3 in grain is well established in the scientific literature, our results have demonstrated that the combination of SPME with headspace sampling is very effective for determining trace levels of PH_3.

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The use of carbon dioxide in big bags and containers for the control of pest in food products

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Abstract

Modified atmospheres (MA) based on high carbon dioxide (CO_2) contents offer an alternative to synthetic chemical fumigation for insect pest control in food commodities during storage and shipment processes. The present study aimed to establish the efficacy of using CO_2 in big bags and containers to prevent pests' development. Four trials were conducted with gastight big bags (900 x 900 x 1000 or 1600 cm). Two of these trials were conducted with polished rice and samples of *Sitophilus oryzae*, one trial with chamomile infested with *Lasioderma serricorne* and one trial with cocoa and samples of *Tribolium confusum* and *Ephestia kuehniella*. Initial contents of CO_2 were higher than 75%, which decreased depending on exposure time (13 to 90 d) and food product. In all four trials the insects present in the infested samples were controlled with the MA. An additional trial was conducted in a 9 m container containing dried herbs in boxes, big bags and other packaging formats. Twelve infested samples of *L. serricorne* and *Plodia interpunctella* were distributed uniformly at the bottom and top of the container. A concentration between 70% and 15% CO_2 was maintained for an exposure time of 18 d. In spite of the decrease in CO_2 content, the treatment was also effective to control all insects present in the samples. Our results confirmed that CO_2 could be applied to food products during the storage in big bags and containers to control the occurrence of pests.

Keywords: Modified atmosphere, Carbon dioxide, Pest control, Stored-product pests.

1. Introduction

Food commodities can be affected by insect pests during the storage period and therefore contamination due to storage insects or their remains may be present in the final product. On the other hand, there are increasing restrictions on the use of pesticides and on the number of active chemical compounds officially registered for pest control. Therefore, implementation of alternative methods of control is necessary. Among alternative methods, Modified Atmosphere (MA) treatments are safe and environmentally friendly ways of controlling pests that affect a large number of raw and manufactured food products. MA treatments have been adopted as feasible alternative treatments since the Montreal Protocol decided to phase out the use of methyl bromide. MA with high carbon dioxide (CO_2) content has been tested for many years for the control of various different pest species (Fleurat-Lessard, 1990; Adler et al., 2000; Navarro, 2006). The use of CO_2 offers several advantages for the food industry: there is no accumulation of toxic residues after the treatment in the final product, no safety interval following treatment is necessary for consumption of the product and it is currently approved as a food additive, E-290 (FAO and WHO, 2010). However, high CO_2 MA requires the use of gastight structures to keep the correct gas concentration throughout the treatment. The objective of the present work was to demonstrate the efficacy of using high CO_2 MA in gastight bags specifically manufactured for this application and in a 9 m container to prevent insect pests' development during the storage of rice, cocoa beans, chamomile and different dried herbs.

2. Materials and methods

Five trials were conducted: two with polished rice in big bags and samples of *Sitophilus oryzae* (L.), one with chamomile in big bags infested with *Lasioderma serricorne* (F.), one with cocoa beans in big bags and samples of *Tribolium confusum* du Val and *Ephestia kuehniella* Zeller, and the last one with various dried herbs in a 9 m container with samples of *L. serricorne* and *Plodia interpunctella* (Hübner).

Insect species tested in this study were obtained from stock colonies maintained at the IRTA (Cabrils, Barcelona). *Sitophilus oryzae* was reared on brown rice, *P. interpunctella* on wheat bran, brewer's yeast and glycerine, and *L. serricorne*, *T. confusum* and *E. kuehniella* on wheat flour and yeast. Rearing was conducted in a climatic chamber at 25±1ºC, 75±10% r.h. and at a photoperiod of 16:8 h (L: D).

Standard big bags (900 x 900 x 1000 cm for rice and cocoa beans trials and 900 x 900 x 1600 cm for the chamomile trial) of woven polypropylene with an internal plastic liner were used for the MA treatment (AG Protectpack S.L.) (PRO COEX PACK 3.85 TRIK multy layer) (Figure 1). The experiments were conducted with food grade Carbon Dioxide CO_2 (S.E. Carburos Metálicos S.A. - Air Products Group).

Figure 1 Chamomile in big bags (900 x 900 x 1600 cm).

2.1. Rice in big bags.

Two trials were conducted with polished rice. In the first trial, 750 g of brown rice infested with eggs of *S. oryzae* were distributed throughout the top, middle and bottom levels of a big bag filled with polished rice. In the second trial, 125 g of infested rice were distributed in the big bag. The big bags were then purged with CO_2 and kept in a warehouse at ambient conditions. Gas concentrations were determined with a gas analyzer (Abbiss, TOM-12), during the exposure, to verify the contents of CO_2 and O_2 inside the big bags. At the end of 48 d in trial 1 and 90 d in trial 2, the bags were opened and insect samples were collected from each big bag. The samples were placed in a climatic chamber at 25±1ºC and 75±10% r.h. until the emergence of adults. The number of *S. oryzae* adults which emerged was then recorded. In both trials, a control treatment with a big bag of rice infested with the same number of samples of *S. oryzae* was conducted in order to compare the results obtained with and without CO_2.

2.2. Cocoa beans in big bags

The trial was conducted with 2 gastight big bags which were purged with 680 kg of cocoa beans. Samples, with 50 g of wheat flour and yeast infested with 50 eggs of *E. kuehniella* and 500 eggs of *T. confusum* were prepared using paper envelopes. Three samples with *T. confusum* and three samples with *E. kuehniella* were distributed at each level of the big bag (bottom, middle and top). One of the big bags was then purged with CO_2 and the not treated was kept as a Control. The big bags were kept in a warehouse at ambient conditions for 13 d. Gas concentrations were determined with a gas analyzer (Abbiss, TOM-12) at the start and at the end of the exposure time, to verify the contents of CO_2 and O_2 inside the big bag. At the end of the treatment, big bags were opened and all samples with the insects were collected and placed in the climatic chamber for 7 wk. Afterwards, the number of emerged adults of *T. confusum* and *E. kuehniella* were counted.

2.3. Chamomile in big bags

The trial was conducted with 28 gastight big bags of chamomile infested with a natural population of *L. serricorne* and purged with CO_2. In nine randomly selected big bags, gas content was determined at the start and after 16, 18, 21, 22 and 24 d of exposure, with a gas analyzer (Abbiss, TOM-12) to verify CO_2 and O_2 levels inside the big bags. At the end of the MA treatment, four samples of approximately 100 g each of chamomile were collected randomly and placed in the climatic chamber for 2 mo. Three Control samples of 100 g of chamomile, not treated with CO_2 were used in order to assess insect infestation. The number of insect adults emerged was recorded in all samples at the end of the trial.

2.4. Dried herbs treatment in container

The experiment was conducted in a 9 m container connected to a cylinder of CO_2. The container had 11 pallets with various dried herbs such as chamomile and fennel in boxes, big bags and in other packaging formats. Samples with 80 g of wheat flour and yeast infested with, approximately, 50 eggs and 50 larvae of *L. serricorne*, and with 20 g of wheat bran infested with, approximately, 100 larvae of *P interpunctella* were prepared using paper envelopes. Six infested samples of *L. serricorne* and 6 of *P. interpunctella* were distributed uniformly at the bottom and top of the container. The container was purged with the MA until CO_2 content reached 70%, approximately. Gas content inside the container was measured with a gas analyzer (Abbiss, TOM-12) to verify CO_2 and O_2 levels throughout the exposure time. Due to a decrease in the concentration of CO_2, additional CO_2 was introduced inside the container during the trial. After 18 d of exposure, the container was opened and the samples with the insects were placed in a climatic chamber for 2 months. Control samples of each insect which were not treated with CO_2 were maintained in a climatic chamber in order to assess insect infestation. The number of live insects was recorded at the end of the experiment.

3. Results and discussion

3.1. Rice in big bags

In the trial 1, the CO_2 and O_2 contents within the sealed big bag just after it was purged with the MA were 100% and 0.8%, respectively. Twenty one d after dosing, CO_2 content decreased to 59% and O_2 content increased up to 10%. At the end of the 48 d treatment, CO_2 content decreased to 45% and O_2 content increased up to 14%. At the end of the trial, *S. oryzae* adults were only recorded in samples of the Control big bag, since they were on the top layer of the big bag where the number of *S. oryzae* adults was higher (Table 1).

Table 1 Rice in big bags. Trial 1. Number of S. *oryzae* adults after 48 d of exposure to CO_2. Samples were collected at three different levels (top, middle and bottom).

Big bag	**Level**	**Sample**	**Number of *S. oryzae***
Treated (CO_2)	Top	1.1	0
		1.2	0
		1.3	0
	Middle	2.1	0
		2.2	0
		2.3	0
	Bottom	3.1	0
		3.2	0
		3.3	0
Control	Top	1.1	160
		1.2	118
		1.3	61
	Middle	2.1	36
		2.2	17
		2.3	26
	Bottom	3.1	7
		3.2	4
		3.3	0

In the second trial, when the big bag was purged, the CO_2 content reached 78% and residual O_2 remained around 0.7%. After 45 d, CO_2 content decreased to 45% and O_2 increased to 10%. At the end of the 90 d exposure, CO_2 content was reduced to more than half compared to the initial values. The O_2 content increased to 12%. No insects survive in the big bag treated with CO_2, while in the Control big bag a large number of insects were recorded in all three levels sampled (Table 2).

Table 2 Rice in big bags. Trial 2. Number of *S. oryzae* adults after 90 d of exposure to CO_2. Samples were collected at three different levels (top, middle and bottom).

Big bag	Level	Sample	Number of *S. oryzae*
Treated (CO_2)	Top	1	0
	Middle	2	0
	Bottom	3	0
Control	Top	1	56
	Middle	2	22
	Bottom	3	27

3.2. Cocoa beans in big bags

Just after the big bag was purged, CO_2 and O_2 contents were 99% and 0.6%, respectively. At the end of the treatment, 13 d after dosing, CO_2 content decreased up to 10% and O_2 concentration increased to 16%. At the end of the trial, no insects of any species tested survived in the treated big bag. Conversely many insects were alive in the samples of the control big bag (Table 3).

Table 3 Cocoa beans in big bags. Number *T. confusum* and *E. kuehniella* adults after 13 d of exposure to CO_2. Samples were distributed at three different levels (top, middle and bottom).

Big bag	Level	Sample	Number of *T. confusum*	Number of *E. kuehniella*
Treated (CO_2)	Top	1.1	0	0
		1.2	0	0
		1.3	0	0
	Middle	2.1	0	0
		2.2	0	0
		2.3	0	0
	Bottom	3.1	0	0
		3.2	0	0
		3.3	0	0
Control	Top	1.1	255	39
		1.2	235	38
		1.3	290	24
	Middle	2.1	129	33
		2.2	84	34
		2.3	245	37
	Bottom	3.1	79	35
		3.2	49	37
		3.3	106	33

3.3. Chamomile in big bags

The CO_2 and O_2 contents in nine of the randomly selected big bags were in average 89% and 1.4%, respectively. Between 18 and 24 d after dosing, CO_2 content was still higher than 82% and O_2 content remained below 3%. At the end of treatment time, no adults emerged in the samples that were treated with CO_2. On the contrary, 2 and 1 *L. serricorne* adults emerged in 2 samples of the control treatment without CO_2 (Table 4).

Table 4 Chamomile in big bags. Number of *L. serricorne* adults in samples with a natural occurring infestation, after 21 d of treatment with CO_2.

Big bag	Level	Sample	Number of *L. serricorne*
Treated (CO_2)	Top	1	0
	Top	2	0
	Top	3	0
	Top	4	0
Control	Top	1	0
	Top	2	2
	Top	3	1

3.4. Dried herbs treatment in container

The initial CO_2 content inside the container reached 70%. However, the level of CO_2 declined during the exposure to 15% and the residual O_2 content increased to 18%. Therefore, it was necessary to add gas to maintain the CO_2 content inside the container. However, treated samples with *L. serricorne* and *P. interpunctella* distributed in the container did not contain any live insects at the end of the trial (Table 5).

Table 5 Dried herbs in container. Number of *L. serricorne* and *P. interpunctella* adults after 18 d exposure to CO_2.

Big bag	Level	Sample	Number of *L. serricorne*	Number of *P. interpunctella*
Treated (CO_2)	Top	1	0	0
		2	0	0
		3	0	0
	Bottom	1	0	0
		2	0	0
		3	0	0
Control	-	1	98	72

The results confirmed that the use of high CO_2 MA in gastight big bags is a feasible alternative to control the occurrence of pests on rice, cocoa beans various dried herbs during their storage. Levels of CO_2 in the sealed big bags remained quite constant throughout the period of exposure and control efficacy was verified in all cases tested. The use of containers for the treatment of food commodities with high CO_2 MA should be implemented in order to keep gas contents above the required levels and to guarantee a high level of control.

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Pre-Mix and on-site mixing of fumigants

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Abstract

Pre-Mix or On-Site mixing, a common practice with liquid insecticides, has benefits of increased synergy with fumigants. Both Pre-Mix and On-Site mixing have some issues with compatibility (e.g., active ingredient chemical stability; material compatibility; reaction with other ingredients). In addition to its fumigant properties, carbon dioxide (CO_2), has a synergistic effect on other fumigants and reduces flammability. The general consensus on the amount of CO_2 to improve efficacy is in the range 5%-20%. The early recognition of the benefits of CO_2 to overcome the flammability of potential fumigants by Jones (1933), initiated safe and more effective fumigant mixtures: examples are ethylene oxide (12.2 vol%), ethyl formate (14.4 vol%) and propylene oxide (8.3 vol%).

The main advantage of gaseous phosphine (PH_3) use is reduced generation time and uniform PH_3 distribution in hours not days. Other advantages of gaseous PH_3 include accurate metering of PH_3 fumigation levels and reduction of Occupational Health and Safety exposure to PH_3. Gaseous PH_3 in a Pre-Mix with liquid CO_2 or mixed On-Site with gaseous CO_2 or N_2+Air to reduce the PH_3 level below Lower Explosive Level of 16,000 ppm. A critical impurity of CO_2 when mixing with PH_3 is oxygen which must be less than 0.01% to avoid the formation of the polymer, $(P_2H_4CO_2)_n$. Large quantities of PH_3 are used to treat grain storage up to 280,000 tonne [CBH, WA] and multiple 30,000 tonne silos at Dalian, China (1.4 Mt facility). Reports indicate synergy with a mixture of propylene oxide (C_3H_6O), sulfuryl fluoride (SO_2F_2) and CO_2. This mixture is an attractive candidate for a Pre-Mix or On-Site mixing of a non-flammable synergized mixture with reported 100% efficacy for all insect life stages.

Keywords: Fumigant mixtures, Synergised, Non-flammable, On-site mixing.

1. Introduction

Generally fumigants are simple gases/gas mixtures without the complications of reactive adjuncts (e.g., surfactants) used to formulate emulsified concentrate liquid insecticides formulations.

Pre-Mix fumigants have benefits of ease of use but may have shelf-life stability issues. On-Site mixing of individual gases has benefits of flexibility and avoids any long-term storage compatibility issues.

Gaseous PH_3 was made commercially available with the patented non-flammable PH_3+CO_2 mixture (Ryan and Latif, 1989). This progressed to the PH_3+Air On-Site Mixing patent (Ryan and Shore, 2005). A critical issue with fumigant gas metering and dispensing is flammability. Flammability is solved by mixing volatile fumigants with inert gases or rapid dilution in turbulent air flow.

The fumigant gas, phosphine (PH_3), is used as a solid (e.g., aluminium phosphide: AlP), pure PH_3 and non-flammable mixtures of PH_3 in CO_2 or N_2. The AlP tablets are formulated to react slowly with atmospheric moisture which allows time for the very flammable PH_3 to diffuse away from the tablet, the gas mixture in air is diluted to levels below the flammability limit. There is no issue with the non-flammable 2.6 vol% PH_3 in CO_2 or 2.0 vol% PH_3 in N_2. The 100% PH_3 can be mixed onsite with an inert gas (e.g., CO_2 or N_2) or rapidly mixed into a turbulent air stream (best done externally to the fumigation space as a safety measure). The PH_3/air mix is non-flammable when PH_3 is less than 16000 ppm (about 22.2 g PH_3/m^3 in air). The early recognition of the benefits of CO_2 to overcome the flammability of potential fumigants by Jones (1933), initiated safe and more effective fumigant mixtures – e.g., ethylene oxide (12.2 vol%), ethyl formate (14.4 vol%) and propylene oxide (8.3 vol%).

The inert gas of choice CO_2, has benefits of requiring three times (3x) less gas cylinders compared to N_2 and is a synergist which improves the efficacy of fumigants. The general consensus on the amount of CO_2 to improve efficacy is in the range 5% - 20%.

One specific issue with gas metering and dispensing of phosphine is polymer formation. Dispensing of gaseous phosphine requires accurate metering using flow meters and gas mixing equipment. The formation of polymers within metering equipment can cause blockages resulting in malfunction. The implication of this is to ensure oxygen is at low levels in PH_3 Pre-Mix and PH_3 On-Site mixtures. It is critical to eliminate air from dispensing equipment by conducting pre- and post-purging of dispensing equipment.

The ultra fine particle polymer which causes blockages and malfunction of dispensing equipment is also very combustible (easily ignited by a trace amount of white phosphorus, P_4, impurity). There is variation in the structure and the colour of the polymers formed and is related to the PH_3 mixture (canary yellow polymer is associated with 100% PH_3 while an orange-brown polymer is found in PH_3/CO_2 mixtures). The structure of the polymer in a PH_3/CO_2 mixture was identified as $(P_2H_4CO_2)_n$ by Gallagher et al. (1996).

The solvent-propellant property of liquid CO_2 was the basis of a patent by Ryan et al. (1978) where active constituents are dissolved in liquid CO_2 at high pressure (50 bar) and contained in an industrial high pressure gas cylinder fitted with a "dip" tube to enable withdrawal of the liquid mixture. Existing Pre-Mix high pressure liquid CO_2 pesticide formulations in industrial gas cylinders are limited in choice and flexibility.

On-Site mixing using low pressure gaseous CO_2 has potential for a wide range of volatile pesticides. On-Site mixing has more flexibility and lower cost in dispensing mixtures of volatile and fumigant chemicals. GasApps Australia P/L (Kings Park, NSW, Australia) has been using On-Site mixing of PH_3 and CO_2 for over 10 years with SIROFLO, the flow-though fumigation technique for bulk grain which has typical exposure time of 18 days (CSIRO developed SIROFLO which revolutionised fumigation of grain stored in "leaky" storages). Each year Australia uses more than 10 t of gaseous PH_3 in SIROFLO fumigation of "leaky" grain storages. This gaseous PH_3 application is shared between the PreMix (PH_3/CO_2) and 100% PH_3 industrial gas cylinder products. The flammability of the 100% PH_3 is overcome by On-Site mixing with CO_2.

The objective of this review is to report on the On-Site mixing of PH_3 with CO_2 and eventual dilution with air to the fumigation level.

2. Materials and methods

The Australian grain industry depends mostly on the fumigant phosphine for insect disinfestations. Historically, the pendulum has swung from the magic days of "malathion and tin sheds" (i.e., residual grain protectants) to non-residual phosphine fumigation. There is movement now from metallic phosphide tablets (AlP) to gaseous phosphine which is marketed as 100% PH_3; 2.6 vol% PH_3/CO_2 and 2.0% PH_3/N_2. Gaseous phosphine formulations require to be diluted in air which at a minimum requires pre- and post-purging of dispensing equipment. The 100% PH_3 is On-Site mixed with an inert gas (e.g. CO_2 or N_2) or rapidly mixed into a turbulent air stream (again, pre- and post-purging is critical to avoid flammability issues, prevent polymer formation and for OH&S reasons).

SIROFLO flow-through fumigation installation has a circulation fan which delivers controlled low pressure (500 Pa) air flow through a network of PVC pipes usually connecting multiple grain storages. The fan maintains a constant flow through any grain storage under treatment. The flow through any individual storage is controlled by individually designed metering orifices. The PH_3 is metered into the air stream and quickly mixes prior to entry into the selected grain storage. Exposure time is 18 d and fumigation is often carried out at unmanned rural sites. The PreMix (PH_3/CO_2) high pressure industrial gas cylinder mixture using a regulator and flow controller is suited to the long-term metering of PH_3 into the continuous flow-through air stream. The flow rate is one volume change per day for the selected "leaky" grain storage to maintain an efficacious PH_3 level. The low concentration (~120 ppm PH_3) is compensated by an exposure time of 18 d.

An alternative to the PreMix is On-Site mixing of PH_3 which requires a higher level of equipment reliability because of the dual role of continuous mixing plus metering the exact PH_3 level required for a successful fumigation. These fumigations are often carried out at unmanned isolated rural locations.

3. Results

The On-Site mixing technique developed by GasApps Australia P/L uses a dual chamber pressure equaliser where both PH_3 and CO_2 are maintained at equal pressure in chambers separated by a regulator diaphragm. Pressure in the dual chambers is equalised by the regulator diaphragm moving on a pivot, which controls gas flow into both chambers. The chamber pressure equalises to the lowest inlet gas pressure. The outlet flow of each gas is controlled by separate metering orifices fitted into the exit of each chamber. The area of the CO_2 orifice required for a mixture of 2.8% PH_3 in CO_2 is thirty-eight times the area of the PH_3 orifice i.e., a 1-mm-diameter PH_3 orifice needs a matching 6-mm-diameter orifice for CO_2.In this way any mixture containing 2.8% PH_3 in CO_2 or less is non-flammable when mixed in any quantity with air. The selected PH_3 in CO_2 mixture is further diluted into a low pressure airflow which can be connected to multiple grain storages. The pressure equaliser mixer, which operates continuously for 18-d exposure time unattended, has performed reliably.

Trials have established that the initiation of polymer formation can be avoided if oxygen levels in the dispensing equipment are less than 100 ppm (all CO_2 cylinders used must be certified less than 0.01% O_2).

The On-Site mixing technique developed by GasApps Australia P/L has been used to treat millions of tonnes of grain. Current annual usage of this technique by GasApps P/L is ~3 t PH_3 which is equivalent to the treatment of 3 million of grain at a concentration of 1 g PH_3/t (720 ppm PH_3).

The On-Site mixed PH_3 and CO_2 is diluted to ~120 ppm PH_3 into the flow-through air with a flow of approximately one volume change per day in the grain storage being treated. The treatment volume is calculated from the volume of the storage independent of amount of grain stored. This treatment continues for an exposure time of 18 days.

4. Discussion

The innovative, dual chamber pressure equaliser, gas mixer adopted for On-Site mixing of PH_3 and CO_2 is a proven reliable device. The PH_3 and CO_2 are maintained at equal pressure and the mixing ratio can be selected by the choice of the fixed orifice fitted in the outlets of the dual chambers. The dual chamber pressure equaliser has performed exceptionally well with continuous duty cycles. The dual chamber pressure equaliser was initially preferred because of its ability to deliver a wide choice of mixed gases and long-term continuous use reliability. An earlier innovation tested was a dual piston pumps mixer with piston volumes in a ratio of 40:1 (2.5 vol% PH_3/CO_2).

The On-Site mixing of PH_3 and CO_2 for the treatment of grain in “non gastight” storages using the flow-through fumigation technique requires continual dosing of PH_3 over a ~3 wk period at isolated and often unmanned grain terminal facilities is an established and growing application technique. The SIROFLO flow-through technique ensures that an effective concentration of PH_3 is maintained in the storage irrespective of losses due to winds or any leakage. The On-Site mixing of PH_3 and CO_2 at the Xizui Grain Terminal, Dalian, China (Newman et al., 2000), was an earlier milestone. This green field site has multiple 30,000 tonne vertical grain storages which are gastight and the initial site storage capacity was 1.4 million tonne. The mixing was carried out using gaseous PH_3 in cylinders and a bulk refrigerated liquid CO_2 tank.

The ongoing application of PH_3 is growing in volume as the required effective concentration continues to be raised to overcome insect tolerance. The trend to 100% gaseous PH_3 has the benefit of reducing the number of cylinders of PH_3 required on site by a factor of thirty-five times (35x). While there are additional benefits of CO_2, there is a trend to minimise the transport of heavy industrial gas cylinders. The On-Site mixing of PH_3 and Air is attractive and it is a growth application. There is potential for the use of On-Site Inert Gas Generators including the old-style burner type (bonus of elevated CO_2, however, it has associated high moisture levels) and the more sophisticated PSA or membrane N_2 generators.

