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Julius-Kühn-Archiv

Pieter A. Oomen, Helen Thompson (Editors)

Hazards of pesticides to bees

11th International Symposium of the ICP-BR Bee Protection Group

Wageningen (The Netherlands), November 2-4, 2011

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

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INTERNATIONAL COMMISSION FOR PLANT-BEE RELATIONSHIPS

Bee Protection Group

11TH INTERNATIONAL SYMPOSIUM

HAZARDS OF PESTICIDES TO BEES

WAGENINGEN, THE NETHERLANDS
November 2-4, 2011

Place
The Symposium was held at the
Hotel *De Nieuwe Wereld*
Marijkeweg 5, 6709 PG Wageningen
The Netherlands

Organising Committee

Pieter A. Oomen (ICPBR, NL), Chairman
Helen Thompson (FERA, UK), Secretary
Gavin Lewis (JSC International Ltd, UK), Vice-Chairman
Anne Alix (Ministère d'Agriculture, F / Dow AgroSciences UK Ltd)

Local organizers

Pieter A. Oomen
Claudia Jilesen (nVWA)
Jacoba Wassenberg (Ctgb)
Adindah Visser (symposium assistant)

ICP-BR / ICP-PR

The International Commission for Plant-Bee Relationships (ICP-BR) was founded in 1950 by the Swiss scientist Anna Maurizio whose outstanding work was mainly devoted to bees and their relationships with plants. Since 1980 this commission – which is affiliated to the International Union of Biological Sciences (IUBS) – has regularly organized in Europe working sessions on the harmonization of methods for testing the toxicity of pesticides to bees. About the time of this symposium, end of 2011, the ICP-BR decided to widen its scope from bees to pollinators, and to rename itself to International Commission for Plant Pollinator Relationships, ICP-PR.

ICP-BR has developed the scientific process preceding decisions from European administrative authorities, EPPO (European and Mediterranean Organisation for Plant Protection) and OECD (Organisation for Economic Cooperation and Development). ICP-BR Bee Protection Group symposia have acquired considerable authority in the area of legislation and regulation concerning bee protection related to the use of plant protection products, bringing together the European expertise of national authorities, industry and research. Operating through the EPPO honey bee sub-group, it has produced the testing methodology and risk assessment guidance currently used under Regulation (EC) 1107/2009.

The Bee Protection Group held its first meeting in Wageningen in 1980 and over the subsequent 32 years has become the established expert forum for addressing the risk of pesticides to bees. It has operated by reaching consensus amongst a wide range of experts active in this field drawn from industry, regulatory authorities and research institutes across the European Union (EU).

ICP-BR symposia Honey Bee Protection

1st symposium	Wageningen NL	1980
2nd symposium	Hohenheim D	1982
3rd symposium	Harpenden UK	1985
4th symposium	Řež CZ	1990
5th symposium	Wageningen NL	1993
6th symposium	Braunschweig D	1996
7th symposium	Avignon F	1999
8th symposium	Bologna I	2002
9th symposium	York UK	2005
10th symposium	Bucharest RO	2008
11th symposium	Wageningen NL	2011

Preface

The managed pollinator protection and health working group (the Bee Protection Group) of the International Commission on Plant Pollinator Relations (ICPPR) (yes, the name was officially changed at the general assembly that took place in conjunction with the International Pollination Symposium in Cholula, Puebla, Mexico in July, 2011) is the most active working group. Through its years of activity, it has provided leadership for the European Plant Protection Organization's concerns for pollinators and pollination, and for the ICPPR as a world-wide body. In the past decade or so there have been major changes in emphasis as more kinds of managed pollinators have become used around the world, new kinds of pesticides have been developed and deployed in agriculture, and international concern for the plight of pollinators and pollination in all ecosystems has risen. This 11th Symposium of the Bee Protection Group continues the traditions of keeping abreast of the needs for pollinator protection. The organizers and speakers are to be congratulated for the forward thinking and synthetic agenda that is reflected in this proceedings.

Around the world, concern for regulatory issues for pollinator health and protection have been, or are being reviewed. The first 19 items in these proceedings cover the gamut from reviews of current situations to the effects of relatively new classes of insecticides, notably the neonicotinoids that have been so much in the news. Pesticide pathways to pollinator and bee toxicity and hazard are mostly quite well understood, but the route through plant guttation is newly recognized. Bees must imbibe water. Guttation water from plants exuding systemic insecticides is a clear hazard.

Varroa destructor is responsible for the deaths of thousands of colonies of honeybees in many parts of the world. Its capacity to spread virus diseases exacerbates the problem. Thus, the testing of various acaricides is crucial to beekeeping as is covered in a single, valuable, contribution.