The recycling of forgotten volatile flammable liquid as potential new fumigants continues and most are candidates for On-Site mixing with inert gases. The recent paper by Muhareb et al. (2009) reported

synergy with a mixture of propylene oxide (C_3H_6O), sulfuryl fluoride (SO_2F_2) and CO_2. This combination is a candidate for a Pre-Mix and On-site Mixing to deliver a non-flammable synergized mixture with reported 100% efficacy for all insect life stages.

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ETHOXOFUME 1000 (EtO): methyl bromide alternative update

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Abstract

Ethylene oxide (C_2H_4O = EtO) is made from the oxidation of ethylene and over 15 million tonnes are produced annually. For over 80 years EtO has been used as a sterilant / fumigant. EtO is lethal to bacteria, viruses, moulds, insects and their eggs. Historically EtO was used in the fumigation of bulk grain. EtO is still widely used in "cold" sterilization of medical devices and instruments. With the precondition of destroying vented EtO at the completion of fumigation, EtO could be a niche methyl bromide (CH_3Br = MeBr) alternative. EtO is toxic by inhalation with an LD_{50} of 330 $mg.kg^{-1}$ EtO is classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC). Occupational Limits: TLV-TWA (1 ppm); OEL (UK)-LTEL (5 ppm). EtO is a colourless, highly flammable gas (Lower Explosive Limit (LEL) = 3 vol% in air) which liquefies at 10.9°C. To reduce flammability EtO is mixed 12 vol% EtO in carbon dioxide (CO_2). Onsite mixing of EtO and Air is an option, however the EtO must be kept below 54 $g.m^{-3}$ (3 vol%) – higher doses of EtO would require on-site mixing with CO_2 or N_2. Quarantine fumigations using ETHOXOFUME 1000 are carried out using vacuum chambers to treat non-food import and export commodities. On completion of the fumigation the EtO/Air mixture can be exhausted using a high pressure fan and destroyed in a "burner" where it is converted to CO_2 and H_2O. The Ct product for the control of various species of insects show that EtO on a weight basis ($g.m^{-3}$) has better efficacy than MeBr. A conservative recommended dose rate of 48 $g.m^{-3}$ results in a concentration of 1.2 vol% for MeBr and 2.7 vol% for EtO (this is less than the LEL of 3 vol%).

Keywords: Ethylene oxide, Fumigant, Sterilant, Insecticide, Incineration

1. Introduction

With the Montreal Protocol listing of methyl bromide (MeBr), the search continues for alternative fumigants. The usage for MeBr is now restricted to Quarantine and Pre-Shipment (QPS) i.e. the fumigation of import and exports. In addition to QPS there are some Critical Use Exemptions (CUE) but these are now minimal. Pre - Montreal Protocol the usage of MeBr for stored product fumigations was in excess of 16,000 tonnes. The advantages of MeBr include low cost, short exposure time (hours not days), practically non-flammable and well documented efficacy. It has been difficult to find alternative fumigants to match these advantages.

There has been some effort evaluating existing volatile liquids and gases that could be considered as alternatives to MeBr. The barrier to introduce new chemicals is the high capital cost of chemical synthesis plant. Toxicology studies for new chemicals can also add significantly to pesticide registration costs.

With the exception of EtO most of the current fumigants cannot match MeBr on the critical issues of cost and efficacy. While gaseous phosphine (PH_3) is very attractive with regards to treatment costs, the PH_3 exposure time for bulk grain storage is at least 3x more than the 24 h required for MeBr.

EtO is a long term sterilant / fumigant however its current usage is the sterilisation of medical devices and quarantine fumigation. EtO is lethal to bacteria, viruses, moulds, insects and their eggs. Historically EtO was used in the fumigation of bulk grain. EtO is still widely used in the "cold" sterilization of medical devices and instruments. Current EtO treatments are carried out in vacuum chambers where the EtO is contained under vacuum and the aerated EtO is destroyed post treatment.

EtO is produced industrially by oxidation of ethylene with oxygen at ~250°C over a catalyst comprising metallic silver supported on alumina (>15 million tonnes of ethylene oxide is produced annually). EtO is the chief precursor to ethylene glycol (automotive coolant and antifreeze) and other high-volume

chemicals (surfactants, ethanolamine etc). EtO is manufactured in tonnage quantities in Australia and also imported in one tonne, stainless steel, pressurised drums.

EtO is toxic by inhalation with an LD_{50} of 330 $mg.kg^{-1}$. Laboratory animals exposed to ethylene oxide for their entire lives have had a higher incidence of liver cancer. EtO is classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC). Occupational Exposure Limits: TLV-TWA (1 ppm); OEL (UK)-LTEL (5 ppm).

The US EPA Risk Management Decision (16 April 2008) stated "the benefits of continued use of EtO outweigh the associated occupational risks provided risk mitigation measures specified by the Agency were adopted". With preconditions of strict OH&S practices and destruction of vented EtO at the completion of fumigation, EtO could be a niche methyl bromide MeBr alternative.

EtO is a highly flammable gas (LEL=3% v/v in air) which liquefies at 10.9°C. Jones and Kennedy (1930) solved the EtO flammability issue by mixing 12 vol% EtO in CO_2. The internationally recognised non-flammable mixture is 9 vol% EtO in CO_2. The study of flammability of fumigants was published by Jones (1933) which detailed non-flammable fumigants including EtO (12 vol%), ethyl formate (14 vol%) and propylene oxide (8 vol%) in CO_2.

2. Materials and methods

Fumigation application options for EtO include EtO/CO_2 (existing commercial non-flammable mixture of 9% EtO in liquid CO_2), EtO/Vacuum (existing vacuum chambers are currently used for quarantine fumigations) and EtO/Air (potential application has precedents with the onsite mixing of PH_3/Air).

Fumigation application using EtO/CO_2 has the benefit of using a commercial non-flammable mixture of 9% EtO in liquid CO_2. Historically non-flammability was determined at 12% EtO and for decades the commercial non-flammable mixture was 10% EtO. Currently the internationally recognised non-flammable mixture is 9% EtO in liquid CO_2 (wt% & vol% are identical for EtO & CO_2 mixtures). The disadvantage of the non-flammable EtO/CO_2 is the number of industrial gas cylinders required to fumigate large grain storages.

Fumigation application using EtO/Vacuum also has the benefit of using proven existing technology and equipment. Fumigations using ETHOXOFUME 1000 (EtO), under specific directions by the Australian Quarantine Inspection Service (AQIS), are conducted at approved facilities. These EtO fumigations are carried out using vacuum chambers to treat non-food import and export commodities. AQIS Guideline: "Ethylene Oxide treatment code: T9020" - specifies an initial minimum vacuum of 50kPa, 1200

$g.m^{-3}$ EtO, 5 h exposure at 50°C. Most EtO documented fumigation quarantine schedules recommend a Ct less than 500 $g.h.m^{-3}$ however for snails in cargo the rate is increased a factor of 5 to ~2500 $g.h.m^{-3}$. The current AQIS recommended dosage of 1200 $g.m^{-3}$ for 5 h i.e. a Ct of 6000 $g.h.m^{-3}$ was reduced from 1500 $g.m^{-3}$ for 4 h after the recommendation of Ryan et al. (2004) on flammability issues. It is understood that the very high AQIS dose is required to ensure the effective fumigation of specially challenging commodities requiring fumigation especially coated (paint, resins, plastic) woods and other commodities. The additional sterilising properties of EtO are a bonus associated with using this sterilant/fumigant.

Disadvantage of vacuum application is the capital investment in vacuum chambers and associated equipment and the small capacity of chambers relative to grain storage.

Fumigation application using EtO/Air is not yet a proven technique. The onsite mixing of EtO and Air must be kept below the 3 vol% lower flammability limit. This could be achieved by the addition of EtO to recycled air from the space being fumigated and maintaining the EtO concentration less than 54 $g.m^{-3}$ (3 vol%). Although, higher EtO levels could be entertained, the oxygen level in the storage would need to be lowered – this option would require partial purging with onsite N_2 generation. Forced draught recirculation using high pressure fans has the benefits of achieving uniform distribution; allowing the addition of EtO to compensate for losses associated with leaks / sorption and extracting EtO to enable destruction on completion of fumigation. EtO is easily burnt by venting via an incinerator / thermal oxidiser / catalytic converter. On completion of the fumigation the EtO/Air mixture can be exhausted using the high pressure fan and destroyed in a "burner" where it is converted to CO_2 and H_2O. Other EtO capture and destruction options include converting to ethylene glycol by scrubbing with 5% aqueous sulphuric acid or adsorption onto activated carbon followed by subsequent destruction or burial.

EtO is a sterilant and has potential to sterilise imported grains (devitalisation of grain/exotic weed seeds and elimination of pathogens) and sterilise soil (microbes, insects, nematodes, weeds). High levels required for grain sterilisation would need to be conducted in modified atmospheres (lower oxygen with onsite generated N_2 to avoid flammability issues). While CO_2 is the preferred diluent for non-flammable mixtures, the transport cost of CO_2 to remote grain storage sites eliminates any benefit over on-site N_2 generation. The safe use in soil may be possible as the high EtO solubility in water allows "in-line" fumigation using water as the carrier. The reaction of EtO with water forming ethylene glycol should ensure minimal release of EtO to the atmosphere in soil fumigation applications.

3. Results

Reported Ct fumigant concentration x time product for the control of various species of insects (Monro, 1969) show EtO and MeBr have similar efficacy. While the Ct favours MeBr (M.Wt. = 94.94) this is more than equalised by the higher (2x) concentration achieved using ethylene oxide (M.Wt. = 44.05) e.g. a dose rate of 48 $g.m^{-3}$ results in a concentration of 1.2 vol% for MeBr and 2.7 vol% EtO.

The high concentration achieved by EtO neutralises any differences so the recommended dose rate for methyl bromide and ethylene oxide should be similar (it should be possible to use 30% lower EtO dose than MeBr). The recommended 48 $g.m^{-3}$ (2.7 vol% EtO) dose is attractive as it achieves an EtO level below the flammability level in air and this concentration should be efficacious for stored product insects in less than 24 h exposure time.

While ethylene oxide doesn't have ozone depletion issues, it is a known carcinogen and OH&S issues require significant more aeration than methyl bromide. As with existing sterilisation practice and registered label recommendations the aeration clearance level for ethylene oxide is the TLV i.e. 1 ppm.

Existing EtO sterilisation chambers are fitted with thermal oxidisers to ensure the EtO vented is less than 1 ppm. EtO readily burn to form CO_2 and H_2O. On-going testing is evaluating prototype toxic gases burning and pyrolysis in high temperature aggregate beds. While high temperature pyrolysis is shown to be effective with EtO, other issues include the development of portable equipment to service multiple locations and the need to accommodate large air dilution required to reach the 1 ppm TLV level.

4. Discussion

The sterilant / fumigant EtO, a known carcinogen, could have niche fumigation-sterilisation applications if the aerated gas post fumigation could be destroyed. EtO, a universally produced industrial chemical (>15 million tonne pa) has potential as a stored product fumigant.

Reported Ct product of fumigants for the control of various species of insects (Munro, 1969) show EtO and MeBr have similar efficacy (possible to use 30% lower dose using EtO vs. MeBr). Reported Ct product suggests 48 $g.m^{-3}$ as a maximum dose rate for stored product pests. Bond (Table 161984) gives Ct for eleven fumigants (including MeBr and EtO) for eight economic stored product pests.

EtO is toxic by inhalation with an LD_{50} of 330 $mg.kg^{-1}$. EtO is classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC). Occupational Limits: TLV-TWA (1 ppm);

As the cost and efficacy is comparable to MeBr, the outstanding issue is the safe application of EtO and development of portable high temperature thermal oxidisers to destroy aerated EtO to ensure there is less than 1 ppm emitted.

This approach should satisfy the US EPA who sees benefits in the continued use of EtO provided risk mitigation measures are adopted

Acknowledgements

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Destruction of toxic and environmental harmful gases

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Abstract

Environmental and Occupational Health and Safety or OH&S issues have influenced the focus to capture / destroy toxic and environmental harmful gases expelled from fumigated storage. These storages are aerated to Threshold Limit Value (TLV) levels at the completion of fumigation to ensure it is safe for human re-entry. Most fumigants by design are toxic and some have additional environmental hazards. The once universal fumigant, methyl bromide (CH_3Br), while restricted by the Montreal Protocol because it is an ozone depletor, is still used commercially in tonnage quantities. The use of CH_3Br is now mostly restricted to Quarantine Pre-Shipment (QPS) fumigations. The CH_3Br dose level is approximately 10.000 ppm and the recommended maximum respiratory level is 5 ppm. The universal fumigant gas, phosphine (PH_3), a very toxic gas, has a recommended TLV of 0.3 ppm. The sterilant, ethylene oxide (EtO), a known carcinogen, could have niche fumigation-sterilisation applications if the aerated gas could be destroyed. The use of heat to decompose toxic gases can involve the extracted contaminated air being fed into a burner where the hot zone destroys and decomposes the vulnerable fumigant gas. While flame burning at high temperature pyrolysis is 100% effective with EtO, most fumigants will form acidic by-product. Other issues include the need to re-locate capture and destruction equipment to service multiple locations and the large dilution required to reach TLV levels. A current alternative consists of adsorbing fumigants on activated charcoal. The spent activated charcoal requires chemical processing and or burial. There are cost issues with the initial purchase of the activated charcoal and post-treatment chemical processing of the adsorbed fumigant. Another disposal option is the burial of the spent charcoal in landfill sites. The ongoing safe use of fumigants is critical to important export industries especially the large volume bulk grain and export timber market.

Keywords: Fumigants, Capture, Destruction, Incineration, Acidic reactants.

1. Introduction

International border protection requires elimination of the introduction of quarantined pests and pathogens. As an aid in this endeavour, imports and exports are subjected to mandatory quarantine and pre-shipment fumigations using toxic gases.

The Australian Quarantine and Inspection Service (AQIS) manage quarantine controls at Australian borders to minimise the risk of exotic pests and diseases entering the country. AQIS also provides import and export inspection and certification to help retain Australia's highly favourable animal, plant and human health status and wide access to overseas export markets. In addition to quarantine requirements, high volume fumigations are carried out by bulk grain and food manufactures for stored-product insects in grain and processed foods.

Fumigants by their nature and properties are toxic gases and vapours. Imports and exports are subjected to mandatory quarantine and pre-shipment fumigations using toxic gases (methyl bromide, phosphine, sulfuryl fluoride and ethylene oxide).

Occupational Health and Safety (OH&S) and Environmental requirements are an issue and are best achieved by removal of fumigants at the completion of the fumigation. The removal of fumigant is usually carried out by the fumigators who take positions up-wind and vent the fumigant to atmosphere by lifting the tarpaulin sheets. The use of forced air from electric fans are sometimes used. With grain silos, aeration fans if available, can be used to speed up the venting of fumigants. Earlier industry practice of fume extraction allowed toxic gas concentration of four hundred times (400x) the Threshold Limit Value (TLV) to be vented, which in the case of CH_3Br is 2000 ppm. This high level was justified when using a

stack vent and relying on dilution in the atmosphere. Today, when approval is sought, the concentration required by Australian authorites, is usually the TLV, ie 5 ppm with CH_3Br.

Capture and removal of the toxic fumigants ensure safe working conditions and avoid contamination of the environment. The fumigant gas recapture system requires the aeration of the fumigated commodity and subsequent capture of the diluted fumigant from the contaminated air to reduce the risk of human exposure to employees, bystanders, transporter and un-packers.

In addition to OH&S concerns there is the environmental issue of ozone depletion with the fumigant, methyl bromide. A number of countries have regulated the mandatory capture of methyl bromide and other fumigants. The current "degassing" method consists of adsorbing fumigants on activated charcoal. The spent activated charcoal requires chemical processing and or burial. There are cost issues with the initial purchase of the activated charcoal, post-treatment chemical processing of the adsorbed fumigant or burial of the spent charcoal in landfill sites. The ongoing safe use of fumigants is critical to important export industries especially the large volume bulk grain and export timber market.

The innovation described in this paper is a thermal oxidizer system fitted with a solid-state-acidic reactor to eliminate any acidic gases formed in the burning process.

The technique used includes the use of a blower sucking air and fumigant from the fumigation space. The make-up air is controlled to maintain a negative pressure in the fumigation space, which has the beneficial properties of assisting desorption of the fumigant and maintaining a weather-proof seal when using tarpaulins in stack fumigations. The blower discharges the fumigant/air mixture into a burner that will heat the air, burn and decompose the fumigant. At elevated temperature the fumigants will break down into products of decomposition and combustion, some of which will be acid gases. The burner discharges the hot gas (surplus air, nitrogen, products of decomposition and products of combustion) into a hot solid alkaline aggregate which will react with acid gases.

2. Materials and methods

Field trials were carried out on FCL (full container load) shipping containers undergoing stack quarantine fumigations. The following equipment was fitted onto fumigated containers requiring aeration: high pressure blower: - GAST Model # R5325A-2 (static pressure = 14.9 kPa; airflow rate = 8 m^3/h), incinerator: - 0.3 m x 1.5 m steel, burner: propane flare, absorbent: 130 mm deep aggregate bed on steel wire mesh support. Special attention was required to keep combustible materials (tarpaulin, rubber hose tyres and plastic pipe) away from the incinerator; and to reseal the system after opening for the lighting of the burner. A length of copper tubing was used to sample the hot gas emitting from the top of the incinerator and to deliver a cool gas sample to the analyser instrument. High methyl bromide readings were analysed using an Interferometer and a Photo Ionisation Detector (PID) used for methyl bromide levels less than 1000 ppm.

Up to nine containers are the norm but for this field trial a stack of four containers (72 m^3 x 4 = 288 m^3) were used. The containers were sealed by a tarpaulin and made gastight using "sand snakes" at the point where the tarpaulin touches the concrete pad. The fumigation space needs to be "gastight storage" to ensure the minimum Concentration x time (Ct) product required for a successful fumigation. This helped in producing a negative pressure during the trial.

The concentration of the methyl bromide fumigant initially was approximately 10.000 ppm (1.0 vol%) which decreases over the fumigation exposure time (in some situations the concentration may be maintained at a constant level). At the completion of the fumigation the methyl bromide fumigant level needs to be cleared to be below the TLV of 5 ppm.

Usually forced air draft is used to aerate the fumigated containers. For the field trials the high pressure blower was used to create a partial vacuum. This resulted in a more controlled flow which had the additional benefits of more uniform extraction of the fumigant and shrink-fitting of the fumigation tarpaulin sheets. The flow was approximately 3 air changes/hour and the dilute fumigant / air mixture was passed into a flame and then into a heated aggregate bed.

The flame and bed act as a "thermal oxidiser" converting the methyl bromide to hydrobromic acid (HBr) which can be removed using alkaline absorbers. Because of the high temperature, solid aggregates absorbers are preferred. The flame can produce temperatures up to 2000°C but would normally be

regulated to the decomposition or auto ignition temperature of the fumigant (methyl bromide auto ignition temperature is 537°C).

3. Results

As the early prototype was not capable of being pressurized, the initial trials were conducted with a shallow 130 mm deep solid adsorbent bed to allow gas flow through the bed.

The level of methyl bromide dropped from 3000 ppm to 25 ppm in less than one hour which was satisfactory as it included the warm-up time for the bed.

The heating of the bed was not uniform as indicated by variations in colour indicating channelling in such a shallow bed. The maximum heating was concentrated above the flare burner. The burner test was limited to one hour and the aeration was completed by standard fumigation practice.

4. Discussion

The innovation of using a blower to create a partial vacuum assisting the aeration of fumigated shipping containers was of interest to quarantine fumigators. The blower would allow fumigators to run aeration unattended overnight without concern of wind driven rain causing water damage to FCL contents. The partial vacuum ensured a stretched and water proof seal on the containers being aerated.

The use of a blower allows the attachment of a burner and absorber to capture and destroy the fumigant emissions. The initial test quickly reduced the methyl bromide emission from 3000 ppm to 25 ppm and it is expected that further tests will reduce methyl bromide levels to 5 ppm and fix any acid gas.

Future testing will include improved temperature control of the mixed gases, uniform flow through deeper adsorbent beds and the selection of effective aggregate absorbents.

The current aeration of containers after fumigation is very open and makes no effort to capture the residual fumigant. The proposed system is a closed one with the capture of the fumigant, and by-products within the system. Because of the high temperature involved in the thermal oxidation, some care is required in the sealing and operation of the system.

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Phosphine generator trial using external air dilution

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Abstract

A commercial phosphine (PH_3) generator manufactured by Beijing Liangmao Technology Development Company Limited, China was used in a fumigation trial on 5000 tonne bunker storage. The generator's production of PH_3 is controlled by the rate of dosing conventional aluminum phosphide (AlP) tablets into 100 kg water in the reaction chamber.

Because PH_3 is flammable and explosive if certain a concentration is reached in air, it is usually mixed with carbon dioxide (CO_2) in the weight ratio of 50:1. This trial's objective was to minimise the amount of CO_2 and dilute the generated PH_3 with air. The PH_3 generated is purged from the reaction chamber by a continuous low flow of regulated CO_2 gas. The PH_3-rich CO_2 purge stream was fed into the suction inlet of an external venturi. A high pressure fan was used to produce the air flow through the venturi to create the vacuum to suck in and dilute the PH_3 rich stream from the generator. The PH_3 was diluted in the air-flow to ensure a non-flammable concentration of less than 1% (10.000 ppm) and thence fed directly into the bunker storage. The trial successfully demonstrated the viability of generating phosphine in this manner, and excellent results in PH_3 concentration and distribution was achieved in very short time periods. The capacity of the generator was limited to 8 kg of AlP tablets, equivalent to 2.667 kg of PH_3, or sufficient to fumigate 2667 m^3 of grain at a dosage rate of 1 g/m^3. A much larger version of the machine would be required to fumigate medium size bunker storages in the 10.000 to 15.000 tonne range. Notwithstanding, the generator offers an attractive package combining the speed and convenience associated with the use of gaseous PH_3 with the low cost of AlP formulations.

Keywords: Phosphine fumigant, On-site generator, Metallic phosphide, Non-flammable

1. Introduction

The preferred fumigant for stored product insect in bulk grain is phosphine because it is the lowest cost fumigant when generated from solid metallic phosphide products (usually aluminium phosphide: AlP). The major hazard concern with phosphine is its wide range of flammability with a lower flammability limit of 1.8% in air. Another issue with PH_3 is its approximate 5 day exposure time which is extended by the 1-2 days required to generate PH_3 gas from AlP tablets reaction with atmospheric moisture.

PH_3 was mostly generated in-situ from AlP tablets until the commercial availability of PH_3 as a compressed gas [either as 100% or non-flammable mixtures of PH_3 in CO_2 (2.6 vol%) or nitrogen: N_2 (2.0%)].

On-site PH_3 generators allow the quick release of PH_3 from AlP tablets by dropping the tablets into water; however this exacerbates the flammability issue. One solution is to purge with large volumes of CO_2, however the additional number of cylinders required adds cost and complications in remote locations (N_2 cylinders can be substituted for CO_2 however this required three times the number of gas cylinders). While some CO_2 cylinders are required for pre-purging and post-purging the reactor, the required number is significantly reduced by mixing the generated PH_3 with air prior to release into the storage to be fumigated. While related techniques can be used, this trial used a venturi which allowed the intimate mixing of PH_3 and air to a non-flammable level of less than 10.000 ppm prior to discharge into the grain storage.

2. Materials and methods

A commercial PH_3 generator manufactured by Beijing Liangmao Technology Development Company, China was used in the fumigation of 5000 tonnes of wheat. The generator's production of PH_3 is controlled by the rate of dosing conventional Aluminum phosphide (AlP) tablets into 100 kg water in the reaction chamber. The maximum rate of dosing is 24 tablets/minute (24 g of PH_3/minute = 72 g of AlP/minute). The recommended capacity of the generator was 8 kg of AlP tablets, equivalent to 2.667 kg of PH_3, or sufficient to fumigate 2667 m^3 of grain at a dosage rate of 1 g/m^3.

Because PH_3 is flammable and explosive over 1.8% [18000ppm] PH_3 it is usually mixed with CO_2 in the weight ratio of 50:1 (2% by weight PH_3 in CO_2 is non-flammable). This trial's objective was to minimise the amount of CO_2 and dilute the generated PH_3 with air. The PH_3 generated is purged from the reaction chamber by a continuous low flow of regulated CO_2 gas. The PH_3-rich CO_2 purge stream is fed into the suction inlet of an external venturi. A high pressure fan was used to produce the air flow through the venturi to create the vacuum (1 kPa) to suck in and dilute the PH_3 rich stream from the generator. The PH_3 was diluted in air flow to a non-flammable concentration of less than 1% (10,000 ppm) prior to delivering this air to the bunker storage.

The measurement of the PH_3 was achieved with commercial instruments used by field fumigators [MiniWarn – Draeger; Silo Chek – Canary Co]. Dilution equipment was prepared which was capable of ten times dilution in air of any PH_3 samples greater than 1000 ppm to keep within the range of the electrochemical instruments used.

3. Results

The trial successfully demonstrated the viability of generating phosphine in this manner, with excellent results in PH_3 concentration and distribution of non-flammable PH_3 gas was achieved in very short time periods (~4 hours, Figures 1 and 2). The generator was operated at its maximum dispensing rate of 24 tablets/minute (24 g of PH_3/minute = 72 g of AlP/minute). In excess of 50 samples were taken to monitor the PH_3 level in the air flow into the bunker grain storage. The cumulative PH_3 produced is graphically presented in Figure 1, and shows 80% is obtained within 3 h and 100% after 6 h. The PH_3 concentration (ppm) profile over a seven 7 h period is shown in Figure 2. The peak PH_3 concentration measured was 4400 ppm and the irregular peak (at 2 h) was the result of temporary blockage in the tablet dispenser. The "blip" at the end of the 7 h period was the result of a mineral acid addition to determine residual PH_3.

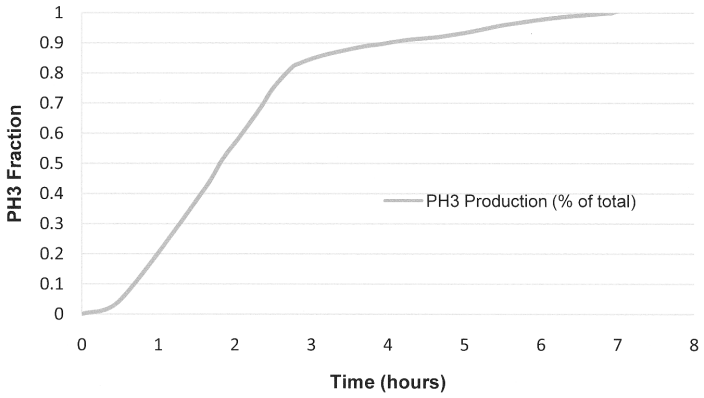

Figure 1 PH_3 generator Trial (PH_3 Production - % of total).

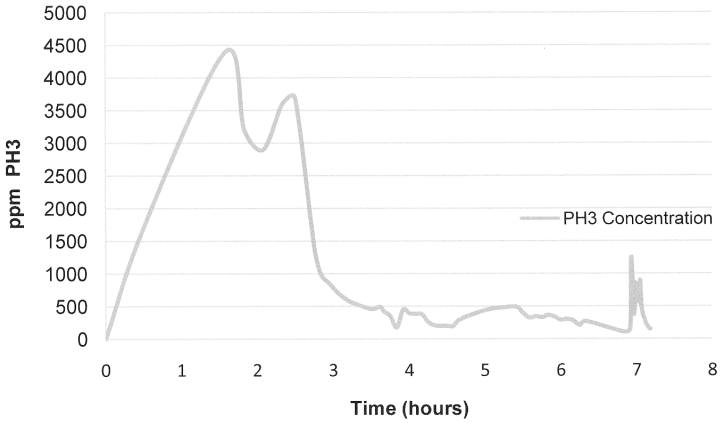

Figure 2 PH_3 generator trial (PH_3 Concentration).

The CO_2 purge was ~3 kg/h and PH_3 generation was ~0.6 kg/h i.e. a ratio of CO_2 to PH_3 of 5:1 which is a tenfold reduction in CO_2 consumption.

4. Discussion

The time required to generate PH_3, while an order of magnitude improvement on generation from AlP exposure to atmosphere moisture, needs to be further reduced. The capacity of the PH_3 generator was 8 kg of AlP tablets, is limited and required "time-out" to prepare additional batches where required.

The field trial demonstrated the need for a larger PH_3 generator (5x times the capacity of the unit tested) and reduction of the PH_3 generating time.

The purge ratio of CO_2 to PH_3 of 5:1, a ten-fold reduction in CO_2 consumption required for a non-flammable mixture, will need to be maintained but preferably reduced using the proposed larger generator (15 kg PH_3).

The much larger version of the PH_3 generator is required to fumigate medium size bunker storages in the 10.000 to 15.000 tonne range. Preference is for the re-design unit to incorporate "real time" removal of spent tablet residues with addition of a filter to minimise the scale up required for the reaction chamber.

Notwithstanding, the existing generator offers an attractive package combining the speed and convenience associated with the use of gaseous PH_3 with the low cost of AlP formulations.

Acknowledgements

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Alternative fumigants to methyl bromide for the control of pest infestation in grain and dry food products

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Abstract

The primary aim of the current study is to evaluate the potential use of the known isothyiocyanates (ITC) as compared to a new ITC isolated from *Eruca sativa* (salad rocket) as fumigants for the control of stored products insects. The biological activity of methyl iodide (CH_3I), carbon disulphide (CS_2), benzaldehyde (C_7H_60) and essential oils were also evaluated. The toxicity of the various fumigants was assessed against adults and larvae of a number of major stored-product insects. ITCs are potential candidates because only very low concentrations are needed for the control of stored-product insects. It should be mentioned that *Eruca sativa* is used worldwide as a food supplement. Methyl thio-butyl ITC the main bioactive component in this plant has high toxicity against insects, but lower mammalian toxicity as compared to other active ITCs. Comparative studies with CH_3I, CS_2 and C_7H_60 showed that the first was the most active compound against stored-product insects followed by the second and the third. CH_3I was found less sorptive and to be less penetrative in wheat than CS_2. The activity of some essential oils was also evaluated. In this context, we should keep in mind that a general consensus is very difficult to achieve in order to introduce broad-spectrum fumigants like methyl bromide or phosphine. Because of this, alternative fumigants could be developed against particular species of insects or to be used for specific food product commodity.

Keywords: Fumigants; Isothiocyanates; Methyl iodide; Carbon disulfide; Benzaldehyde.

1. Introduction

Insect damage in stored grain and other durable commodities may amount to 10-40% in developing countries, where modern storage technologies have not been introduced (Raja et al., 2001). Fumigation is still one of the most effective methods for the protection of stored grain and dry food from insect infestation. At present, mainly two fumigants are still in use: methyl bromide and phosphine. The first one is being mostly phased out in developed countries due to its ozone depletion effect (WHO, 1995; Shaaya and Kostyukovsky, 2006). Phosphine is mainly used today, but there are repeated reports that a number of storage pests have developed resistance to this fumigant (Rajendran and Karanth, 2000). Therefore, there is an urgent need for new strategies to focus on a search for alternative fumigants for the control of stored-product insects. In this paper we present a comprehensive study to evaluate the potential use of isothiocyanates (ITCs), methyl iodide (CH_3I), carbon disulfide (CS_2), benzaldehyde (C_7H_60) and essential oils (EOs) for the control of stored-product insects.

ITCs were chosen for this study because of the pesticidal properties of the chemicals (Fenwick et al., 1983) and the potential use of methyl ITC as fumigant for wheat (Ducom, 1994).

CH_3I is known as a potent insecticide. CS_2, according to Winburn (1952), was one of the most effective grain fumigants as viewed from efficiency and low cost. C_7H_60, occurs in kernels of bitter almonds, has low toxicity to mammals and it is used in topical antiseptics. The bioactivity of essential oils, the major volatile in aromatic plants, and their constituents has been well studied against a large number of stored-product insects. (Regnault-Roger and Hamraoui, 1995; Raja et al., 2001). In our laboratory, by screening a large number of EOs we could isolate two very active fumigants obtained from Lamiaceae plants. The main component of one of the oils is pulegone. The other is not yet identified.

2. Materials and methods

The tested stored-product insects were laboratory stains of *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *Trogoderma granarium* Everts and *Plodia interpunctella* (Hübner).

The ITCs were obtained by putting 100 g ground seeds into round bottom flask containing buffer solution (1% ascorbic acid). The flask is held in a water bath at 70°C for two hours to facilitate the hydrolysis of sinigrin to ITC by the enzyme myrosinase which is found inside the seeds. The second step is steam distillation with use of the Dean-Stark apparatus (Leoni et al., 1997). The yellow upper layer is then separated and extracted with petroleum ether. Finally, the petroleum ether is evaporated under a stream of air. The unknown ITC obtained from the seeds of *Eruca sativa* was identified as methylthio butyl isothiocyanate by gas chromatography (GC), nuclear magnetic resonance (NMR) and infra-red (IR) spectroscopy. CS_2, CH_3I and C_7H_60 were purchased from Sigma Chemical Company, St. Louis, MO, USA. The essential oils from the aromatic plants were obtained from freshly harvested leaves and stems by steam distillation.

Two types of bioassays were performed to evaluate the activity of the fumigants. The first screening of the compounds was space fumigation in glass chambers of 3.4-L capacity (Shaaya et al., 1991). The highly active compounds were then assayed in simulation glass columns 10 cm in diameter × 120 cm in height, filled to 70% by volume with wheat (11% moisture content). The insects were introduced in cages, each holding 20 insects of the same species together with food. Groups of four cages were suspended by a steel wire at different heights from the bottom of the column. Insect were exposed to fumigants for 3 to 72 h, removed from fumigation, held for 7 d and the mortality assessed.

3. Results

3.1. Toxicity of isothiocyanats (ITCs)

Mustard family (Brassicaceae) seeds contain ITCs, volatile essential oils that are known to possess insecticidal activity. By screening a number of various species of Brassicaceae seeds it was possible to isolate from seed oils of *Sinapis arvensis*, *Eruca sativa* and *Diplotaxis* spp. an unknown ITC at concentrations of 98%, 92% and 33%, respectively. Later, this compound was identified as methylthio butyl ITC. In space fumigation, the biological activity of this compound was compared with four common ITCs, namely, allyl, methyl, butyl and ethyl. Allyl and methyl ITCs were found to be the most potent against adults and larvae of four and three stored-product insects respectively. A concentration of 1 $\mu L \cdot L^{-1}$ air and exposure time of 4 h was enough to kill all the tested adult insects, except for *T. castaneum,* which was found much more susceptible to these two ITCs (Table 1).

Table 1 The fumigant toxicity 7 d after treatment with four active isothiocyanates compared with methylthio-butyl ITC isolated from the plant *Eruca sativa* (space fumigation).

			Mortality (%)						
			Larvae			Adults			
Compound	**Conc. (µL/L)**	**Exposure time (h)**	***Tribolium castaneum***	***Trogoderma granarium***	***Plodia interpunctella***	***Sitophilus oryzae***	***Rhyzopertha dominica***	***Oryzaephilus surinamensis***	***Tribolium castaneum***
Allyl ITC	1.0	3.0	-	-	-	100	100	100	100
	1.5	3.0	23	84	100	-	-	-	-
Methyl ITC	1.0	2.5	-	-	-	100	100	100	100
	1.5	3.5	100	81	100	-	-	-	-
Methylthio-butyl ITC	1.0	3.0	-	-	-	100	89	100	0
	1.0	3.0	100	100	87	-	-	-	-
Ethyl ITC	1.0	3.0	-	-	-	100	100	100	0
	1.5	3.0	20	6	100	-	-	-	-
Butyl ITC	1.5	3.0	-	-	-	65	43	68	25
	1.5	3.0	5.5	23	7	-	-	-	-

Third instar larvae were used; Mortality in control less than 5%

3.2. Efficacy of CH_3I, CS_2 and C_7H_60 as fumigants

In space fumigation, CH_3I was very effective against all insect stages tested. Exposure to a concentration of 3 to 5 $\mu L \cdot L^{-1}$ for 3 h was lethal and caused 100% mortality of all stages of the test insects, except for *T. granarium* larvae (Table 2). Adults of *T. castaneum* were found the most tolerant followed by *R. dominica, S. oryzae* and *O. surinamensis*. In the case of larvae, *T. granarium* was most tolerant, followed by *T. castaneum* and *P. interpunctella* (Table 2).

Table 2 Mortality 7 d after treatment with CH_3I, CS_2 and benzaldehyde against stored-product insects in space fumigation.

			Mortality (%)						
			Adults					Larvae	
Compound	Exposure Time (h)	Conc. (µL/L)	*Sitophilus oryzae*	*Rhyzopertha dominica*	*Oryzaephilus surinamensis*	*Tribolium castaneum*	*Tribolium castaneum*	*Trogoderma granarium*	*Plodia interpunctella*
CH_3I	3	3	94	85	100	65	40	-	100
		4	100	100	-	95	77	58	-
		5	-	-	-	100	100	70	-
CS_2	24	5	72	53	23	0	-	-	-
		7	100	100	74	10	-	-	100
		9	-	-	100	100	100	60	-
Benzal-dehyde	24	1.5	79	16	39	0	-	-	-
		3	100	100	100	65	0	0	-

CS_2 = Sp. gravity 1.26; Third instar larvae were used; mortality in control less than 5%

CS_2 was less effective than CH_3I and needed a concentration of 7-9 $\mu L \cdot L^{-1}$ air for one day to achieve total mortality of all the test insects for *T. granarium* larvae. In the case of CS_2, adults of *T. castaneum* were found to be the most tolerant, followed by *O. surinamensis, R. dominica* and *S. oryzae.* The larvae of *T. granarium* were more tolerant than *T. castaneum* (Table 2).

C_7H_60 was less active than CH_3I and CS_2 in space fumigation bioassays. A dosage of 3 $\mu L \cdot L^{-1}$ air and exposure time of 1 d caused 100% adult mortality of all the tested adult insects except for *T. castaneum* which caused only 65% kill (Table 2).

In glass columns filled to 70% wheat CS_2 penetrated better than CH_3I, but needed a higher concentration to achieve total mortality (Table 3). It should be mentioned that for methyl bromide fumigation the recommended concentration is 30 to 50 g/m^3.

Table 3 Toxicity of CH_3I and CS_2 to adult stored-product insects at different depths in 120 cm-high columns filled with 70% wheat by gravity.

				Mortality (%)			
Compound	Conc. (µL/L)	Exposure Time (h)	Depth in grain (cm)	*Rhyzopertha dominica*	*Oryzaephilus surinamensis*	*Sitophilus oryzae*	*Tribolium castaneum*
CH_3I	5	24	20	100	100	100	100
			120	10	10	30	0
		72	20	100	100	100	100
			120	95	75	80	0
CS_2	20	48	20	100	100	100	100
			120	100	0	30	10
		72	20	100	100	100	100
			120	100	100	100	100

Mortality in control less than 5%

3.3. Studies with essential oils (EOs).

The essential oils (EOs) of aromatic plants families are volatiles that can be easily extracted by hot water vapors. The main components of the EOs are monoterpenes and to a lesser extent, sesquiterpenes (Brielmann et al., 2006).

In order to isolate bioactive EOs, we screened a large number of EOs extracted from aromatic plants and isolated their main constituents by methods cited in Shaaya et al., 1991, 1994, 1997. Using space fumigation methodology two EOs obtained from *Lamiaceae* plants, were found to be the most potent fumigants as compared with all other essential oils obtained from a large number of aromatic plant species tested against stored-product insects. The main component of one of the EOs was pulegone, and of the other is not yet totally identified, and it is called SEM76. In space fumigation, these two volatiles

caused total mortality of all adults tested at very low concentration of 0.5 $\mu L \cdot L^{-1}$ air and exposure time of 24 h. For comparison we tested also limonene which represents most of the other EOs tested (Table 4).

In glass columns filled to 70% volume with wheat showed that SEM76 at a concentration of 70 $\mu L \cdot L^{-1}$ air (equivalent to 70 $g \cdot m^{-3)}$ and 7 d exposure time caused 100% kill of adults of *S. oryzae* and *O. surinamensis* but not of *T. castaneum and R. dominica* (Table 4). Supplementation of 15% CO_2 (200 $g \cdot m^{-3}$) caused reduction in the effective volatile concentration. The concentration of 50 $\mu L \cdot L^{-1}$ air was enough to cause 96-100% kill of all adult insects tested (Table 4).

Table 4 Fumigant toxicity of SEM 76 and Pulegone in space fumigation and columns with and without CO_2.

Fumigation	Compound	Conc. (µL/L)	Mortality (%)			
			Rhyzopertha dominica	*Oryzaephilus surinamensis*	*Sitophilus oryzae*	*Tribolium castaneum*
Space	SEM 76	0.5	100	100	100	87
		1	100	100	100	100
	Pulegone	0.5	100	100	100	100
	Limonene	0.5	27	27	24	0
Columns	SEM 76	70	100	100	70	66
		70+15% CO_2	100	100	100	100
		50+15% CO_2	100	100	100	96

4. Discussion

Our findings showed that ITCs are potential candidates because only very low concentrations are needed for the control of stored-product insects. *Eruca sativa* (salad rocket) is used worldwide as a food supplement and methylthio butyl ITC, the main bioactive component in this plant, has lower mammalian toxicity as compared to the other active ITCs tested. The lower toxicity makes this fumigant a promising candidate for the disinfestations of grain and dry food products.

Comparative studies with C_7H_60, CH_3I and CS_2 showed that CH_3I was the most toxic compound to stored-product insects, followed by CS_2 and C_7H_60. CH_3I was found less penetrative in wheat then CS_2. CH_3I is toxic to humans and its use in food as a fumigant therefore is limited. CS_2 is flammable and can be used mainly as a supplement to increase the activity of other fumigants. In fact, a mixture of trichloroethylene, carbon disulphide and carbon tetrachloride (Calandrex^R) in a ratio 64:26:10, respectively, was developed by us and was found to be effective against stored-product insects (Polacek et al., 1960). C_7H_60 has low toxicity to mammals, but it is less effective against stored-product insects than all other fumigants tested. These three compounds may play a role mainly as supplements to increase the activity of other fumigants.

Our findings, as well as those of other researchers, suggest that certain plant essential oils and their active constituents, mainly terpenoids, have potentially high bioactivity against a range of insects and mites. They are also highly selective to insects, since they are probably targeted to the insect-selective octopaminergic receptor, a non-mammalian target (Kostyukovsky et al., 2002). The worldwide availability of plant essential oils and their terpenoids, and their use in cosmetics and as flavoring agents in food and beverages, is a good indication of their relative safety to warm-blood animals and humans. The ultimate goal is the introduction of these phytochemicals with low toxicity, which comply with health and environmental standards, as alternatives to methyl bromide and phosphine for the preservation of grain and dry food.

We should keep in mind that is very difficult to achieve the introduction of broad-spectrum fumigants like methyl bromide or phosphine. In this context, alternative fumigants could be developed against particular species of insects or to be used for a specific food product commodity.

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Life stage and resistance effects in modelling phosphine fumigation of *Rhyzopertha dominica* (F.)

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Abstract

Resistance to phosphine in insect pests of stored grain is a serious problem and there is a world-wide need for the development of sustainable resistance management strategies. Here we introduce results from a new mathematical model of resistance development that includes all life stages, rates of oviposition, natural mortality and mortality under fumigation in relation to resistant genotype. The example we discuss is phosphine resistance in the lesser grain borer, *Rhyzopertha dominica* where resistance is known to be controlled by two major genes that are close to recessive in expression, so that resistance is not fully expressed unless both resistant genes are present and homozygous. An example of a scenario where this model could be used concerns the repeat application of phosphine in a situation where control of all life stages has not been achieved. We determined a critical interval within which a second fumigation must occur to stop a rapidly recovering population of resistant genotypes. Such scenarios can be readily investigated using this approach to provide the grain industry with resistance management options and strategies.

Keywords: *Rhyzopertha dominica*, Population dynamics, Stored wheat, Phosphine fumigant, Low concentration

1. Introduction

The Australian grain industry has a zero tolerance to the presence of insects in stored grain, and phosphine fumigant is the primary method of control. The genetic resistance of *Rhyzopertha dominica* (F.) to phosphine has driven research to determine how fumigation strategies may effect the proliferation of resistant genotypes, and conversely how to control infestations that contain phosphine resistant pests when increasing the applied dosage may not be practicable. A deterministic two-locus genetics model has been developed using a mating table and a novel death rate under fumigation (Lilford, 2009). The model has had some success in determining the dynamics of the population of *R. dominica* with various levels of resistance to phosphine under phosphine fumigation of varying dosages.

Resistance to phosphine is an inherited trait and two major genes are responsible for the strong phosphine resistance in *R. dominica* (Collins et al., 2002). These two genes act in synergism to display a significantly increased resistance to phosphine compared to any one of the resistance genes on their own (Schlipalius et al., 2008). Phosphine resistance is incompletely recessive at both genes (Schlipalius et al., 2008). That is, individuals that are heterozygous have a phenotypic response similar to susceptible individuals.

Lilford (2009) demonstrated that a two-locus model yielded different conclusions compared to a one-locus model; the single locus approach greatly exaggerates the rate of increase of the strongly resistant individuals compared to the other genotypes.

A greater tolerance to the phosphine fumigant has been observed in the egg and pupa stages (Hole et al., 1976). The Lilford (2009) model does not incorporate any life stage characteristics such as oviposition or stage-specific tolerance to phosphine, therefore a model incorporating life stages was developed to investigate *R. dominica* population dynamics in a more realistic fashion.

For the purposes of the current model, we have assumed that eggs and pupae may be up to three times more tolerant to phosphine than adults. Incorporating life stages into the current model provides us with the opportunity to investigate fumigation strategies that can optimise eradication or be identified as ineffective, in situations where control is not complete and eggs and pupa of the pest survive. In this paper, we outline aspects of the models of interest, and give some preliminary results into the effects of multiple fumigations with the life stage model.

2. Methods: Modelling R. dominica populations in grain silos

The following discussion is concerned with the rates used in the mathematical models. Because the generations are overlapping, the populations are modeled as a continuous function of time. There are thirty-six dependent variables, corresponding to the nine genotypes for each of the four life stages. Therefore, the model consists of thirty-six differential equations with terms corresponding to the complex interactions between genotypes and life stages.

2.1. Genotypical resistance

Similar to Lilford (2009), we model the population of these nine genotypes (i,j each taking values susceptible (s), hybrid (h) or resistant (r) by using an eighty-one times nine mating table (Lilford, 2009) to determine progeny genotype. This mating table permutes every combination of male and female mating, and determines the rate of progeny in a particular genotype when multiplied by the birth rate.

During fumigation, the birth rate (that is; eggs laid per unit time) is considered to be zero, the death rate becomes that due to fumigation and the model becomes a simple exponential relation, that is;

$$N_{ij}(t) = N_{ij}(0).e^{a_{ij}t} \quad (1)$$

where the death rate under fumigation α_{ij} is different for each genotype $\{i, j \in s, h, r\}$ and is dependent on the concentration of phosphine used. The dosage is predominantly dependent on time and concentration of phosphine, and we use Haber's rule (Bunce and Remillard, 2003) to determine a ratio of time and concentration for a toxic effect of phosphine. Haber's rule is $C^n t = k$, where C is concentration, t is the needed time to breathe/absorb the gas in order to produce the toxic effect, k is a constant relating concentration and time for the toxic effect of phosphine on *R. dominica* (in this case), and n is called the toxicity index. Daglish (2004) gives the toxicity index for phosphine and *R. dominica* as n=0.8673 and this value is used in the following calculations.

Collins et al. (2002) determined phosphine concentrations to reduce the population of a particular resistant genotype of *R. dominica* by 50% over a 48-hour fumigation. Using these phosphine concentrations (labeled LC_{50} in the reference but C_H here) and Haber's rule with the toxicity index for phosphine, we derive constants k for each genotype's resistance to phosphine

$$k = 48C_H^n \quad (2)$$

We then take these k values relating concentration and time to toxicity for a particular genotype to get a relation in terms of times (T_H) to kill 50% of the population as follows

$$T_H = \frac{k}{C^n} \quad (3)$$

and then determine the hourly death rate by substituting the time from equation (3), and the fact that the population at this time is half of the initial population (that is $N(t) = N(0)e^{\alpha.T_H} = N(0).0.5$) into equation (1), rearranging and taking logs to get the death rate for varying concentration of phosphine as follows:

$$\alpha(C) = \frac{C^n \ln 0.5}{k} \quad (4)$$

The per capita death rate for each genotype then is determined by the Haber's k constants (determined in equation (2)) and the concentration of phosphine applied. The resistance to phosphine is directly related to the magnitude of k. These values are given in Table 1.

Table 1 Haber's constant for each genotype (s = susceptible, h = hybrid, r = resistance) as derived in Lilford (2009) from data determined in Collins (2002) and Daglish (2004) at 25C and 60% relative humidity. These are used to determine specific death rate under fumigation for genotypes.

Genotype *i, j*	Haber's constant k
ss	0.2088
sh	0.417
sr	1.674
hs	0.2088
hh	0.537
hr	0.537
rs	4.0908
rh	4.0908
rr	50.019

2.2. Life stages and producing offspring

The interest in life stage effects was partly due to the fact that only adults can lay eggs, and also the observation that there is some resistance to phosphine in the egg and pupa life stages. In particular, eggs are resistant to the fumigant and the most numerous life stage, and adults are the easiest to kill (Hole et al., 1976). In this case, a likely scenario after a suboptimal fumigation is that no adults are left, but there are eggs. Egg numbers will initially decrease when this occurs, as there are no adults to lay them, and eggs die naturally or become larvae at a certain rate. So, when there are no adults, how long does it take for egg numbers to reach a minimum, and can this be exploited to maximise the chances of complete control? Figure 1 graphically shows the four life stages as states and shows rates of movement between the states, and rates of movement out of the states that reduce the entire population (death).

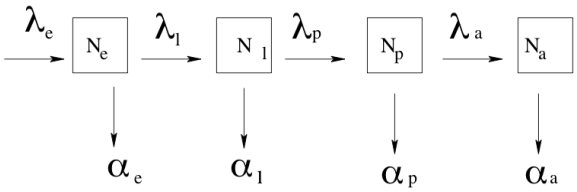

Figure 1 The transition diagram showing rates of population movement between different life stages (λ) and out of the system (α).

2.2.1. Time spent in each life stage

The information presented in Table 2 has been taken from Potter (1935). From Table 2 we can see that out of a beetle's average life span of 100 days, it is only able to lay eggs for less than half of this time.

Table 2 The average time spent in each life stage and a total life span at a temperature of 26°C and relative humidity of 65%, based on Potter (1935).

Description	Dulation (days)
The average time for a laid egg to hatch into a larva	15.5
The average time taken for a larva to develop into a pupa	36
The average time taken for a pupa emerge as an adult	6.5
The average time spent as an egg laying adult	42
Total average life span	100

2.2.2. Determining life stage rates

Table 2 gives expected times in each life stage. However, we need to determine rates into and out of each life stage as per Figure 1, where $\lambda_i, i \in \{e,l,p,a\}$ (egg, larva, pupa, adult) describes the rate into stage *i* from the previous stage, and $\alpha_i, i \in \{e,l,p,a\}$ describes the natural (that is; independent of fumigation) attrition rate out of each life stage. Table 2 was used to determine some of the rates in Figure 1 as follows. For subscript $i \in \{e,l,p,a\}$, let $N_i(t)$ be the number of beetles in life stage i at time t and λ_i be the rate of increase in $N_i(t)$. Now, assuming an exponential distribution for population numbers would imply that the mean time spent in any life stage is given by

$$E(X_i) = \frac{1}{\lambda_i} \quad i \in \{e,l,p,a\} \quad (5)$$

Taking the expected values as the average time spent in the life stages given by Table 2, we can rearrange equation (5) to determine rates from the times in Table 2. However, we also need data for the natural attrition rates (such as the percent of non-viable eggs laid) for each stage. This information specific to *R. dominica* could not be found, so parameters for the Alfalfa weevil were taken from Kuhar et al. (2000) as an approximation. Table 3 combines this information to give the rates used in the model. Under fumigation we assume that there are additional deaths due to the effect of phosphine. The per capita death rate under fumigation is given by adding the fumigation death rate specific to the genotype to the natural death rate specific to the life stage.

Table 3 Transition rates (to 4 decimal places) used in life stage model.

Per capita rate	Symbol	Value (units = per day)
Female adult lays an egg	λ_e	8.3333
Going from egg to larva	λ_l	0.0645
Going from larva to pupa	λ_p	0.0278
Going from pupa to adult	λ_a	0.1538
An egg dies	α_e	0.0363
A larva dies	α_l	0.0095
A pupa dies	α_p	0.0952
An adult dies	α_a	0.0375

2.2.3. Initial populations

We start with an initial total population of 1000, however all the populations for each genotype and life stage are proportional to the total initial population. The model does not rely on whole numbers and so is not affected by the size of the initial population, provided it is positive. This means that if we start with a total initial population of 10,000, the results will all be ten times the result from starting with an initial population of 1000. We do need to disperse the initial total population among the genotypes and life stages. Values for the initial genotype population ratios were taken from Lilford (2009), and the initial ratio of members in the different life stages was determined by running a numerical model for *R. dominica* life stages over a long period of time, and noting the population ratios when they remained stable (Table 4)

Table 4 Ratios of different life stages and genotypes (s = susceptible, h = hybrid, r = resistance) in population

Genotype	Ratio in population (%)	Life stage	Ratio in population (%)
ss	14	Egg	91.05
sh	10	Larva	5.97
sr	4	Pupa	0.65
hs	18	Adult	2.33
hh	17		
hr	6		
rs	6		
rh	21		
rr	4		

3. Results

In this section, we investigate some typical fumigation scenarios to demonstrate how the model might be applied in practice. First, we plot results for a single suboptimal fumigation and the subsequent recovery of *R. dominica*. Second, we plot results for two separate fumigations, interrupted by a certain period and the recovery. Additionally, we explore how long this interruption period can be, how altering the period of the interruption influences the development of resistance, and the ability to control infestations.

One suggestion is that after a fumigation where adults and larvae have been eradicated, but there are still eggs and pupae, the population is shocked into a state of decreasing egg numbers, and as the egg population is the hardest to eradicate, there may be an advantage with starting a second fumigation by the time the egg population reaches a minimum. Figure 2 shows the life stage dynamics with an initial population of 99% eggs, and 1% pupa. These proportions were assumed feasible (under the initial ratios given in Table 4) in a scenario where all larva and adults have been killed by fumigation. In Figure 2, initially the egg numbers decrease as there are no adults left to lay them, but as time goes by, eggs hatch and pass through the larval and pupa stages to adult. Eventually eggs begin to be laid again, and there is a definite recovery in egg numbers around the 6th day. Pupae numbers increase slightly by the 20th day, even though there are no larvae at time zero, and it takes an average of 36 days (by Table 2) for larvae to develop into pupae. This intuitively unrealistic effect occurs because times in Table 2 are averages, the larvae numbers increase quickly, and the more larvae that exist, the greater the possibility that some will develop rapidly into pupae.

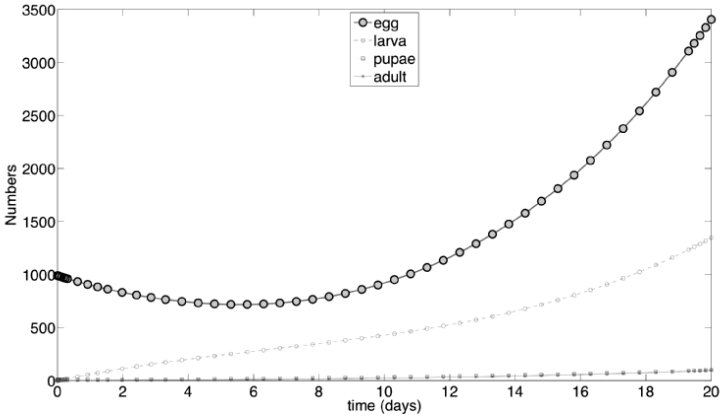

Figure 2 Life stage model showing eggs, larva, pupa and adults with initial population of eggs and pupa only.

Multiple staggered fumigations are simulated with a suboptimal concentration of phosphine as would be the case in a poorly sealed silo that is unable to maintain concentrations for extended periods. The concentration of 0.003 mg/L was chosen to be lower than the labelled dose and not able to eradicate resistant *R. dominica*, but still able to eradicate susceptible *R. dominica* (Collins et al., 2002). The model uses a temperature of 25°C and relative humidity of 60% during fumigation, and 26°C and 65% when fumigation is turned off. These were the conditions when the data for the corresponding rates was obtained, and are consistent with the conditions generally found in grain silos.

Figure 3 represents modelled results of the *R. dominica* population over 70 days where there is a single fumigation and represents a situation where adequate concentration has not been applied due to leakage. This fumigation was maintained for the first 31 days. The model run in Figure 3 gives four different graphs, one for each life stage. The model determines population numbers for nine different genotypes (of varying resistance) of *R. dominica*, portrayed as different types of line in each graph, with the most resistant genotypes as thicker lines with large circle markers. The fumigation period is represented by the shaded area. The fumigation period of 31 days is twice the average period for an egg to develop to the next larval stage (Table 2). After the fumigation, the recovery is different for each of the life stages, with the larva life stage having the greatest initial rate of growth. As would be expected, the resistant genotypes rr, rh and rs all show strong comparative recovery with rh reaching the highest population. Strains hh and hr recover less strongly, and hs does not recover. The susceptible genotypes ss, sh and sr do not recover over the 70 day run.

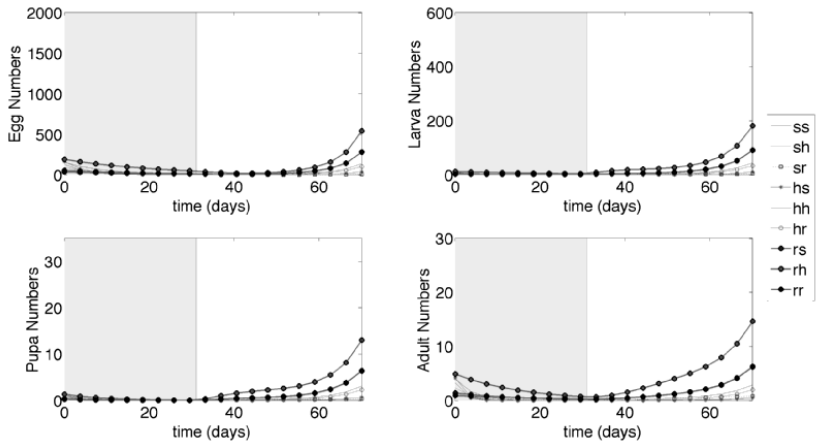

Figure 3 Fumigation at 0.003 mg/L (shaded area) for 31 days.

Figure 4 is a run of the same *R. dominica* population model as depicted in Figure 3 (nine genotypes and four life stages) and the run is also 70 days, but the fumigation has been split into two fumigations of 15.5 days performed 6 days apart. The 15.5 days is taken from Table 2 as the average time an egg takes to turn in to a larva, and 6 days was chosen from Figure 2 as the time taken for *R. dominica* egg numbers to reach a minimum after all adults and larva have been killed. The total duration and concentration of the fumigations run in the two shorter fumigations is the same as in the longer fumigation. Results of the model indicate that there is no observable difference in the total number of insects alive at the end of the 70-day period between the continuous and staggered fumigations. The rate of growth between fumigations is greatest in the larval stage once again. This is related to the population sizes of the immediately previous life stage.

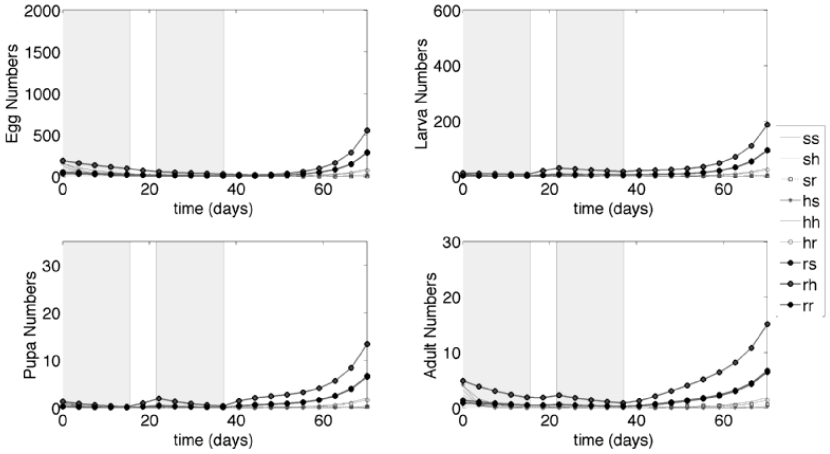

Figure 4 Genotype and life stage model with 2 staggered fumigations (shaded area) 6 days apart.

Figure 5 represents results from a third run of the genotype and life stage model over 70 days with a staggered fumigation with a much longer interval of 26 days between fumigations. The fumigation duration and concentrations were the same as in fig 4. This time, the rate of recovery of the resistant genotypes rr, rh and rs (thick lines with large circle markers) is very rapid. The rh genotype egg number going from around 500 to 2000 in a matter of 13 days. Compare with approximately 50 to 550 in Figure 3, with one long continuous fumigation, and approximately 40 to 550 when the fumigation is staggered with an interval of 6 days between fumigations.

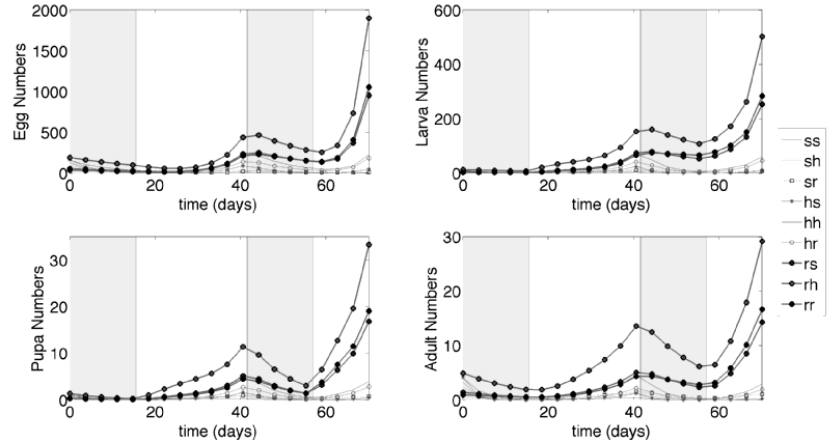

Figure 5 Genotype life stage model with 2 staggered fumigations (shaded area) 26 days apart.

4. Discussion

Analysis of the runs of mathematical models with life stage effects presented here has given effects of different fumigation strategies at low concentrations. The relation between the time a fumigation is run, the time between fumigations and the speed of recovery indicates that a longer interval between fumigations manifests in a faster rate of increase in insect numbers with a high proportion of resistant species after fumigation has ceased.

Our staggered fumigation results indicate a safe period where the population is shocked into dormancy by the first fumigation, and during this period the population is at the greatest risk from phosphine. The

concentration of fumigant in both the first and second fumigation is the same suboptimal concentration in the results presented here to illustrate that the short interval caused no difference compared with the continuous fumigation, but the implication is that if a leak leading to a suboptimal fumigation is discovered, there is a critical period within which the leak must be fixed and a second fumigation of labelled concentration applied to eradicate the pest. Otherwise, there is a risk of increasing proportions of resistant genotypes of pest in the grain that are impossible to eradicate. This critical period is the time taken for egg numbers reaches a minimum after the first fumigation has killed all adults. In results not presented here, the model verified that a second fumigation of labelled dose within this critical period would eradicate the pest.

Presented here are preliminary investigations that illustrate the value of including life stage detail in an effort to determine more effective strategies in controlling pests.

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New applications of hermetic storage for grain storage and transport

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Abstract

Recent evolution of pesticide-free post harvest hermetic storage for dry commodities as now used in 38 countries is described, and its current application in the storage of grain and other commodities is discussed and illustrated. Results of studies carried out on the protection of seeds, rice, maize, wheat, pulses, cocoa and coffee are presented. Plastic structures suitable for long-term storage systems have been developed and applied. The growing number of types of flexible hermetic containers for various needs, especially in tropical conditions, is documented. Types of hermetic containers include: 1) Portable containers of 60-kg to 2-tonne, called SuperGrainbags™ which are suitable for seed storage in bulk; 2) Bulk storage for household or farm use and daily withdrawal of grain of 1-tonne called Grainsafe II™; 3) Flexible enclosures of 5- to 1000-tonne capacity termed storage cubes or Cocoons™, designed for storage at the farmer-cooperative and small trader level or larger commercial and strategic storage facilities; 4) Hermetic Bunker storage for long-term storage in bulk of 10,000- to 20,000-tonnes; 5) TranSafeliner™ provide quality preservation, insect control, and prevention of condensation during shipment of bagged commodities in containers across intercontinental distances. Recent applications for wheat storage in bunkers in Jordan, and cocoa storage in West Africa, and the growing use of hermetic liners for intercontinental shipments of a variety of commodities, are described. Key performance parameters for safe storage in the face of elevated humidity and temperature in preventing growth of molds and the survival of insects for dry and wet commodities are discussed. Economic analysis is provided for representative applications, including rice, maize and cocoa.

Keywords: Hermetic storage, Modified atmospheres, Quality preservation, Storage insect control, Flexible storage structures,

1. Introduction

A new approach to cocoa bean storage, “hermetic storage” (HS), has been investigated in both laboratory and field studies to protect them in storage and in transit, and is now in commercial use in Africa, Asia and Latin America. Hermetic storage is a type of modified atmosphere (MA) that has now been applied for the protection of stored agricultural commodities including cocoa beans as well as coffee, rice, maize, pulses and seeds (Navarro et al., 1984; 1993; Navarro, 2006). It is also called "sealed storage” or “air-tight storage” or “sacrificial sealed storage” or “hermetic silo storage”. This method takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the MA by reducing the O_2 and increasing the CO_2 concentrations through respiratory metabolism. It has been shown that hermetic storage allows safe storage for periods ranging from weeks to many months, as well as during shipment across intercontinental distances with storage losses typically well below 1%.

Fumigants are still widely used for pest control in stored products, but non-chemical and environmentally user-friendly methods of pest control in the post harvest sector are becoming increasingly important. Methyl bromide (MB) will be phased out in developing countries by 2015, because of its contribution to stratospheric ozone depletion (UNEP, 2002). In contrast; phosphine remains popular, particularly in developing countries, because it is easier to apply than MB. However, some insects have developed resistance to phosphine in some countries over the last decade (Savvidou et al., 2003).

Storage problems prevail in the presence of adequate oxygen and temperature. In the presence of high relative humidity molds develop to cause quality deterioration resulting in an increase in free fatty acids (FFAs), rancidity and mycotoxins. These postharvest problems are eliminated through the lethal effect of

a low O_2 and high CO_2 atmosphere produced through respiration processes of biological agents. Under hermetic conditions, stored commodities with intermediate moisture contents generate modified atmospheres due to the respiration of the microflora and the commodity itself. The objective of this paper is to provide data on the novel approach of using hermetic storage based solely on biogenerated MA as an insect control, and quality preservation method for stored cocoa beans, coffee, seeds and other stored agricultural products.

2. Storage structures and enclosures developed for hermetic storage

Modern HS systems use special low permeability flexible plastic enclosures. These hermetic storage containers have evolved to store a variety of dry commodities in the range of 60 kg to 20.000 tonnes. They became commercially available starting in the early 1990's, and today are in use in more than 38 countries in a variety of configurations.

A few specialized applications require rapid disinfestation, such as in 3 days for dried figs (Ferizli and Emekci, 2000). In these, oxygen levels are reduced rapidly, either by purging with CO_2 (Gas - Hermetic Fumigation "G-HF"), or by applying a significantly high vacuum (Vacuum - Hermetic Fumigation "V-HF"). In either case, the process can quickly reduce oxygen content to below 1% to 2% (Navarro et al., 2002; Villers et al., 2008).

The most widely used form of HS is the Cocoon™ (Fig. 1A). It is manufactured in capacities ranging from 5 tonnes to 300 tonnes. Cocoons are made from specially formulated flexible 0.83 mm thick PVC with permeability to oxygen of 400 cc/m^2/day and to water vapour of 8 gm/m^2/day. A newer type of Cocoon called the MegaCocoon™ has more recently been introduced for larger scale storage of up to 1050 tonnes, with initial installations in Sudan (Fig. 1B).

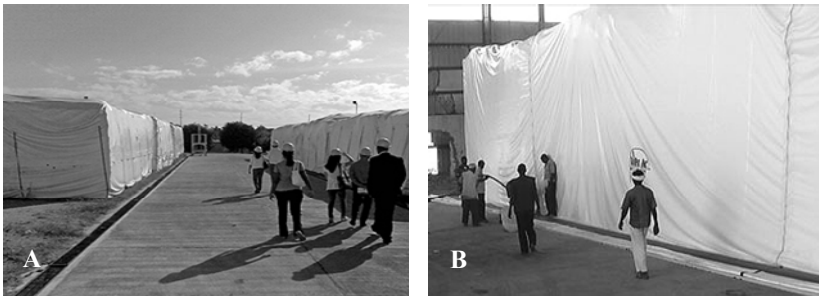

Figure 1 **A:** Corn, Outdoor storage in Cocoons™, Philippines, 2008 (left). **B:** 1050 Tonne MegaCocoon™, 2009 (right).

For smaller unit containers of 60 kg to 90 kg capacity, a transportable SuperGrainbag™ (SGB) is used (Fig. 2A). The SGB is a 3-layer coextruded plastic with thickness of 0.078 mm, 3 cc/m^2/day permeability levels for oxygen and for water vapour of 8 g/m^2/day. Using the same material, the SuperGrainbags-HC™ has become available for use with mechanized loading, which handles up to a 2-tonne capacity for bags or bulk storage (Fig. 2B).

Figure 2 **A:** 60kg SuperGrainbag with Corn seeds. **B:** SuperGrainbag -HC™ storing paddy inside woven protective polypropylene bag.

To protect bagged or bulk commodities against damage and deterioration when shipping across intercontinental distances in 20- and 40-foot standard shipping containers the TranSafeliner™ was introduced in 2008 (GrainPro, 2008) (Fig. 3).

Figure 3 TranSafeliner™ with coffee, Guatamala

The TranSafeliner provides hermetic storage during transport in a standard shipping container using the same type of ultra low permeability co-extruded plastic used in the SuperGrainbags shown in Figure 2 (Villers et al., 2008).

3. Current applications of hermetic storage

3.1. Hermetic storage of rice and rice seeds

As a result of extensive studies at IRRI (Rickman and Aquino, 2004) and later by PhilRice (Sabio et al., 2006), over the last 10 years, the benefits of storing both rice and rice seeds in hermetic storage are now well understood and in widespread use, particularly in Asia (Villers et al., 2006). The Cocoons shown in Figure 1 are used by the National Food Authority of the Philippines, to safely store rice paddy for up to one year. Hermetic storage applications for rice and/or rice seed are currently found in such countries as: Cambodia, East Timor, Indonesia, India, Pakistan, Philippines, Sri Lanka, and Vietnam (Montemayor, 2004).

3.2. Hermetic storage of wheat and barley

Hermetic storage of wheat in "Hermetic Bunkers" with capacities ranging from 10.000 to 20.000 tonnes was first introduced in the early 1990's, as shown in Figure 4. Hermetic storage of wheat, stored at or below its critical moisture content of 12.5%, provides storage without significant degradation of quality, including maintenance of baking qualities, for up to 2 years (Navarro et al., 1984; 1993). In Cyprus such Bunkers allowed quality preservation of barley for 3 years, with total losses of 0.66% to 0.98%, and with germination remaining above 88% (Varnava and Muskos, 1997).

Figure 4 20.000 tonnes of Wheat in Hermetic Bunker, Jordan 2009

3.3. Hermetic storage of dry maize

Cocoons are widely used in Rwanda, Ghana and the Philippines for storing both shelled and unshelled maize, in capacities ranging from 50 to 1050 tonnes (Figure 1). Similar quality preservation results were obtained for maize when stored in 60 kg capacity SuperGrainbags. The large flexible hermetic storage units are generally used at the village level, but also as strategic reserves to prevent famine at the district level (Navarro, 2006; Montemayor, 2004, Navarro et al., 1995).

3.4. Preservation of high moisture maize

Under humid and warm conditions harvested grains should be dried to safe moisture levels that inhibit the activity of microorganisms. Drying to these moisture levels is often not economical for farmers in developing countries. Laboratory studies were carried out on the effect of various moisture contents on the quality of maize grains in self-regulated modified atmospheres during hermetic storage (Weinberg et al., 2008). Shelled maize of 26% moisture content was stored in a Cocoon™ under hermetic conditions for 96 days to demonstrate the effectiveness of maintaining its quality prior to subsequent drying or processing into feeds or ethanol. The high moisture maize in the Cocoon™ initially had 59 ppb of aflatoxin, which increased to 90 ppb after one week of storage and remained at that level for 96 days (Arnold and Navarro, 2008). Feeding trials indicated that wet maize can be safely stored for extended periods of time without significant increase in aflatoxin, and without significant changes in starch.

3.5. Hermetic storage of pulses (beans)

Beans in storage are subject to invasive pests such as *Callosobruchus maculatus* (F.) and *C. chinensis* (L.), which are controlled through hermetic storage. In Rwanda and Ghana, storage of beans in Cocoons of 20 to 150 tonne capacity has permitted groups of farmers to hold their crops off the market while waiting for more favorable market prices (MINAGRI, 2006).

3.6. Hermetic storage of coffee

Field data from Costa Rica shows that preventing the penetration of external humidity alone has proved sufficient to protect coffee bean quality for up to 9-months (Aronson et al., 2005). Coffee is now stored commercially in portable SuperGrainbags, or in larger Cocoons for storage to preserve quality, and also, for long transit-time shipments in shipping containers without refrigeration, using SuperGrainbags, or TranSafeliners™. Hermetic coffee storage of green coffee beans is now practiced in Costa Rica, East Timor, Ethiopia, Jamaica, Hawaii, Peru, and the continental United States.

3.7. Preservation of stored cocoa beans

According to the Transport Information Service (TIS) (2009), moisture content higher than 8% corresponds to the rapid mold growth threshold of 75% relative humidity. It is thus recommended to insist on a water content of 6% or less when transporting cocoa beans in containers.

Data was shown for insect control and for quality preservation of stored cocoa beans by employing a novel approach through the use of biogenerated modified atmospheres as a methyl bromide alternative. The respiration rates of cocoa beans at equilibrium relative humidities of 73% at 26ºC in hermetically sealed containers depleted the oxygen concentration to <1% and increased the carbon dioxide concentration to 23% within six days. A hermetically sealed flexible structure containing 6.7 tonnes of cocoa beans at an initial moisture content of 7.3% (70% equilibrium r.h.) was monitored for oxygen concentration and quality parameters of the beans (Navarro et al., 2007). The measurements showed a decrease in oxygen concentration to 0.3% after 5.5 days (Figure 5). No insects survived the oxygen depleted biogenerated atmosphere. These encouraging results reveal the possibility of utilizing biogenerated atmospheres in integrated pest management (IPM) for quality preservation (by preventing the development of FFA, molds, and mycotoxins), and insect control of cocoa pests (Jonfia-Essien, 2008a; 2008b).

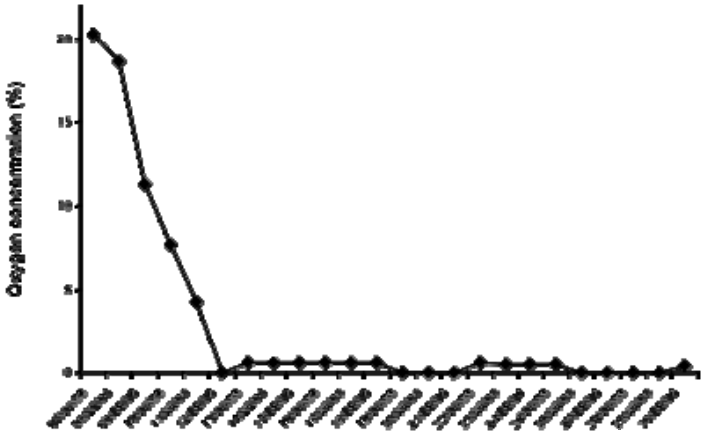

Figure 5 Changes in oxygen concentration in cocoa beans, Makassar, Indonesia, 2005

4. Other commodities

Hermetic storage is currently used for such commodities as sorghum, and rice bran. A 2009 trial (unpublished) in Kerala, India, suggests possible hermetic storage application to tea storage by maintaining constant humidity.

5. Conclusions

Hermetic storage was shown to be a successful storage method for the protection of the commodities replacing fumigants for insect control and for quality preservation of stored products. Hermetic storage is achieved in specially constructed plastic structures suitable for long-term storage systems, as well as intermediate storage of cereals, pulses, coffee and cocoa have been developed and applied. Flexibility, transportability, ease of erection, simplicity of operation and maintenance and durability are distinct advantages. Their availability in various sizes, capacities and forms can suit a wide range of requirements to fit several levels of operation.

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Mortality time of immature stages of susceptible and resistant strains of *Sitophilus oryzae* (L.) exposed to different phosphine concentrations

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Abstract

The mortality time on egg, larvae and pupae of four strains with resistance factor 1, 69, 160 and 295 to phosphine of *Sitophilus oryzae* (L.), which were expressed in R_1, R_{69}, R_{160} and R_{295} in this report, respectively, were investigated with stable concentrations of 100, 300, 500, 700 and 900 mL m^{-3} of phosphine in a well sealed fumigation chamber. The mortality time on all immature stages was about 10 d for strain R_1, more than 15 d for all resistance strains exposed to 100 mL m^{-3} of phosphine. Mortality time on egg and larvae of R_1 was 9 and 6 d at 300 and 700 mL m^{-3}, respectively. But it was only 4 d and 2 d for pupae of R_1 at 700 and 900 mL m^{-3}, respectively. The mortality time on immature stages of R_{69} was 12 and 5 d with the 300 and 700 mL m^{-3}, respectively. And that on immature stages of strain R_{160} and R_{295} was 15 and 10 d with phosphine of 300-700 mL m^{-3}, respectively. With the fumigant of 900 mL m^{-3}, the full death time were 5 d for larval of all strains, 5d for pupae and egg of R_1 and more that 8 or 9 d for pupae and egg of three resistance strains. The egg and pupae of *S. oryzae* were the most tolerant stages to phosphine both for susceptible and resistance strains.

Keywords: *Sitophilius oryzae*, Immature stage, Phosphine, Mortality time

1. Introduction

The importance of phosphine usage to control stored-grain insect pests has increased due to international phasing out of the fumigant methyl bromide and the difficult to develop new fumigants in recent years and in the future. Phosphine has several advantages that have made it attractive for use in the grain industry. It is relatively easy to apply (compared with other fumigants), versatile and inexpensive, with international acceptance as a near residue-free treatment (Emery et al. 2003) or readily available without restrictions. High-level resistance to phosphine reported in Bangladesh (Tyler et al., 1983) and later in India and other countries (Zettler, 1993; Rajendran and Narasimhan, 1994; Chaudhry et al., 1997; Daglish and Bengston, 1998; Zeng, 1999; Collins et al., 2002; Wang et al., 2004; Pimentel, 2009), threatens the useful life of this fumigant and causes control failures in many species and cases. To protect the long-term use of phosphine in the grain storage industry and continue to market low residue product in the world grain trade it is important to manage development of phosphine resistance in stored-grain insects (Newman, 1998). Knowing the mortality time or full death time on insect population during fumigation is a key for the resistance management. The exposure time to the fumigant is more important than its dosage in many cases (Annis, 1993). The concentration and exposure time product are usually different owing to variable concentration, insect species, strains for same species or population (Price, 1985) and different stages in a species (Hole et al., 1976). Exposure time needed to control insects using phosphine is becoming longer due to resistance. For instance it was 7 d (Taylor and Harris, 1994; Bengston et al., 1997; Rajendran et al., 2001; Rajendran and Muralidharan, 2001), later 8 d (Rajendran and Gunasekaran, 2002; Collins et al., 2005), 6-9 d (Price and Mills, 1988; Liang, et al., 1999; Collins et al., 2002) and more than 7 d (Sayaboc, et al., 1998) for resistance *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) under the concentration of 0.2, 0.3, 0.5 and 0.7 g m^{-3}. There are several reports about phosphine resistance on other insect species (Daglish et al., 2002; Wang et al., 2006). It was recommended that exposure time should be more than two, three and four weeks relatively with 100 to 350 mL m^{-3} of phosphine concentration according to the China national recommended standard on phosphine fumigation (Wang et al., 2002). But, in some cases, when even applying these recommended standards, insect survival was still encountered. Furthermore, the mortality time in insects is useful reference to successful fumigation while maintaining phosphine at effective level. Although *Sitophilus*

oryzae (L.) (Coleoptera: Curculionidae) is one of the world's most serious pests in stored grain, there are a few data on the practical significance of phosphine resistance on this species. An Australian susceptible strain, a homozygous resistant strain exhibiting a level of resistance common in Australia and an unselected field strain from China with a much stronger resistance were investigated (Daglish et al., 2002). The objective of the present work was to study the mortality time on egg, larvae and pupae of a susceptible strain and three different levels of resistant strains of *S. oryzae* to phosphine, from three provinces of China.

2. Materials and methods

2.1. Insects

Four strains of *S. oryzae* with different levels of resistance to phosphine were received from Department of Employment, Economic Development and Innovation, Queenland, Australia (QDEEDI), and three grain depots in China. All strains were maintained without further exposure to phosphine in the Stored Product Insect Research Laboratory, Henan University of Technology, Henan, China. Resistance factor was examined followed the standard FAO test method to phosphine (Anonymous, 1975). Resistance factor of Strain LS_2 from (QDEEDI) was ×1 (as reference to susceptible strain) and marked with R_1 here, collected in 1965 from Brisbane, south-east Queensland (Daglish et al., 2002). That of Chinese strain SCXD from Xindu Grain Depot in Sichuan Province, was ×69 and marked with R_{69};. strain CQTL from Tongliang Grain Depot in Chongqing City was ×160 and marked R_{160}. ; of strain HBSY from Shiyan Grain Depot in Hubei Province was ×295 and marked R_{295}. These populations were reared on wheat (13% m.c.) in glass jars under controlled conditions (28±1ºC, 70 ± 5 % r.h.).

2.2. Fumigation chamber

The fumigation was carried out in a rectangle chamber (dimensions of 60×35×40 cm) that was made of armor plate except for transparent top side which was made of plexiglass. There was one operating opening on one vertical side that could be sealed with rubber glove. A sampling cylinder was inserted on another vertical side that can be sealed by two screwed caps which were 80 mm in diameter and 200 mm in length. The insect cages can be taken out through this cylinder during fumigation that avoids the fumigant leaking. The size of insect cage was 10 mm in diameter and 70 mm in length. The airtightness of the chamber was maintained by an airproof mat bolted between rectangle bin and transparent top. The fumigant in the chamber could be re-circulated and monitored by an electronic phosphine monitor with a pump and two rubber pipes controlled by valves. The phosphine monitor could detect phosphine concentration in a range of 0-1000 mL m^{-3} and in precision of 0.01 mL m^{-3} (model HL-210, Xinjialiang Co., Beijing, P.R. China). A supersaturated solution of sodium chloride in a Petri dish placed on the bottom in the chamber was used for maintaining 70% r. h. The pressure decay time at 500 Pa was more than two min for the chamber.

2.3. Phosphine, monitoring and concentration control

The phosphine source was generated from zinc phosphide in acidified water based on FAO method (Anonymous, 1975). The fumigant was injected using a gastight syringe through the recirculation rubber pipes. The phosphine concentration was determined by the monitor after the insect cages and chamber were ready for the test. There were six fumigation chambers maintained at constant concentrations of 0, 100, 300, 500, 700 and 900 mL m^{-3} of phosphine. Phosphine supplementation was necessary if there was a decay in the concentration after a daily check.

2.4. Fumigation of eggs

Five thousand two-week-old adults were delivered into three kg of wheat (14% m.c.). Eggs of the same age were selected from infested kernels. Fifty kernels with egg plugs dyed red with acidic fuchsine solution were put into the cages for exposure to the fumigant. Three replication of egg cages were taken out at 3 d intervals during 12 d fumigation. The fumigated and control wheat contained eggs were dissected with penknife after each checking time. The rate of hatch was counted through dissection.

2.5. Fumigation of larvae

Infected wheat seeds with dyed egg plugs were reared until insects reached the larval stage. Fifty kernels with larvae were placed into the cages for each different fumigation regime. Three cages that served each for a replicate were taken out at 5 d intervals during 12 d fumigation. The fumigated larvae in the kernels

were incubated under controlled conditions until the day there was no new adult emergence. The rate of pupation was counted after kernels dissection.

2.6. Fumigation of pupae

Insects inside the infested wheat seeds, identified by the dyed egg plugs were reared to pupa stage. Fifty pupa kernels were placed into the cages for exposure to each different fumigation regimes. Three cages that served each for a replicate were taken out in 2 d interval during 8 d fumigation. The fumigated pupae in the seed were incubated under controlled conditions until the day there was no new adult emergence. The mortality of pupae was determined by dissecting the infested kernels.

2.7. Statistical methods

The statistical analysis was performed using DPS 3.11 software and Microsoft Excel 2003.

3. Results

3.1. Mortality time on eggs

The mortality time on eggs of different strain of *S. oryzae* was expressed by the eclosotion rate of adult through egg reared after a series of fumigations (Fig. 1).

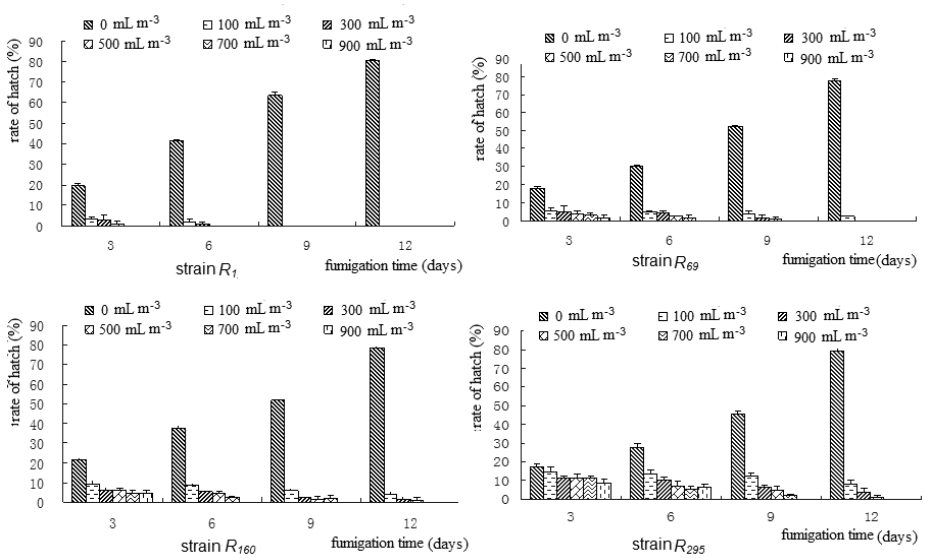

Figure 1 Rate of egg hatch of *Sitophilus oryzae* strains exposed to different phosphine concentrations.

There was more than 80% eclosion rate of adult from kernel infected by eggs for all strains in control. Eclosotion rate of control for all strains increased with time. There were no obvious differences in of egg hatch rates among the four strains at each similar time. There was a sharp decreasing in adult eclosion rate for all strain at any fumigated concentration; there was no adult emergence for any longer times or higher concentrations. It indicates that phosphine can kill hidden eggs by penetrating kernels and/or the egg plug.

The least mortality time for strain R_1 were 3 d at 700 mL m^{-3} of fumigant, 6 d at 500 mL m^{-3}, 9 d at 100 mL m^{-3} and more. The least mortality time for strain R_{69} were 6 d at 900 mL m^{-3}, 9 d at 700 mL m^{-3}, 12 d at 300 mL m^{-3} and more. The mortality time for strain R_{160} was 6 d at 900 mL m^{-3} and 12 d at 700 mL m^{-3} and more. The mortality time for strain R_{295} was 9 d at 900 mL m^{-3} and 12 d at 700 mL m^{-3} and more. The resistance factor was larger and mortality time longer at similar concentrations for different strains. The mortality time was shortened with increased phosphine concentration for the same strain of the insect.

3.2. Mortality time on larvae

The mortality time on larvae of different strains of *S. oryzae* was obtained according to the full mortality of tested insects in wheat seed. The mortality (Fig. 2) was checked by seed dissection after rearing and complete eclosion of adults. Figure 2 indicates that there was less than 5% mortality of larvae for unfumigated kernels infested by eggs of all strains. There was an increase in larva death rate for fumigated kernels of all strains at any tested phosphine concentration and exposure time. Phosphine can kill the larvae hidden in the seed through the penetrating kernel and/or egg plug in a short time. With 100 mL m^{-3} phosphine, the mortality time was 10 d for R_1 and 15 d for the three resistance strains. There seems to be no difference in mortality times among of resistance strains. With 300 mL m^{-3} phosphine, mortality time was 5 d for strain R_1, 10 d for R_{69} and 15 d for R_{160} and R_{295}. at least five days was required to cause mortality at concentrations above 300 mL m^{-3} for R_1, above 700 mL m^{-3} for strain R_{69}, 900 mL m^{-3} for R_{160} and R_{295}. Ten days was required for above 300 mL m^{-3} for strain R_{69}, and above 500 mL m^{-3} for R_{160} and R_{295} The effect of resistance on extending mortality time was clearly demonstrated to control larvae (Fig. 2).

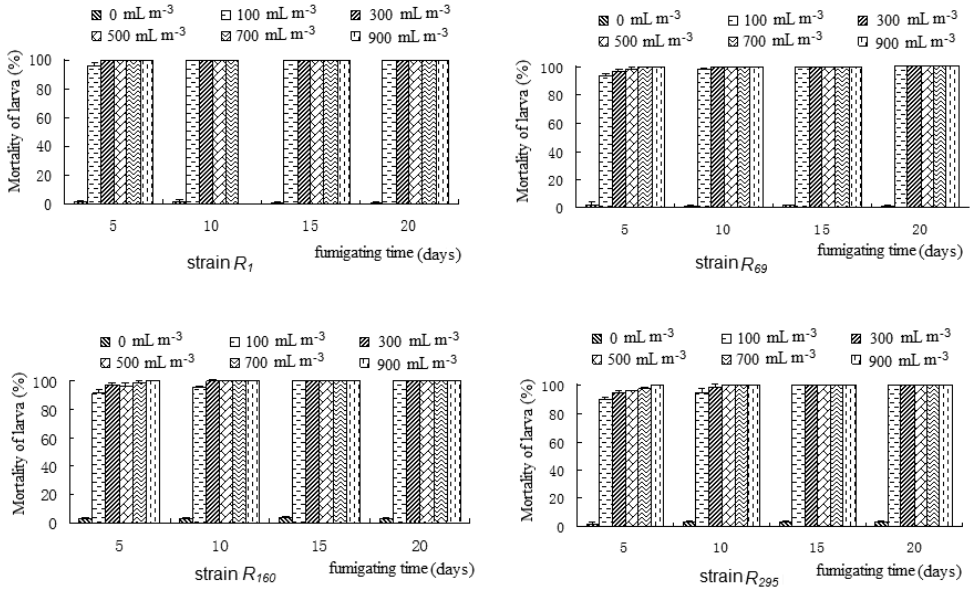

Figure 2 Mortality of larva of four *Sitophilus oryzae* strains exposed to different phosphine concentrations.

3.3. Mortality time on pupae

The mortality time on pupae of different strains of *S. oryzae* was detected according to the mortality of insects in wheat seed. The death numbers (Fig. 3) were checked by dissecting each kernel after incubation and adult emergence was completed. For R_1 the mortality times were 2 d in 900 mL m^{-3} of phosphine, 4 d in 700 mL m^{-3}, 6 d in 500 mL m^{-3}, 8 d in 300 mL m^{-3} and 10 d in 100 mL m^{-3}. The exposure time became shorter with the increase in concentration. The mortality time for pupae of resistance strains was longer than that of strain R_1, obviously. The time was postponed with the resistance level in the same concentration. The higher concentration made the mortality time shorter in the tested range of phosphine.

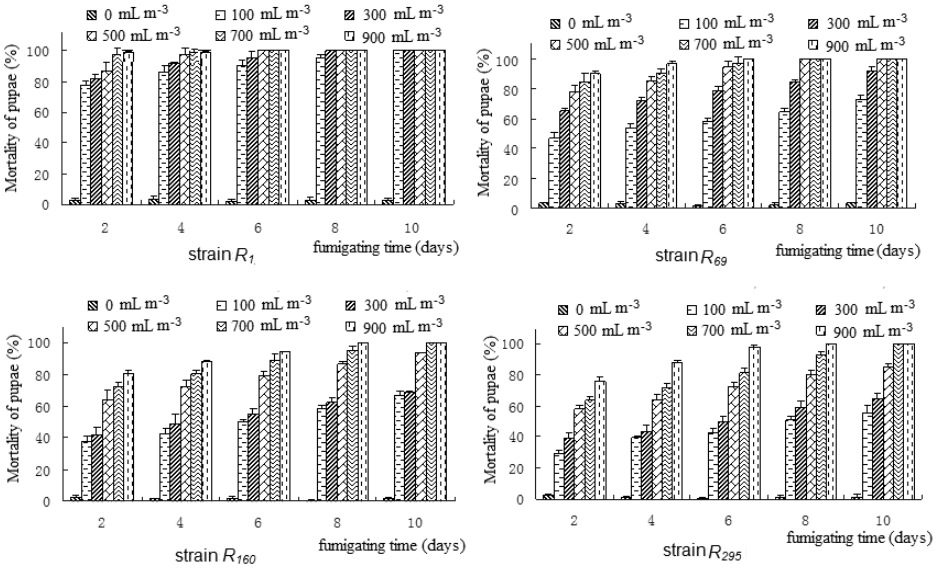

Figure 3 Mortality of pupa of four *Sitophilus oryzae* strains exposed to different phosphine concentrations.

3.4. The effect of developmental stages on the mortality time

For the comparison on different developmental stages of *S. oryzae*, the mortality time was shown in Table1. Table 1 indicates that the order of tolerance to phosphine was from egg to pupae for all strains.

Table1 The mortality time (days) for different strains and life stages exposed to five phosphine concentrations.

strain	stage	Mortaly time (d)				
		100 (mL m^{-3})	300 (mL m^{-3})	500 (mL m^{-3})	700 (mL m^{-3})	900 (mL m^{-3})
R_1	Egg	9	9	6	6	6
	larvae	10	5	5	5	5
	pupae	10	8	6	6	6
R_{69}	Egg	—	12	12	9	6
	larvae	15	10	10	5	5
	pupae	—	—	8	8	6
R_{160}	Egg	—	—	—	12	6
	larvae	15	15	10	10	5
	pupae	—		10	10	6
R_{295}	Egg	—	—	—	12	9
	larvae	15	15	10	10	5
	pupae	—	—	10	10	8

"—":There still were some survivals at the tested concentration

4. Discussion

Sitophilus oryzae is a major pest of stored grain, but little is known about mortality time at specific concentration of phosphine against this species, particularly in regard to immature stages and phosphine-resistant strains. Daglish et al. (2002) investigated the effects of exposure period and phosphine concentration on mortality of a strain with a resistance factor of ×77, collected from Santai County from Sichuan Province, China in 1998. We examined the mortality time of susceptible and resistance strains at different elevated of phosphine efficacy against immature stages hidden with in wheat kernels. Mortality time could be shortened by increasing in phosphine concentration. The impact of resistance on insect killing was nearly relative to the concentration levels. Population mortality could be achieved with lower

concentrations combining with longer exposure times. Time was more important than concentration, especially in fumigation practice where the dosage of fumigant or cost could be reduced. That validated to equations of the form $C^n t=k$ time again. In all cases $n<1$, indicating that time was a more important variable than concentration (Daglish et al., 2002), is verified again. The egg and pupae of *S. oryzae* were very tolerant of phosphine both for susceptible and resistance strains. Therefore, fumigation must be aimed at tolerant stages in order to control all stages of the population. Although there is nothing inherently different between constant and changing concentration on adults of a phosphine-resistant strain of *S. oryzae* (Daglish et al., 2004), the basic phosphine concentration is necessary for a successful fumigation. The findings of this study will be useful in modifying fumigation recommendations. The fumigating concentration should be higher than existing data in the range of 100 to 350 mL m^{-3} of phosphine for quick killing, especially for management and control high level of the resistance in insect pests control.

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Fumigation activities of ethyl formate on different strains of *Liposcelis bostrychophila*

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Abstract

The psocid, *Liposcelis bostrychophila* is a prevalent insect pest in large grain depots in P.R. China. Our previous research proved that ethyl formate killed psocid adults of susceptible strain within 24 h, and the fumigation efficacy at relatively low temperature was better than that at relatively high temperature. In this paper, fumigation activities of ethyl formate on DDVP and PH_3 resistant strains of *L. bostrychophila* were studied by the sealed jar fumigation method under different ethyl formate concentrations, fumigation times, and temperatures. Results showed that treatment time and concentration significantly affected fumigation effectiveness of ethyl formate against *L . bostrychophila* adults at 30°C. The 50% lethal concentrations (LC_{50}) of ethyl formate against two resistant strains increased as the temperature increased from 20° to 30°C. At 27±0.5°C and 24 h fumigation, the LC_{50} were significantly lower for DDVP and PH_3 resistant strains than that of the susceptible strains and the LC_{50} of DDVP resistant strains were significantly lower than that of PH_3 resistant strains.

Keywords: Fumigation activity, Ethyl formate, *Liposcelis bostrychophila*, Resistant strain

1. Introduction

The psocid, *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae)has a worldwide distribution and is commonly found in various processed and unprocessed dry foods in households, granaries, and warehouses. Apart from causing measurable damage to stored grain (Rees and Walker, 1990), infestations of *L. bostrychophila* can also cause health problems among storage and warehouse workers (Sidik et al., 1986). In P. R. China, *L. bostrychophila* has posed an alarming threat to stored grains, especially in the storage facilities where CA and insecticide combined treatments are commonly practiced (Wang, 1997; Wang et al.,1999). The main chemicals to control *L. bostrychophila* are phosphine and methyl bromide, but resistance of *L. bostrychophila* to chemicals was very serious because of chemicals abuse (Chen et al., 2003). Due to its small size and strong resistance to chemicals, it was easy to miss its presence. Hence, it is becoming more and more difficult to control. Furthermore, methyl bromide will be phased out in the year 2015 in China, so it is urgent to find new fumigants to be as the alternatives of methyl bromide and phosphine. Ethyl formate (EtF) is a promising and environmental friendly fumigant, which was registered as dry fruit fumigants in 2002 in Australia (Ren and Mahon, 2006). The previous research proved that EtF killed *L. bostrychophila* adults of susceptible strain within 24 h, and the fumigation efficacy at relatively low temperature was better than that at relatively high temperature (Li et al., 2006). The purpose of this research was to evaluate the fumigation activity of EtF on the adults of the DDVP and PH_3 resistant strains of *L. bostrychophila* at different temperatures, fumigation times and EtF concentrations to provide data for developing EtF as an alternative of phosphine to control resistant strains of *L. bostrychophila.*

2. Materials and methods

2.1. Insects

Stock colonies of susceptible strain of *L. bostrychophila* were collected from a simulative warehouse at Chongqing Key Laboratory of Entomology & Pest Control Engineering, Southwest University, Chongqing, China. The insects were reared on an artificial diet consisting of whole wheat flour, brewer's yeast and milk powder (10:1:1) in a temperature controlled room at 27 ± 0.5°C, in complete darkness. Cultures were set up in glass bottles (250 mL) with a nylon screen cover and kept in desiccators (5000 mL), in which the relative humidity (r.h.) was controlled with saturated NaCl solution to maintain

75-80% r.h.. The resistant strains of *L. bostrychophila* to dichlorvos (DDVP-R) and phosphine (PH_3-R) were created as follows: At monthly interval, the booklice were treated with DDVP acetone solution of appropriate concentrations and about 75% mortality was maintained in each treatment. After 85 treatments, the strain that exhibited 22.36-fold resistance to DDVP was obtained and used as the DDVP-R. As to phosphine resistant strain, at monthly intervals, the booklice was treated with phosphine and about 75% mortality was maintained each time. After 85 treatments, the strain with 4.51-fold resistance was regarded as booklice resistance strain to phosphine (PH_3-R).

2.2. Fumigants

Ethyl formate (>98.00%) was produced by Shanghai Chemical Reagent Group of China. Dichlorvos 80% (DDVP) was from Chongqing Pesticides Co. and phosphine (Aluminium phosphide >56%) from Shangdong Jining Pesticide factory.

2.3. Effect of fumigation time and EtF concentration on the efficacy of EtF against L. bostrychophila

The 1-L glass jars were used in the fumigation. At 30°C, the EtF concentrations of 10, 12, 14 and 16 µL/L, and fumigation times of 12, 24, 36, 48 and 60 h were adopted. The detailed methods were as follows. Thirty 5-day-old adults were collected for each treatment. Adults were placed in a plastic box (diameter 2 cm, Length 1 cm), wrapped in nylon gauze and then the wrapped plastic box was placed at the bottom of a 1-L jar. The filter paper containing a known quantity of EtF was placed in the glass jar and a plastic film was used to seal the jar. The jars were placed in the incubator set at controlled temperature in the dark. Each treatment was replicated three times. Controls groups were kept without EtF fumigation. Mortality was checked after fumigation.

2.4. LC_{50} of L. bostrychophila at different fumigation time and temperatures

The same fumigation method as described in section 1.2.1. was adopted. The fumigation times were designed as 24 and 48 h. The temperatures were 20°, 25° and 30°C, respectively. Five to seven EtF concentrations were used for each resistant strain. For DDVP resistant strain, for 24 h exposure time at 20°C, EtF concentrations were 6, 7, 8, 9, 10, 11 and 12 µL/L; at 25°C, EtF concentrations were 7, 8, 9, 10, 11, 12 and 13 µL/L; at 30°C, EtF concentrations were 8, 9, 10, 11, 12 and 13 µL/L. For 48 h exposure time, at 20°C, EtF concentrations were 6.5, 7, 7.5, 8, 8.5, 9 and 9.5 µL/L; at 25°C, EtF concentrations were 7, 8, 9, 10, 11 and 12 µL/L; at 30°C, EtF concentrations were 7, 8, 9, 10, 11, 12 and 13 µL/L. For PH_3 resistant strain, under 24 h fumigation, at 20°C, EtF concentrations were 7, 7.5, 8, 8.5, 9, 9.5 and 10 µL/L; at 25°C, EtF concentrations were 10, 11, 12, 13, 14, 15 and 16 µL/L; at 30°C, EtF concentrations were 10, 11, 12, 13, 14, 15 and 16 µL/L. Under 48 h fumigation, at 20°C, EtF concentrations were 6, 6.5, 7, 7.5, 8, 8.5 and 9 µL/L; at 25°C, EtF concentrations were 9, 9.5, 10, 10.5, 11, 11.5 and 12 µL/L; at 30°C, EtF concentrations were 11, 12, 13, 14, 15, 16 and 17 µL/L. EtF concentration designs were based on the corrected mortalities of 16% ~ 84%. Each treatment had three replications.

2.5. LC_{50} comparison of L. bostrychophila strains to ethyl formate

The same fumigation method as the above in 1.2.1. was adopted. Under the conditions of 27±0.5°C and 24 h fumigation, LC_{50} were measured for DDVP and PH_3 resistant and susceptible strains. For DDVP-R, EtF concentrations were 7, 8, 9, 10, 11, 12 and 13 µL/L. For PH_3-R, EtF concentrations were 10, 11, 12, 13, 14, 15 and 16 µL/L. For susceptible strain, EtF concentrations were 11, 12, 13, 14, 15, 16 and 17 µL/L. Each treatment had three replications.

2.6. Data analysis

All the data concerning mortality were corrected by using Abbott's formula (Abbott, 1925). Mortality data were transformed using arcsine ($x^{0.5}$) and ANOVA was carried out using SPSS software. Duncan's multiple range tests was used to test the difference significance and IRM software (developed by Southwest University) was used to obtain LC_{50} values and regression equations.

3. Results

3.1. Effect of fumigation time and EtF concentration on the EtF efficacy

Under the conditions of different fumigation time and EtF concentration, the toxicities of EtF against *L. bostrychophila* DDVP resistant strain were listed in Table 1. The corrected mortality increased gradually as the fumigation time increased at fix EtF concentration. The concentration of 14 µL/L EtF led to 90%

corrected mortality within 36 h of fumigation. When the fumigation time was the same, high EtF concentration increased EtF toxicity against *L. bostrychophila*. The concentration of 16 µL/L of EtF caused more than 90% corrected mortality within 24 h fumigation and 100% corrected mortality within 48 h fumigation. Two-way ANOVA showed fumigation time affected the corrected mortality of *L. bostrychophila* DDVP resistant strain significantly (F = 165.175; df = 4, 40; P = 0.0000), so did the EtF concentration (F = 256.835; df = 3, 40; P = 0.000). However, the interaction between EtF concentration and fumigation time influenced the corrected mortality insignificantly (F = 1.297; df = 12, 40; P = 0.258).

Table 1 Effectiveness of ethyl formate against *L. bostrychophila* of DDVP-R strain at different exposure times and concentrations at 30°C.

	Corrected mortality (%)			
Treatment time (h)	**10µL/L**	**12µL/L**	**14µL/L**	**16µL/L**
12	14.47±2.23 a	33.00±5.77 a	46.67±3.33 a	60.33±3.33 a
24	41.33±4.33 b	68.00±1.00 b	82.00±1.00 b	95.67±2.96 b
36	45.33±3.93 b	76.67±2.03 bc	90.00±1.73 c	97.67±2.33 b
48	56.67±2.03 c	80.00±1.73 c	94.33±1.33 c	100±0.00 b
60	64.67±2.33 c	89.00±1.00 d	100±0.00 d	100±0.00 b
F	40.487	46.222	136.351	26.367
df	4,10	4,10	4,10	4,10
P	0.000	0.000	0.000	0.000

Note: The data shows the average of three replicates. Data in the same column followed by different letters show significant difference at 0.05 level by Duncan's multiple range test.

The toxicity of EtF against PH_3 resistant strain was listed in Table 2. The efficacy of EtF against *L. bostrychophila* increased as the fumigation time and EtF concentration increased. Fumigation time (F = 35.741; df = 4, 40; P = 0.0000) and EtF concentration (F = 76.757; df = 3, 40; P = 0.000) affected the corrected mortality significantly, but the interaction between them insignificantly (F = 0.841; df = 12, 40; P = 0.635).

Table 2 Effectiveness of ethyl formate against *L. bostrychophila* of PH_3-R strain at different exposure times and concentrations at 30°C.

	Corrected mortality (%)			
Treatment time (h)	**10µL/L**	**12µL/L**	**14µL/L**	**16µL/L**
12	17.67±2.91a	29.00±6.11 a	38.67±5.67 a	62.33±2.33 a
24	19.00±4.16 a	45.33±6.22 ab	62.33±12.33 ab	82.00±6.66 b
36	21.00±2.00 a	51.33±2.96 b	72.00±11.00 bc	83.00±0.00 b
48	32.00±9.00 a	55.67±4.33 b	79.00±6.11 bc	89.00±1.00 b
60	64.33±1.33 b	78.00±6.66 c	91.00±4.16 c	99.00±1.00 c
F	14.324	9.538	5.547	19.152
df	4,10	4,10	4,10	4,10
P	0.000	0.002	0.013	0.000

Note: The data shows the average of three replicates. Data in the same column followed by different letters show significant difference at 0.05 level by Duncan's multiple range test.

3.2. LC_{50}s of L. bostrychophila at different fumigation time and temperatures

The fumigation toxicity of EtF against DDVP resistant strain was listed in Table 3. The table showed that the LC_{50} increased as the temperature increased. Moreover, the LC_{50} was affected by temperature. Within 24 h exposure time, the LC_{50} value was 7.874 µL/L at 20°C, and the LC_{50} value was 10.18 µL/L at 30°C, which meant the EtF efficacy at lower temperature was more effective than that at high temperature.

Table 3 The LC_{50} of ethyl formate against *L. bostrychophila* of DDVP-R strain at different exposure times and temperatures.

Treatment Time (h)	Temperatures (°C)	Regression equation (Y=)	r	LC_{50}(μL/L)	X^2
24	20	-1.599+7.3650x	0.998	7.874±0.15	3.533*
	25	-5.290+10.327x	0.983	9.920±0.13	6.728*
	30	-4.580+9.5050x	0.994	10.18±0.15	1.150*
48	20	-2.691+8.7970x	0.979	7.486±0.11	3.382*
	25	-4.285+9.5130x	0.991	9.463±0.14	2.574*
	30	-3.194+8.2680x	0.988	9.796±0.15	3.582*

For PH_3 resistant strain, the LC_{50} change tendency was the same as that of DDVP resistant strain (Table 4). Based on the regression equations, we found relatively large slope rates, which meant that the susceptibility of both *L. bostrychophila* resistant strain to EtF was consistent and increasing the EtF concentration could improve fumigation efficacy.

Table 4 The LC_{50} of ethyl formate against *L. bostrychophila* of PH_3-R strain at different exposure times and temperatures.

Treatment Time （h）	Temperatures (°C)	Regression equation (Y=)	r	LC_{50}(μL/L)	X^2
24	20	-8.678+9.976x	0.993	8.678±0.11	1.321*
	25	-7.161+0.145x	0.975	11.91±0.15	8.529*
	30	-0.543+0.398x	0.972	14.13±0.40	2.270*
48	20	-1.905+7.792x	0.967	7.695±0.12	5.043*
	25	-2.993+7.778x	0.991	10.65±0.16	2.589*
	30	-6.207+9.926x	0.981	13.46±0.17	4.624*

3.3. LC_{50} comparison of the L. bostrychophila strains to ethyl formate

Table 5 showed that LC_{50} of DDVP and PH_3 resistant strains decreased significantly compared with the LC_{50} of susceptible strain. Meanwhile, LC_{50} for DDVP resistant strain was obviously smaller than that for PH_3 resistant strain, which explained that the two resistant strains under the DDVP and PH_3 pressure were more susceptible to EtF and EtF could be used to control *L. bostrychophila* DDVP and PH_3 resistant strains.

Table 5 Susceptibility of the L. bostrychophila strains of DDVP-R and PH_3-R and susceptible strain to ethyl formate at 27±0.5°C.

strains	LC_{50} (μL/L)	r	Regression equation(Y=)	X^2
DDVP-R	9.9801±0.14	0.987	-5.590+9.927x	6.547*
PH_3-R	12.141±0.18	0.991	-7.261+1.105x	8.427*
S	13.583±0.19	0.989	-4.645+8.586x	6.746*

4. Discussion and conclusions

The resistance of stored grain insect to fumigants has been increasingly serious since fumigants began to be used (Ding et al., 2002). At present, the widely used fumigants are only PH_3 and methyl bromide, but methyl bromide will be phased out in developing countries by 2015 and PH_3 abuse cause serious resistance problems for main stored product insect pests. Hence, scientists from developed and developing countries all over the world are trying their best to explore new alternatives to control insect pests. EtF, as an old fumigant, which has been used as a fumigant for dried fruits for many years, aroused scientist's interest. It was ever reported that EtF could control stored product insect pests effectively (Muthu et al., 1984; Hilton and Banks, 1997). The researchers at SGRL, CSIRO in Australia used EtF to fumigate stored wheat and sorghum in unsealed conditions and their results showed that EtF killed insect pests within several hours (Ren and Mahon, 2006). The fumigation efficacies of EtF on *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) and *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) in the laboratories were also studied and the results also demonstrated that EtF had satisfactory fumigation activities in a short fumigation time (Damcevski and Annis, 2006). The toxicities varied with the stored product insect pest species. In China,

Tang et al. (2006 a, b) researched the fumigation activities of EtF against *S. oryzae* and *T. castaneum* in the laboratory and he proved that EtF killed insects in a short time and the toxicities of EtF were better at lower temperatures than at high temperatures. In a previous research Li et al., (2006) showed that EtF killed psocid adults of susceptible strain within 24 h, and the fumigation efficacy at relatively low temperature was better than that at relatively high temperature. This paper proved that EtF also killed DDVP and PH_3 resistant strains booklice in relatively short time period, and temperature and EtF concentration affected EtF efficacy significantly. EtF susceptibility for two resistant strains that LC_{50} were smaller that of susceptible strain and the LC_{50} between the DDVP and PH_3 resistant strains differed significantly. LC_{50} values for DDVP resistant strain were obviously smaller than that of PH_3 resistant strain, which showed EtF could be considered to be the fumigant to control the booklice which had developed resistance to DDVP. The toxicity data of EtF against other insect pests and its mechanism of action need to be further researched.

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A study of fumigation toxicity of horseradish essential oil against two stored grain insects

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Abstract

Horseradish essential oil is a biological fumigant which was extracted from *Armoracia rusticana* The effects of different concentrations, temperatures and grain types by fumigation using horseradish essential oil were studied in this paper. The oil had significant fumigation efficacy against pests in stored grain under natural conditions. In the absence of grain, a concentration of 2.25 ppm horseradish essential oil could kill *Sitophilus zeamais* and *Rhyzopertha dominica* after 12 h at 25°C . Three ppm of horseradish essential oil could kill all the *S. zeamais* and *R. dominica* on the surface of maize, wheat and paddy during 72 h exposure at 25°C. The mortalities of *S. zeamais* placed under maize, wheat and paddy were 100%, 100% and 98%, respectively, using 24 ppm of horseradish essential oil at exposure for 72 h at 25 °C . The mortalities of *R. dominica* were 100%, 93% and 86%, respectively, under the above conditions. Temperature did not result in significant differences on the fumigation efficacy of horseradish essential oil. Different types of stored grain had a significant influence on the fumigation efficacy of horseradish essential oil. The order of fumigation efficacy were maize, wheat and paddy. We concluded that horseradish oil could be used as a sanitary and environmentally user-friendly bio-fumigant within empty granaries or other storage structures.

Keywords: Horseradish oil, Stored grain pest, Fumigant toxicity, Fumigation

1. Introduction

Almost all countries have been using fumigants to kill pests in stored grain since fumigation is a convenient means to control stored grain pests. Fumigant which can be used in stored grain have special requirements. Therefore only a few fumigants such as methyl bromide and phosphine are being used currently. Due to the involvement of methyl bromide in the depletion of ozone the terms of the Montreal Protocol have classified it as an environmentally controlled substance, and it will be phased-out by 2015 worldwide (MBTOC, 1998). Phosphine is used frequently, but many insects have developed resistance (Benhalima et al., 2004). Finding alternatives to methyl bromide is urgent in the field of stored product (Guo et al., 2004). According to the practices of IPM, an effective fumigation alternative should not only be friendly to the environment and safe to stored grain, but also act effectively against stored-grain pests and have low cost. In recent years, scientists have searched for bio-fumigants. AgraQuest in the United States introduced first fungal fumigant in the world in 2002. This fungal fumigant is a kind of natural volatile substance which is produced by *Mascodor albus* (Wang, 2003). Essential oils extracted from plant can also be used as fumigants in stored grain (Shaaya et al., 1997). Wu et al. (2007) extracted active substances horseradish oil from the horseradish plant and determined the toxicity to stored-grain pests. Horseradish oil was able to kill several species of stored-grain pests. The toxicity of horseradish oil was studied further in this paper, including (1) horseradish oil bioassay in the absence of grain (2) toxicity of horseradish oil to two stored-grain pests in the presence of different grains; (3) toxicity of horseradish oil to two stored-grain pests at different temperatures.

2. Materials and methods

2.1. Insects

All test insects were reared at Wuhan Polytechnic University in electronically controlled incubators 27±1°C and 75±5% r.h. *Sitophilus zeamais* Motschulsky was reared on whole wheat, *Rhyzopertha dominica* (F.) on broken wheat. The wheat to be used was first sterilized at 80°C for 2 h. The moisture content was then adjusted to 13±1%. Adults (7-14 days old) were used in this study.

2.2. Chemicals

Allyl isothiocyanate (85% purity) was provided by Professor Lin Kai-Chun. Acetone was analytical grade (≥97%purity) purchased from Tianjin Basifu chemical Co. Ltd.

2.3. Horseradish oil bioassay in absence of grain

Fumigation in the absence of grain was carried out as described by Xu et al. in 1L airtight jars (Xu et al., 2008). Firstly, 30 test insects were placed in the jar. Secondly, measured quantities of horseradish oil were added to a filter paper (3 ×11cm) which was glued vertically inside the jar. Thirdly, the jar was closed as soon as possible, and the lid was sealed on with parafilm. Finally, the jar was placed in incubators at 25 °C. Each bioassay was carried out with five horseradish oil doses and an undosed control. Each dose or control was repeated three times. Mortality was determined after the treated *S. zeamais* and *R. dominica* were maintained at 25°C for 12 h.

2.4. Horseradish oil bioassay in the presence of grain

Fumigation with grain was carried out as described by He et al. (2008). The adult insects were fumigated in gas-tight 15-L glass desiccators sealed with glass stoppers containing a septum. The 80% of the desiccators' volume were filled by 12% m.c. grain (maize, wheat and paddy respectively). Thirty adult maize weevils and 30 adult lesser grain borers were placed seperately into a ventilated bag. Three replications of each insect were placed under the grain, and three replications were placed on the top of the grain. Horseradish oil was added onto a filter paper in a dish. The container was sealed after the reagent was added. Horseradish oil fumigations were carried at 25±1°C and 75±5% r.h. in electronic controlled incubators with acetone treated as a blank control. The exposure was complete after 72 h, and the desiccators were opened and mortalities were calculated for each replicate.

2.5. Statistical analysis

The mortality results were analyzed statistically using SPSS data processing software (Jia, 2006).

3. Results

3.1. Horseradish oil bioassay in the absence of grain

Horseradish oil was highly toxic to *S. zeamais* and *R. dominica* since low concentration could kill the tested pests rapidly. A concentration of 2.25 ppm horseradish oil killed all *S. zeamais* and *R. dominica* after 12 h exposure at 25°C. The LC_{50} values of horseradish oil to *S. zeamais* and *R. dominica* were 0.64 ppm and 0.69 ppm, respectively (Table 1). The LC_{90} values of horseradish oil to *S. zeamais* and *R. dominica* were 1.60 ppm and 1.71 ppm, respectively (Table 1).

Table 1 Bioassay results of horseradish oil on two test insects after 12 h at 25°C.

Insects	Slope±SE	LC_{50} (95% CI) μg/mL	LC_{90}(95% CI) μg/mL
Sitophilus zeamais	3.16±0.25	0.64 (0.37-0.93)	1.60 (1.11-3.87)
Rhyzopertha dominica	3.27±0.24	0.69 (0.42-0.96)	1.71(1.20-3.61)

3.2. Horseradish oil bioassay in the presence of different grains

Horseradish oil was highly toxic to *S. zeamai*s and *R. dominica* (Tables 2, 3). Three ppm of horseradish essential oil killed all the *S. zeamais* and *R. dominica* on the surface of maize, wheat and paddy for 72 h exposure at 25°C. The mortalities of *S. zeamais* placed under maize, wheat and paddy were 100%, 100% and 98%, respectively, at exposure to 24 ppm of horseradish essential oil for 72 h at 25°C. The mortalities of *R. dominica* were 100%, 93% and 86%, respectively, under the above conditions. The lower mortalities of the pests placed under the grains showed that the presence of grain had a significant influence on fumigation activity. Fumigant efficacy of horseradish oil varied with the type of grain used (Tables 2, 3).

Table 2 Mortalities of adult *Sitophilus zeamais* fumigated with different concentrations of horseradish oil after 72h at 25°C(%), n=3.

		Mortality ± SD (%)				
		Dose (ppm)				
Grain	**Placement in desiccator**	**1.5**	**3**	**6**	**12**	**24**
Maize	top	89.0 ±2.4	100±0	100±0	100±0	100±0
	bottom	75.0±1.9*	88.7±3.5*	100±0	100±0	100±0
Wheat	top	85.0±3.2	100±0	100±0	100±0	100±0
	bottom	68.0±2.7*	82.3±1.5*	98.3±1.5	100±0	100±0
Paddy	top	87.0±4.1	100±0	100±0	100±0	100±0
	bottom	57.0±3.5*	65.3±3.5*	72.3±2.5*	90.0±2.0*	98.2±3.7

*Mortalities of pests is significantly different between top and down of the same grain (P<0.05, Student's t-test).

Table 3 Mortalities adult of *Rhyzopertha dominica* fumigated by different concentrations of horseradish oil after 72h at 25°C(%), n=3.

		Mortality ± SD (%)				
		Dose (ppm)				
Grain	**Placement in desiccator**	**1.5**	**3**	**6**	**12**	**24**
Maize	top	85.0±3.7	100±0	100±0	100±0	100±0
	bottom	70.0±2.5*	56.3±3.5 *	73.7±4.0*	89.0±1.0*	100±0
Wheat	top	86.0±4.1	100±0	100±0	100±0	100±0
	bottom	64.0±2.1*	47.33±3.22*	62.0±3.0*	81.7±1.5*	93.0±1.0
Paddy	top	83.0±5.3a	100±0	100±0	100±0	100±0
	bottom	58.0±2.6*	32.3±2.5*	51.7±3.1*	67.00±1.7*	86.00±1.7*

*Mortalities of pests is significantly different between top and down of the same grain (P<0.05, Student's t test).

3.3. Horseradish oil bioassay different temperatures in the presence of grain

Tables 4 and 5 show the mortalities of *S. zeamais* and *R. dominica* fumigated applying 12 ppm horseradish oil at 16, 24, 32°C. The mortalities of *S. zeamais* and *R. dominica* increased slightly as the temperature increased, but not significantly. This may have been due to the higher concentration of the fumigant used in the test.

Table 4 Mortality adult of *Sitophilus zeamais* fumigated with12 ppm horseradish oil for 72 h at different temperatures. (%)[a]

		Mortality ± SD (%)		
		Temperature (°C)		
Grain	**Placement in desiccator**	**16**	**24**	**32**
Maize	top	100±0a	100±0a	100±0a
	bottom	96.0±3.5a	100±0a	100±0a
Wheat	top	100±0a	100.±0a	100±0a
	bottom	92.3±0.6a*	100±0a	100±0a
Paddy	top	100±0a	100±0a	100±0a
	bottom	86.3±1.5a*	89.0±1.0a*	92.3±2.5a*

[a] Results are the means ± SD (n=3). Means within columns followed by the same letter are not significantly different (P<0.05, LSD Fisher's multiple range test).
* Mortalities of pests is significantly different between top and down of the same grain (P<0.05, Student's t-test).

Table 5 Mortality adult of *Rhyzopertha dominica* fumigated with 12 ppm horseradish oil for 72h at different temperatures (%)[a]

		Mortality ± SD (%) Temperature (°C)		
Grain	**Placement in desiccator**	**16**	**24**	**32**
Maize	top	100±0a	100±0a	100±0a
	bottom	88.7±6.1a*	89.0±6.0a*	90.7±7.5a*
Wheat	top	92.5±10.4a	93.6±8.2a	100±0a
	bottom	78.3±7.5a*	82.3±3.8a*	84.7±5.8a*
Rice	top	98.3±1.9a	99.2±2.9a	100±0a
	bottom	59.7±6.5a*	64.7±3.6a*	66.7±1.5a*

[a] Results are the means ± SD (n=3). Means within columns followed by the same letter are not significantly different (P<0.05, LSD Fisher's multiple range test).
* Mortalities of pests is significantly different between top and down of the same grain (P<0.05, Student's t- test).

4. Discussion

Horseradish, Armoracia rusticana G. Gaertn., B. Mey. & Scherb, is a perennial herbaceous plant in Armoracia genus, which is distributed in eastern Europe, Turkey, Japan, northeast China, north China and other areas with more than 2000 years of cultivation history. Its fleshy root is used for food as a seasoning with special peppery taste welcomed by European, Japanese and Chinese (Delaquis and Mazza, 1995). The isothiocyanates of the horseradish and other cruciferous vegetables vary in their biological activities, such as anti-cancer and tumor, antibiosis and inhibiting platelet aggregation (Zhang et al., 2005). Eighteen essential oils were indentified from 95% of the whole oil in Chinese horseradish.

Of these constituents, allyl isothiocyanate accounted for about 32%, The second constituent was 4 - pentenyl isothiocyanate, which accounted for 26% (Lin et al., 2001). The chemical constitutions of the essential oil from horseradish varied according to the plant distribution area. The essential oil constitutions from the plant in Asia were widely different from that in Europe. But they all possessed allyl isothiocyanate, allyl rhodanate, 4-pentenyl isothiocyanate, butyl isothiocyanate and iso-β-phenyl isothiocyanate (Lin et al., 2001). Wu (2007) determined the toxicity of the pungent essential oils from horseradish to variety of pests, plant pathogens, soil bacteria and nematodes, which indicated that these essential oils could be researched as a potential bio-fumigants. Since a low concentration of 2.25 ppm horseradish oil could completely control *S. zeamais* and *R. dominica* for 12 h exposure at 25°C in the absence of grain, it was suggested that this horseradish oil could be used as a sanitary and environmentally user friendly bio-fumigant within empty granaries or other storage structures.

It had been discussed that grain could absorb and breakdown a fumigant such as ethyl formate (Damcevski and Annis, 1998; Damcevski and Annis, 2006). We also found that the presence of grain decreased the toxicity of horseradish oil to pests. The mortalities of the two pests placed on the top of the grains were 100% after 72 h exposure with the concentration of 3 ppm, but the mortalities of S. zeamais placed under the maize, wheat and paddy were 89, 82 and 65%, respectively, and that of R. dominica were 56, 47 and 32%, respectively, (Table2, 3).

A higher concentration of 24 ppm could kill 100, 100 and 98% of S. zeamais placed under the maize, wheat and paddy, respectively, and that of R. dominica were 100, 93 and 86%, respectively (Tables 2, 3). The higher molecular weight and higher boiling point of the horseradish essential oil might be attributed to the poor penetrability and diffusibility in the grains. Appropriate synergists or gas recirculation equipment could contribute to facilitate diffusion of horseradish oil in the grains.

The type of grain and its amount directly affected the efficacy of horseradish oil. In this paper, horseradish oil gave the best efficacy for maize, good for the wheat and the worst for paddy (Tables 2, 3, 4, 5). This was partly the result of the grain's properties such as size and its surface smoothness. Other factors should also be studied further. We conclude that the horseradish oil could be used with certain grains in small warehouses or the farmers' warehouse under natural fumigation conditions.

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Generation of phosphine gas for the control of grain storage pests

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Abstract

The phosphine generator is a device for rapid production of phosphine (PH_3) gas to be introduced into grain storage. The aluminum phosphide (ALP) tablets are used as raw material and its effective constituent is 56%. When the aluminum phosphide and water are brought into contact a hydrolyzation reaction takes place to produce the phosphine gas. Controlling the reaction temperature, reaction pressure and the dosage of aluminum phosphide immersed in the water, the hydrolyzation reaction can be controlled or the output speed of phosphine generated can be controlled. If phosphine concentration exceeds the explosive limit, the phosphine easy catches fire and causes explosion. The carbon dioxide is an inert gas. Before the high concentration phosphine is released into air, using carbon dioxide dilute it to make the phosphine concentration diminution, until phosphine concentration is very low than the phosphine explosion limit. According to the amount of phosphine generated in unit time, mixing an amount of carbon dioxide with it at the same time, controlling both proportion between phosphine and carbon dioxide can be realized. weight ratio between phosphine and carbon dioxide has been proved to be safe by practice. This ratio is 2% phosphine to 98% carbon dioxide. If the generation of phosphine gas can be controlled, the phosphine generator can be used for phosphine recirculation fumigation at low or high concentrations. The generator is operated outside of the storage structure and the phosphine gas is introduced into the structure, either directly through the gas distributor or through recirculation system which has a recirculation fan.

Keyword: Phosphine, Generator, Fumigation, Grain, Storage, Pests

1. Introduction

Phosphine has been used widely in grain storage globally because it is effective, easy to use, has strong penetration and is inexpensive. In China for the national grain reserves, annually 2500 t of aluminum phosphide are applied, of which 1000 t are applied using phosphine generators. Although the phosphine resistance of pests has been increasing, the following combinations have been applied: phosphine generators in combination of recirculation fumigation in improved gas tightness storages; phosphine generators in combination of recirculation fumigation, under plastic film; phosphine generators in combination of gas distributor for fumigating multiple stacks or containers.

The phosphine generators technology is based on the combination of carbon dioxide and phosphine for fumigation. After mixing phosphine produced by phosphine generator with carbon dioxide in a steel cylinder, the gas is conveyed into the treated premises. This technology has several advantages, such as reducing the potential combustion, increasing gas penetration, improving uniform distribution in grain bulks, reducing the fumigation time, and reducing polluting residues.

2. Phosphine recirculation fumigation technology

Phosphine recirculation fumigation technology has been regarded as an effective and feasible way for pests control in silos and warehouses. It could make phosphine uniform distribution by recirculation fan and recirculation pipe. From its effect for pest's control, people usually took this as a simple, safety, economic and effective way.

Before fumigation, we should be familiar with the air tightness of the grain storage to be fumigated. This is the key factor in the success of the recirculation fumigation. An accepted standard of the air tightness of grain storage consists of pressure decay half life time from 500 Pa to 250 Pa should be 200 sec in large empty silos, and over 60 sec in empty warehouses.

The first part of the equipment is the phosphine recirculation system that is composed of the following five parts: A. Phosphine generator; B. Recirculation fan; C. Aeration pipe on the bottom of the granary;

D. Recirculation pipe on the surface of grain and under plastic sheeting; E. Recirculation pipe on the outside wall of granary. (Fig. 1)

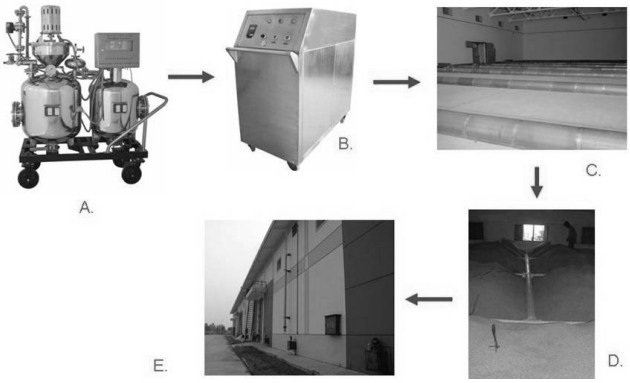

Figure 1 Phosphine recirculation fumigation system. The red arrows show the direction of phosphine movement in the recirculation fumigation system.

The second part of the equipment is the fumigant generator and distribution system (Fig. 2): A. Phosphine generator; B. Gas distributor.

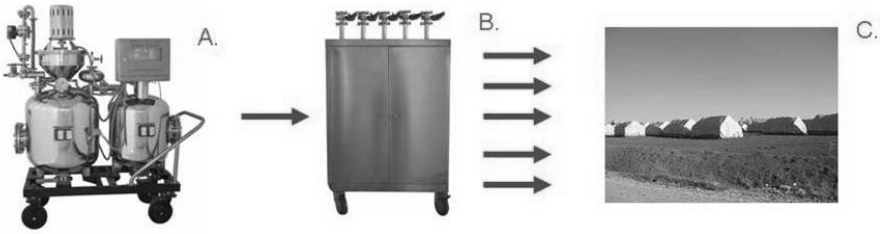

Figure 2 Regulating distributes fumigation system. The red arrows show the direction of phosphine movement in the regulating distributes fumigation system.

3. Operation principle the phosphine generator

The phosphine generator is a device for rapid production of phosphine gas to be introduced into grain storage. The aluminum phosphide is used as raw material. The generator is operated outside of the storage structure and when the recirculation fan is activated, the gas mixture of phosphine and carbon dioxide from phosphine generator is conveyed to the outlet of recirculation fan. The air flow carries the mixture gas into the ventilation system on the bottom of grain storage. By using the recirculation fan the phosphine gas is introduced into the stored grain. Through the ventilation pipeline the phosphine gas can be uniformly distributed.

The recirculation fan that is used in recirculation fumigation system should conform the following parameters: fan power ≤1kW; air pressure ≤1kPa; air flow rate ≤1000 m^3/h; linear velocity of lamina brim ≤ 40m/s. For granaries with improved gas tightness, the exchange air flow rate of the granary should be at least two times per day.

4. Phosphine recirculation fumigation technology under plastic film

It is possible to apply phosphine recirculation fumigation in grain bulks under plastic film after grain surface is covered by plastic film, and recirculation pipes are installed in the grain surface and connected to ventilation pipes. Sealing by plastic film, could prevent phosphine leaking and keep effective concentration for a long time.

5. Phosphine generators in combination of gas distribution system

This system is composed by A. Phosphine generator and B. Gas distributor (Fig. 2) The gas distributor is mainly composed of 5 unit gas flow meters which are specially used for regulating the gas flow of carbon dioxide. Adjusting the 5 unit gas flow meters, the mixture of phosphine and carbon dioxide can be divided into proportional 5 pipes. This set up enables fumigation of several warehouses or several stacks or several containers at the same time.

6. Operation of the phosphine generator

One fumigation process needs 8 kg aluminum phosphide (tablets or pellets), 180kg water, 8 cylinders of carbon dioxide each of 20 kg gas, and 110V or 220V electric source.

The phosphine generator can produce about 2.66 kg of phosphine in 2 h. Before the generator begins to work, air from its internal chamber should be removed by flushing with carbon dioxide. The tablets or pellets of aluminum phosphide are automatically supplied through a timing motor device to a reaction chamber that contains water. The phosphine gas is produced and then carried by carbon dioxide into the storage. Since the phosphine concentration is maintained below 2% in air, there is no fire hazard.

The generator is controlled by a computer. Once programmed, the generator will operate automatically, until the 8 kg aluminum phosphide is processed.

During the generator operation, the aluminum phosphide and water are brought into contact to produce the phosphine gas. Controlling the reaction temperature, reaction pressure, and the dosage of aluminum phosphide immersed in the water, the hydrolyze reaction and the amount of phosphine generated in per unit time can be controlled. Aluminum phosphide is automatically supplied (through a timing motor device) into a reaction chamber that contains water. In this reaction the gas phosphine is generated immediately, and then it is carried by carbon dioxide into the grain storage (Fig. 3).

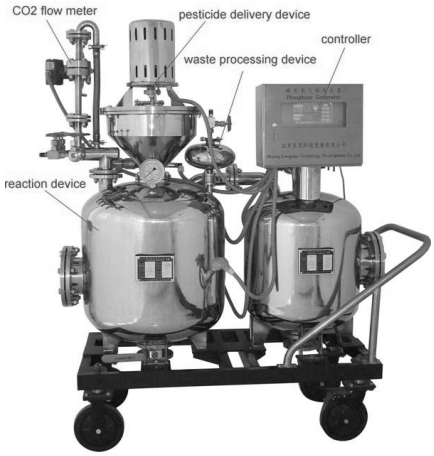

Figure 3 Configuration of the phosphine generator. The configuration of the phosphine generator. It includes: aluminum phosphide delivery device, container, reaction device, container, filtration container, controller, carbon dioxide supply valve, carbon dioxide (CO_2) flow meter and waste processing device, reagent container.

Because the phosphine is flammable and explosive, it should be mixed with carbon dioxide by weight ratio of 98% carbon dioxide and 2% phosphine before the phosphine is released into air.

The cone-shaped container can hold 8 kg aluminum phosphide (tablets or pellets). A timing motor is located on the top of the container and its spiral device rotation speed can be controlled by the phosphine generator. The spiral device supplies aluminum phosphide into the reaction container that can hold 100 kg water User can adjust the carry speed by touch screen on the controller of phosphine generator. The adjustable range is 12~72 g/min. One gram of aluminum phosphide (tablets or pellets) can produce about 0.33 g phosphine, so the capacity of pure phosphine is 4 ~24 g/min. If the immersed speed of the aluminum phosphide into water in reaction container is 72 g/min, after 111 minutes 8 kg aluminum

phosphide is consumed, and the total capacity of phosphine generated in one cycle of fumigation is 2.66 kg.

The aluminum phosphide tablets contain some wax that causes delay in its hydrolyzation reaction. After the total aluminum phosphide is immersed into the water the function of the reaction container is terminated, but carbon dioxide is blown for at least 60 minutes, until the hydrolyzation reaction can be nearly completed.

7. Reaction and filtration container

Before fumigation, water is added into the reaction and the respective filtration container. During the whole process in one cycle of fumigation, the capacity of the reaction container is 100 kg water for reaction reagent, and the capacity of the filtration container is 80 kg water. The water of the filtration container is used for filtration of the foam generated in reaction container; and also for cooling the phosphine and carbon dioxide mixture gas.

When the aluminum phosphide is added into reaction container, it reacts with water at once and generates the gas phosphine immediately. A certain amount of carbon dioxide gas is supplied to mix with the gas phosphine in the reaction container, and then the mixture of the two gases is conveyed into the filtration container. After cooling and filtration, the cool and pure mixed gas can be released into the site to be fumigated or into the gas recirculation pipe.

The other function of carbon dioxide gas is agitating the aluminum phosphide in the reaction container, so that the aluminum phosphide hydrolysis reaction can be accelerated.

The pressure gauge on the reaction container indicates working pressure of the mixture of phosphine gas and carbon dioxide. The pressure transducer is a sensor that can detect the pressure inside the reaction container. If the pressure inside reaction container exceeds the set pressure, the controller sends an alarm signal. The working pressure of the reaction container is 2~5 kPa, that is depending on the gas resistance from the outlet of phosphine generator to fumigation sites. In case of excessive pressure in reaction container, the safety valve is automatically activated for releasing the excessive pressure.

The temperature sensor can detect the temperature inside the reaction container. If the temperature inside reaction container is exceeded, the controller of phosphine generator can give an alarm. The working temperature is 35 to 60°C, depending on the gas generation rate and environment temperature. If the gas generation rate and environment temperature are both higher, the working temperature is also higher and vice versa. The electric heater is controlled by the controller of the generator to heat the water inside reaction container.

8. Controllers

The work of the phosphine generator is controlled by a microcomputer inside the controller of phosphine generator. The working parameters can be set and displayed on a touch screen.

The phosphine generator can be used at low phosphine concentration and long exposure time, or at high phosphine concentration and short exposure time. User can increase or decrease the gas generation rate from 12 to 72 g/min. According to the working speed, the microcomputer calculates the required quantity of carbon dioxide, to ensure the proportion of 2% phosphine to 98% carbon dioxide. The flow rate of carbon dioxide is also showed on the touch screen from 100 to 600 L/min (about 200 to 1200 g/min), equivalent to rate of gas generation from12 to 72 g/min. According the requirement quantity of carbon dioxide, user needs to adjust the carbon dioxide supply valve on steel cylinder by manual for supplying the gas of carbon dioxide to phosphine generator.

The pressure transducer can detect the pressure inside reaction container, which is from 2 to 5 kPa. The temperature sensor can detect the temperature inside reaction container, which is from 35°C to 60°C. If the normal values of these parameters are exceeded, the controller of phosphine generator can send an audible alarm. User can set the rate of aluminum phosphide to be immersed into the water inside reaction container, and the required carbon dioxide gas flow displayed on touch screen.

9. Carbon dioxide supply valve and flow meter

Providing enough carbon dioxide is the key to ensure the safety of fumigation process. The gas flow for the carbon dioxide supply valve should be adjusted 120 L/min (240 g/min), the total gas flow of five units carbon dioxide supply valve should be 1200 g/min. When they are mixed, their weight proportion is 98.04% carbon dioxide and 1.96% phosphine in the gas mixture. The carbon dioxide loaded in steel cylinder is the gas source for the phosphine generator. The carbon dioxide supply valves are set on the steel cylinder. The maximum capacity of one carbon dioxide supply valve is 120 L/min (240 g/min). According to the phosphine generator rate and the quantity of carbon dioxide, the user can decide how many carbon dioxide steel cylinders should be used. For example, if the phosphine generator rate is 72 g/min, the output speed of pure phosphine is 24 g/min, each carbon dioxide cylinder can supply 120 L/min (240 g/min) carbon dioxide gas, user should be using five carbon dioxide cylinder, which can supply 600 L/min (1200 g/min) carbon dioxide, so that the weight proportion between phosphine and carbon dioxide is 2:98.

Frost and ice is usually formed when carbon dioxide is released from carbon dioxide cylinder through a supply valve. Therefore, electric heating is applied on carbon dioxide supply valve to avoid such a phenomenon.

The carbon dioxide gas has two more functions. One is agitation in the reaction container to ensure aluminum phosphide hydrolyzing reaction is complete in the water. The other is in adjusting the temperature of the mixed gas at the outlet of phosphine generator.

10. Reagent container

After the hydrolyzation reaction of aluminum phosphide in water, two kinds of materials are produced: aluminum hydroxide and phosphine. The chemical formula is: AlP+3H2O=Al (OH) 3+PH3. So the waste liquid includes aluminum hydroxide [Al (OH) 3] and the residual water that looks like a watery mud in the reaction container.

Because aluminum phosphide includes a little wax, sometimes the hydrolyzation reaction is not complete, so the waste liquid includes an amount of phosphine. Before releasing the waste liquid from the reaction container, the phosphine gas in the waste liquid must be cleared.

To eliminate the presence of residual phosphine, the waste liquid is treated using a special reagent composed of nitric acid (HNO3), perchloric acid (HClO4) and copper sulphate (CuSO4), at a ratio of 1000 mL, 500 mL, and 70 to 100 g, respectively.

d. After processing, the waste liquid can be changed into a non-poisonous liquid which is safe and can be disposed of by the normal route for industrial waste.

Nitric acid is mainly used to neutralize ammonia in the waste liquid. The oxidant, nitric acid can oxidize low valence phosphorus. Perchloric acid is mainly used as the oxidant which can oxidize low valence phosphorus. Also used to neutralize ammonia in the waste liquid. As the catalyst, copper sulphate (CuSO4) is used to catalyze the reaction between perchloric acid and low valence phosphorus. After adding perchloric acid into copper sulphate (CuSO4), the reaction between perchloric acid and low valence phosphorus can be accelerated.

11. Safety devices in the phosphine generator

The generator may only be operated by certified fumigators who have been specifically trained to use this equipment. If there is insufficient flow of carbon dioxide, or the pressure in the internal chamber is excessive, or the temperature in the reaction chamber becomes too high or too low, or there is power failure, the generator will automatically stop production of phosphine gas. Should any of these conditions develop, an audible alarm will sound.

12. Technical parameters of phosphine generator

Working sites: indoor or outdoor. Environment temperature: 0°C ~ 45°C. Environment relative humidity: 20 ~ 90% r.h. Environment height above sea level: ≤1000 meter. Category of pesticide: aluminum phosphide pesticide, tablets or pellets. Diameter of the tablets or pellets: less then 10 mm. Content of aluminum phosphide in pesticide tablets or pellets: 56%. Maximum weight of aluminum phosphide in once fumigation process: ≤8 kg. Speed of aluminum phosphide tablets immersed into the water of the

reaction container: 12~72 g/min. The detect range of CO_2 flow rate by CO_2 flow meter: 100~ 600 L/min (200 ~1200 g/min). Design pressure of container: 200 kPa. Working pressure of container: 2~5 kPa (Depending on the gas resistance from the outlet of phosphine generator to fumigation sites). Safety valve threshold pressure: 80~90 kPa. Working temperature: 35 ~ 60°C. Noise of equipment: <70dB. Electrical source (AC): 230 V±10% /50 Hz ±1 Total power: 3.7 kW. Power rate of the 5 units carbon dioxide supply valve are 1.5 kW. Power rate of the electricity heater for phosphine generator is 2.0 kW. Power rate of the controller of phosphine generator is 0.08 kW. Power rate of the timing motor 0.12 kW. Size of equipment: length 160 cm × width 70 cm × high 170 cm. Net weight t: 350 kg. Gross weight: 400 kg. Water volume: 180 kg (reaction container: 100 kg, filtration container: 80 kg). Carbon dioxide supply valves: 6 units. Percentage of gas mixture: phosphine 2%; carbon dioxide 98%

13. Technical parameter of carbon dioxide supply valve

Inlet pressure: 0~15,000 kPa. Outlet pressure: 0~500 kPa. Gas flow rate: 0~120 L/min or 0~240 g/min. Voltage of electric heater: 230 V±10% / %/50 Hz±1. Power of electric heater 1 unit: 0.30 kW; 5 unit total power: 1.5 kW. Safety valve threshold pressure: 800~1000 kPa. Cut-off temperature of heat electric relay: 50±5°C.

14. Recirculation Fan

The recirculation fan is used in recirculation fumigation system. When the recirculation fan is in operation, the mixture gas of phosphine and carbon dioxide supplied from the phosphine generator is directly added to the outlet of recirculation fan and the flowing air carries the mixture gas into the ventilation system on the bottom of grain storage. By using the recirculation fan the phosphine gas is introduced into the stored commodity. Through the ventilation system the phosphine gas can be made uniform distribution.

15. Phosphine concentration monitors

Phosphine concentration monitors are used to detect the phosphine concentration during the fumigation process. Its detection range is 0 ~1000 ppm. The electrochemical sensor has been used in the detector that measures the concentration of target gas continuously and sends sound-light alarms once the threshold was achieved (Fig. 4).

16. Phosphine alarm

Phosphine alarm is a specially designed pocket sized instrument to protect workers from the hazards of phosphine gas inhalation during the fumigation process. If the concentration of phosphine around the work site is over 0.3 ppm, it will sound and light the alarm (Fig. 5).

Figure 4 Gas distributor.

Figure 5 Recirculation fan.

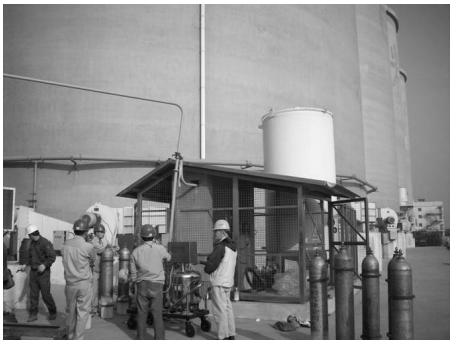

Figure 6 Phosphine recirculation fumigation system on big silo.

Figure 7 Phosphine recirculation fumigation system on big storehouse.

Figure 8 Phosphine recirculation fumigation system on big stack.

Study on the mortality of the stored-grain insects adults in different concentrations of low oxygen

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Abstract

During this research, adults of *Liposcelis bostrychophila* Badonnel, *Oryzaephilus surinamensis* (Linnaeus), *Tribolium confusum* Jacquelin du Val, two strains of *Tribolium castaneum* (Herbst) (from Zhongshan and Yiyang), *Sitophilus oryzae* (Linnaeus) and *Sitophilus zeamais* Motschulsky were first kept in various low oxygen atmospheres whose concentrations were 0%, 1% and 2% for different exposure times, then in the normal atmosphere. The data and trend of mortality changes accompanied by the low oxygen concentration and exposure time were gained after this research. Results indicate that there were obvious inter-specific differences among the test insects in the sensitivity to low oxygen atmosphere, and the sensitivity declined as follows: *L. bostrychophila*, *O. surinamensis*, *T. confusum*, *T. castaneum*, *S. oryza*e and *S. zeamais*. The insects of closely related species presented diverse responses to the low oxygen stress. *Tribolium confusum* and *T. castaneum* showed a very dissimilar sensitivity to the low oxygen atmosphere, but there was no obvious difference between *S. oryzae* and *S. zeamais*. A fast lethal effect on the adults of *L. bostrychophila*, *O. surinamensis*, *T. confusum* and *T. castaneum* was observed when exposed to 0% oxygen. At 1% and 2% oxygen atmosphere, the mortality level of *T. castaneum* exceeded 60% and 80%, respectively. The lethal effect of 2% was more efficient than 1% oxygen, which was the same as *S. oryzae* and S. *zeamais* at 2% oxygen atmospheres. The mortality of *S. oryzae* and S. *zeamais* at 1% and 2% oxygen concentration was higher than at 0%, and the mortality of these two insects might be higher under the condition of a small amount of oxygen. At 1% and 2% oxygen atmosphere, the growth trend of the cumulative mortality of *T. confusum* and *T. castaneum* generally conformed to the classical model of the Logistic formula which indicated the response of insect pests when applying common insecticide. These results are useful to extend the control technology of stored-grain insects with low oxygen universally.

Keywords: Low oxygen; Stored-grain insect; Mortality; Treatment duration

1. Introduction

As a green control technology of stored-grain insects, the low oxygen disinfestation technology has been more and more popular with many companies in China. In order to get a scientific practical application of it, the worldwide studies have focused on the lethal effect of different low oxygen concentrations to kinds of stored-grain insects. As reported, atmosphere where the oxygen content was less than 1%, the lethal time of *O. surinamensis, R. dominica* and *S. oryzae* was more than 1 d, 4 d and 14 d respectively, and that of *T. confusum* and *T. castaneum* was more than 7 d at 20ºC to 29ºC (Banks and Fields, 1994). The stored-grain insects, such as *R. dominica, S. granarius* and *S. oryza*e adults were more tolerant than *C. ferrugineus*, *O. surinamensis* and *T. castaneum* at various low oxygen concentrations (Krishnamurthy et al., 1986; Conyers and Bell, 1996). In China, the disinfestation effect of high-purity nitrogen atmosphere (almost anaerobic) to common stored-grain insect adults was studied (Zhang et al., 2007.). In view of many studies, Annis (1987) made many useful suggestions on the relationship between the oxygen content and exposure time in the application of low oxygen disinfestation technology.

In recent years, with the gradually increasing demand of the green grain storage, there was a large scale application of controlled atmosphere storage technology in China. To reduce the costs of grain storage by the CO_2 controlled atmosphere, the low oxygen storage technology was studied, the control of stored-grain insect with low oxygen technology and the green low oxygen grain storage technology were carried out, and until now some good effects have been achieved in China. Until May 2009 in China, there were more than 20 national grain depots which conducted the application of “Nitrogen-rich and Low-oxygen

Grain Storage Technology" (NLGST), a great progress in green grain storage technology has been made in China. In the present study, adults of *L. bostrychophila*, *O. surinamensis*, *T. confusum*, *T. castaneum*, *S. oryzae* and *S. zemais* were exposed to different low oxygen concentrations and exposure times, then they were kept at normal atmosphere. The mortality of all these pest species was analyzed, with the aim to provide more theoretical support for the promotion of NLGST.

2. Materials and methods

2.1. Culture of the test insects

Sitophilus zeamais and *S. oryzae* sampled from Guangzhou, Guangdong Province were reared on wheat of moisture content 14 ± 2%; *T. confusum* form Puyang, Henan Province, and two *T. castaneum* strains form Yiyang, Hunan Province and Zhongshan, Guangdong Province were reared on a mixture ($w_{\text{whole wheat flour}}/w_{\text{yeast}}$ = 19/1); *O. surinamensis* form Nanyang, Henan Province was reared on a mixture of feeds ($w_{\text{oat}}/w_{\text{whole wheat flour}}/w_{\text{yeast}}$ = 6/3/1); *L. bostrychophila* form Xinshagang, Guangdong Province was reared on a mixture of feeds ($w_{\text{whole wheat flour}}/w_{\text{yeast}}/w_{\text{milk powder}}$ = 1/1/1) passed a screen by 187.5 μm. All of them were maintained at 30 ± 1ºC (75 ± 5 % r.h.).

2.2.1. The experimental equipment

The experimental equipment consisted of the gas source, flux control device, experiment container and pipelines(Zhang et al., 2007). To ensure purity and stability of the gas, we chose high-purity N_2 (GB 8980-88) and medical O_2 (GB 8982-88) as the gas source. The glass desiccators were used for treating test insects with condition of low oxygen atmosphere, and desiccator lids were sealed gastight using Vaseline.

2.2.2. The assessment

Adults of 7-10 d old were chosen as test insects. Batches of 50 insects were placed in each culture dish with a diameter of 75 mm, whose wall was coated with teflon, and the bottom was covered with filter paper. Furthermore, crushed wheat of 1 g/dish was added for *S. oryza*e and *S. zeamais*. *T. castaneum*, *T. confusum*, *O. surinamensis* were kept with 0.1 g/dish while for *L. bostrychophila* was kept with of 0.001 g/dish.

All experiments were repeated three times, and the control group was exposed to the normal atmosphere for the same exposure time as the treatments. All experiment groups were maintained at 30 ± 1ºC (75 ± 5 % r.h.). In order to mitigate their discomfort caused by the container or else, the test insects were placed into the desiccator 24 h before the experiment. After that, according to Table 1, 2 and 3, the test insects were treated for different exposure times at 0%, 1% and 2% oxygen.

Table 1 Exposure time (h) of different test insects in the oxygen concentration of 0% at 30 ± 1°C and 75 ± 1% r.h.

Test insects strains	**Experiment group**						
	A	**B**	**C**	**D**	**E**	**F**	**G**
L. bostrychophila	2	3	4	5	6	7	8
O. surinamensis	3	4	5	6	7	8	9
T. castaneum (Zhongshan)	11	13	15	17	19	21	23
T. castaneum (Yiyang)	10	11	12	13	14	15	16
T. confusum	10	11	12	13	14	15	16
S. oryzae	25	27	29	31	33	35	37
S. zeamais	25	27	29	31	33	35	37

Table 2 Exposure time(h) of different test insects in the oxygen concentration of 1% at 30 ± 1°C and 75 ± 1% r.h.

Test insects strains	Experiment group						
	A	B	C	D	E	F	G
L. bostrychophila	12	14	16	18	20	22	24
O. surinamensis	12	18	24	30	36	42	48
T. castaneum (Zhongshan)	12	18	24	30	36	42	48
T. castaneum (Yiyang)	12	18	24	30	36	42	48
T. confusum	12	18	24	30	36	42	48
S. oryzae	12	18	24	30	36	42	48
S. zeamais	12	18	24	30	36	42	48

Table 3 Exposure time(h) of different test insects in the oxygen concentration of 2% at 30 ± 1°C and 75 ± 1% r.h.

Test insects strains	Experiment group						
	A	B	C	D	E	F	G
L. bostrychophila	23	26	29	32	35	38	41
O. surinamensis	12	24	36	48	60	72	84
T. castaneum (Zhongshan)	12	24	36	48	60	72	84
T. castaneum (Yiyang)	12	24	36	48	60	72	84
T. confusum	12	24	36	48	60	72	84
S. oryzae	12	24	36	48	60	72	84
S. zeamais	12	24	36	48	60	72	84

After treatment in the low oxygen atmosphere, the culture dishes and the test insects were removed from the desiccators together and ventilated for 20-30 min in the normal atmosphere. After that, all groups were maintained in the normal atmosphere at 30 ± 1°C (75 ± 1% r.h.), and added the feeds properly. The mortality changes were daily recorded until they were stable.

3. Results and discussion

3.1. The cumulative mortality and changes of L. bostrychophila adults in three different low oxygen concentrations

The mortality curves of *L. bostrychophila* in the oxygen atmosphere of 0%, 1% and 2% are shown in Figure 1. For 0% treatment, the mortality of *L. bostrychophila* increased fast, and reached 100% after 6 h. In 1% oxygen concentration, it needed 24 h to reach the mortality of 100%. And for 2% treatment group, it needed 48 h. The results indicated that, the lower the oxygen contents the shorter the lethal time.

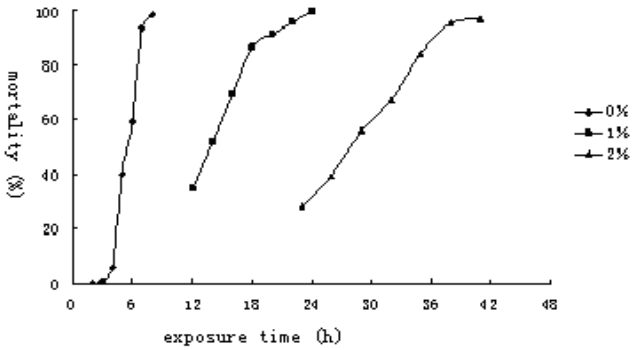

Figure 1 The mortality curve of *L. bostrychophila* adults in three different low oxygen concentrations.

Despite the difference of more than 10 h in the lethal time, the mortality rate of 1% and 2% samples increased almost simultaneously within the mortality of 30%-90%. These results suggest that adults exposed to the above two treatments might have the same sensitivity to the low oxygen. The mortality raise to 85% or more was gradual and slow, which indicated that there was a group of individuals with higher tolerance, and a slower change of the sensitivity to low oxygen than the earlier dead insects. But the relevant reason still needed to be further studied.

As Figure1 showed, the mortality curves of three different concentrations of oxygen separated clearly. These results suggest that *L. bostrychophila* might be very sensitive to changes of the low oxygen concentration, and slight change of oxygen content could greatly affect its lethal impact.

3.2. The mortality curve of O. surinamensis adults in three different low oxygen concentrations

The mortality curve of *O. surinamensis* in the oxygen content of 0%, 1% and 2% was shown in Figure 2. At 0% oxygen, the mortality of *O. surinamensis* increased rapidly, and reached 100% after 6 h. At 1% oxygen, the mortality after 24 h was about 92%. And at 2% oxygen, the mortality was 60% after 24 h. The results showed that, with the reduction of the oxygen content, the duration to the mortality of 100% was significantly reduced. For the treatment of 1% and 2% oxygen, the mortality increased much more slowly than 0%. These two treatments responded similarly at exposure to 12 h, and at longer exposure time the mortality response separated gradually, and the treatment of 1% reached to the mortality of 100% earlier.

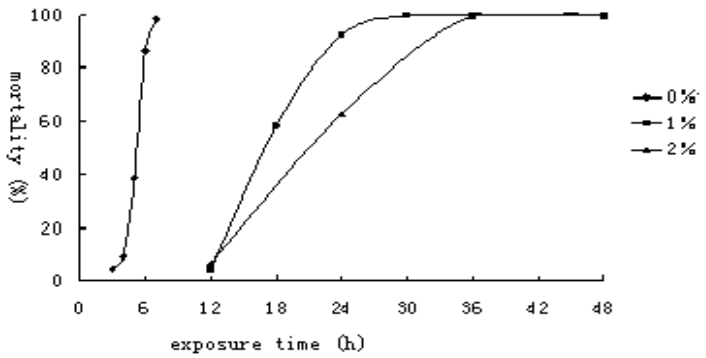

Figure 2 The mortality curves of *O. surinamensis* in three different low oxygen concentrations.

3.3. The mortality of T. confusum in three different treatments of low oxygen

The mortality curves of *T. confusum* at the low oxygen concentration of 0%, 1% and 2% were shown in Figure 3. At the oxygen content of 0%, the morality of *T. confusum* increased within short exposure times, and attained 100% after 16 h. The mortality at 1% and 2% oxygen was less than 20% at shorter exposures than 24 h. Then at exposure of 48 h, the mortality of the test insects reached close to 100%. However, the response of the insects differed obviously to these two low oxygen treatments. After the treatment of 1% oxygen for 36 h, the mortality reached as high as 95%. At 2% oxygen, the mortality gradually increased, reached only 40% after 36 h, and then prolonging the exposure to 48 h, resulted in up to 95% mortality. In addition, for the 1% oxygen, the cumulative morality response of the pest followed the trend of “S” type curve.

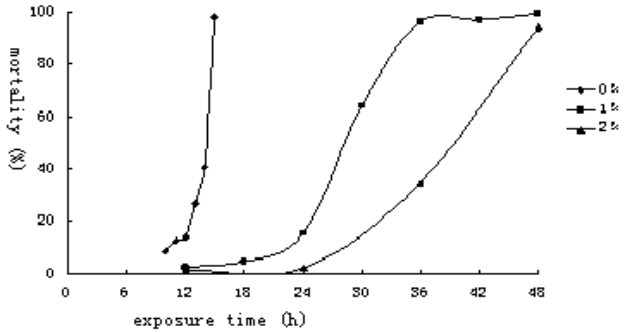

Figure 3 The mortality curves of *T. confusum* at three different treatment of low oxygen.

3.4. The mortality of T. castaneum (Yiyang strains) in three different treatments of low oxygen

The mortality curve of *T. castaneum* (Yiyang) in the low oxygen atmosphere of 0, 1 and 2% under experimental conditions were presented as Figure 4. For the oxygen content of 0%, the morality of the pests increased rapidly, and reached the peak 100% after 12 h. At 1 and 2% oxygen, the mortality raise tardily, and no mortality was observed before 18 h exposure. Then with the extension of the exposure time, the mortality at the two oxygen concentrations began to increase, and after 48 h the two mortality curves reached up to 80 and 100%, respectively. Under the test conditions, the mortality curves of 1 and 2% began from the same starting point, and then gradually increased with near linearity, and there was an incorporated trend with a crossover at 36 h, and the mortality of 2% oxygen exceeded that of 1%.

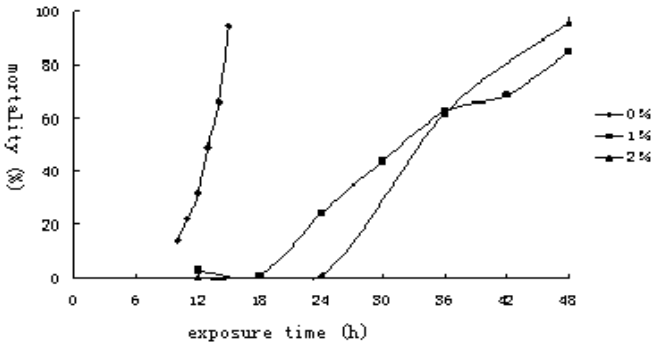

Figure 4 The mortality curves of *T. castaneum* (Yiyang) at three different treatment of low oxygen.

3.5. The mortality of T. castaneum (Zhongshan) in three different treatments of low oxygen

The mortality curves of *T. castaneum* (Zhongshan) in the oxygen content of 0%, 1% and 2% were shown in Fig. 5. For the treatment of 0%, the mortality of the pests increased fast, and went up to 100% after 16 h. The mortality curves of 1% and 2% groups resembled *T. castaneum* (Yiyang). After exposure to 24 h, there was almost no change in the mortality level. When prolonged the exposure time to 34 h, it was found that both of the two oxygen concentrations caused to increased mortality, and reached more than 80% and the two curves were very close to each other. Insect mortality at 1% oxygen ascended slowly with a stable level at 80%, but the 2% oxygen went up to 99% mortality rapidly from 80% in 18 h, and the effective time lasted 48 h, whose mortality of 2% oxygen was strikingly exceeded the 1% oxygen. In addition, for 1% and 2% oxygen, the cumulative mortality of the test insects increased and followed the shape of "S".

The sensitivity to low oxygen of *T. castaneum* (Yiyang) and of *T. castaneum* (Zhongshan) showed many similarities to hypoxia condition. It was found that the mortality was very low after treatment for 18 or 24 h at 1 and 2% oxygen, accelerated when extended to 36 h, crossed at 60 and 80%, afterwards exposure to 2% oxygen resulted in higher mortality than at 1% oxygen. Mortality of *T. castaneum* (Yiyang) reached close to 100% after 48 h (Fig. 4); while the mortality of *T. castaneum* (Zhongshan) changed slowly (Figure 5). The mortality remained steady 80% at 1% oxygen, however, at 2% oxygen after 48 h reached 95%. Furthermore, compared with *T. confusum* (Fig. 3), there was no crossing for 1 and 2% groups, which suggested that there was obvious difference between *T. confusum* (Fig. 3) and *T. castaneum* (Fig. 4 and 5) in the sensitivity to the hypoxia atmosphere, and the same strains had a similar sensitivity, and the reason needed to be deeply analyzed.

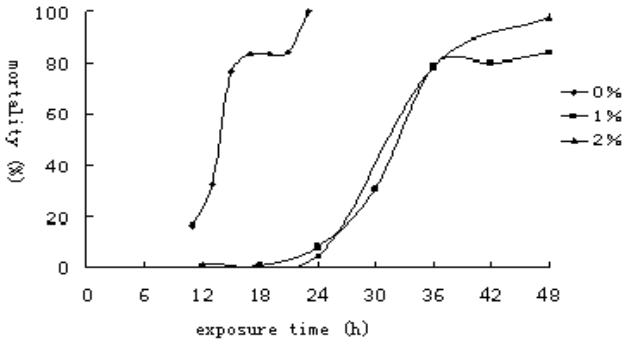

Figure 5 The mortality curves of *T. castaneum* (Zhongshan) at three different treatment of low oxygen.

3.6. The mortality of S. oryzae in three different treatments of hypoxia

The mortality curves of *S. oryza*e in the oxygen content of 0%, 1% and 2% were shown in Figure 6. The results showed that the mortality of the three test groups went up immediately. *Sitophilus oryza*e was less sensitive to hypoxia at 0% oxygen than at 1 and 2%, in which group it needed a longer exposure time for mortality. At 0% oxygen, the mortality was only around 75%, however, the mortality at 1 and 2% oxygen was 82 and 86%, respectively for the same exposure times. That is to say, under the condition of 1 and 2% oxygen, *S. oryza*e were more sensitive than at 0% oxygen within the mortality range of 10 - 80%.

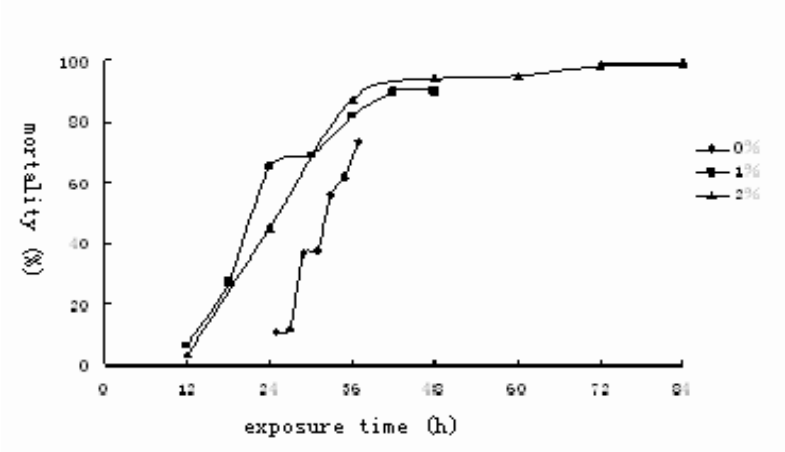

Figure 6 The mortality curves of *S. oryza*e at three different treatment of low oxygen.

There was a merged trend in the mortality curves at 1 and 2% oxygen. Moreover, the growth became very slow after the mortality of more than 90%. For the mortality at 2% oxygen, the exposure time to achieve from 86% to nearly 99% mortality lasted for 44 h from the 36 h to the 80 h. This indicates that some individuals were very tolerant to the hypoxia atmosphere, and we should pay attention to these high-tolerant individuals to monitor that whether *S. oryza*e can be used as the tolerant insect species to the hypoxia condition.

3.7. The mortality of S. zeamais in three different treatments of low oxygen

The mortality curves of *S. zeamais* in the oxygen content of 0, 1 and 2% were shown in Figure 7, which were similar to *S. oryza*e. For the treatment of 0%, the mortality was less than 75% after 36 h, which was slightly higher than 1, and 2% oxygen that reached 87% mortality. At 1 and 2% oxygen, the mortality curve fit well together from 0% to 60%, then began to separate after that. For 2% oxygen, the duration in which the mortality increased from 87 to 99% lasted for 48 h from the 36 h to the 84 h. These results suggested that similar to *S. oryza*e, there were also *S. zeamais* individuals tolerant to low oxygen atmosphere.

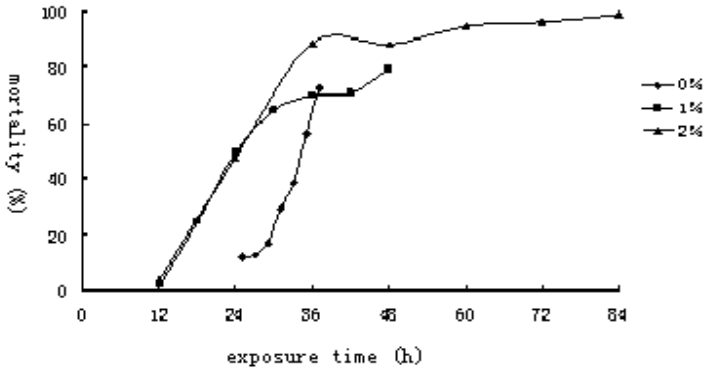

Figure 7 The mortality curves of *S. zeamais* at three different treatments of low oxygen.

The mortality curves of *S. zeamais* and *S. oryza*e to the low oxygen treatment presented similarities in response (Fig. 6 and 7), which was different from *T. confusum* and *T. castaneum*. In addition, the mortality at 1 and 2% oxygen, was higher than 0% oxygen. That is to say, the tolerance of *S. oryza*e and *S. zeamais* to the oxygen concentration at 0% was stronger and more specific, which is attributed to the spiracular closing mechanism of the adult beetles. At the lowest oxygen concentration the beetles strongly close their spiracles and in this way they prevent water loss, whereas at higher oxygen levels 1 and 2% they enable ventilation through their spiracles and death is caused due to desiccation (Navarro, 1978)

4. Conclusions

1. The sensitivity of five storage insect pests to the hypoxia treatment of 0, 1 and 2% oxygen were observed in declining order as follows: *L. bostrychophila*, *O. surinamensis*, *T. confusum*, *T. castaneum*, *S. oryza*e and *S. zeamais*, which presented a significant diversity.
2. The trend of the cumulative mortality of *T. confusum* and *T. castaneum* at 1 and 2% oxygen atmosphere generally conformed to the classical model of Logistic formula which indicated the mortality growth of pests when applying common insecticide.
3. 0% oxygen concentration has a quick lethal effect on the adults of *L. bostrychophila*, *O. surinamensis*, *T. confusum*, and *T. castaneum*.

4. The adults of closely related species showed a diverse tendency to the hypoxia stress. The mortality response of *T. confusum*, *T. castaneum* varied widely; however, *S. oryza*e and *S. zeamais* responded almost in identical trend.
5. In 1 and 2% oxygen concentration atmosphere, the mortality curves of crossed at 60 and 80% mortality, and the lethal effect of 2% oxygen was more efficient than that of 1%. Furthermore, under the same conditions *S. oryza*e and *S. zeamais responded similarly*.
6. The mortality of *S. oryzae* and *S. zeamais* in 1 and 2% oxygen concentration was higher than 0% oxygen, and in other words the mortality of these two pests might be higher in the presence of a small amount of oxygen

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