With major changes in the kinds of pesticides that have come into common use has come the need for refining test methodologies in laboratory to field-scale assessments. These proceedings, with 11 contributions in this area, serve to emphasise the importance of this sort of research. Closely coupled to test methodologies is the need to monitor pollinator poisoning incidents. There are 6 contributions that address honey bee poisoning incidence and how it can be monitored.

Although most of the symposium emphasises the importance of honey bees, other managed pollinators were not ignored. No fewer than 12 contributions address other pollinators, mostly bees from around the world. South American, African and European situations are discussed. The relatively new technology of using pollinators to disseminate biological control agents against crop pests and pathogens is presented in two contributions, one related to the adverse effects of pesticides on bee-biovectoring technology and the other on safety for the pollinator-vector of the biocontrol agents in formulation.

The symposium conclude with a plenary session to summarise and synthesise the information presented in the six sessions described briefly above, and presented in the individual contributions.

These proceedings will lead the way to future and evolving considerations in the ever pressing need for information on how to mitigate the effects of chemicals on honeybees for their own sake as valued micro-livestock and as pollinators. On the broader front, the need to protect other managed and wild pollinators and the pollination services they provide is now more evident than ever before.

Peter G. Kevan, Ph. D., FRES, FRSC

Chair ICPPR & Scientific Director of the Canadian Pollination Initiative

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Introduction to the 11th Symposium of the ICPBR Bee Protection Group

Pieter A. Oomen
Chairman and local organiser

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I wish you welcome to this 11th symposium of ICPBR Bee Protection Group, welcome to this 3rd symposium in Wageningen, and welcome to the 114 participants from 18 countries. Welcome to representatives from Government authorities, Universities and Research Institutes, Pesticide Industry and Ecotoxicological Test Consultancies, Beekeepers Organisations, private persons:

Tab. 1 Participants to this 11th ICPBR Bee Protection Group symposium accordingto their country of origin and according to the kind of organisation they represent.

Country	Participants
Algeria	1
Austria	1
Belgium	6
Brazil	6
Canada	1
Finland	1
France	15
Germany	25
Italy	8
Japan	1
Kenya	5
Norway	1
Saudi Arabia	1
Slovenia	2
Spain	2
Switzerland	4
The Netherlands	18
UK	16
Total	114

Represented organisation	Participants
Authorities	16
Beekeepers	8
Consultancy	6
Ecotoxicology	20
Government	10
ICPBR	2
Industry	24
International	2
Universities	26
Total	114

The main aim of these symposia is to finally achieve good protection of bees and other pollinators from harmful effects of pesticides by means of legal regulation of pesticide use, by enforcement of regulations, and by monitoring of the effects in practice. Earlier aims of these symposia, harmonisation of testing methods and risk assessment approaches, has been largely realised but still these remain aims of our group. In practice we want to realise these aims through:

- Exchange of information, know-how, methodology
- Signalling new and unexpected problems and solutions
- Harmonising testing methods
- Stimulating international coordination
- Recommendations to national and international authorities in pesticide registration
- All our discussions are or should be science based, using scientific arguments only. No politics in these discussions! Forget your own specific interests; this conference is about the common interest of protecting bees and pollinators only
- In practice of our Bee Protection Group we use to elaborate solutions for specific problems in dedicated working groups that report to this symposium.
- Solutions then are submitted to international organisations: EPPO, OECD, European Commission. And now EFSA as well.

This symposium brings together nearly all expertise in Europe on pesticide risks to bees, and we welcome the representatives from outside Europe (in this symposium from Algeria, Brazil, Canada, Japan, Kenya, Saudi Arabia) to participate in this expertise. We are happy to welcome among this wide expertise the chairman of the current EFSA Working Group on Pollinators, Mr. Robert Luttik, who is invited to give an update of the remit and work of the EFSA group.
The programme comprises three days of presentations and discussions, including half a day for a social programme in order to stimulate the informal contacts between all participants. Half a day is dedicated to reports from our specialised working groups and discussions about their proposals. And on the last day we welcome the FAO project on wild pollinators in the tropics with participants from Brazil, Kenya and the Netherlands that aims to develop practical options to reduce pesticide risks to wild pollinators. The specialised working groups that are developing proposals for practical solutions in realising our protection aims are:

- Risks posed by dusts (coordinator: Rolf Forster)
- Assessment of risks posed by guttation (coordinator: Jens Pistorius)
- Acceptability of effects in field studies (coordinator: Gavin Lewis)
- Acceptable levels of control and toxic reference mortality from in-cage and field tests (coordinator: Christine Vergnet)
- Risk to honeybee larvae (coordinator: Roland Becker)
- Design of post registration monitoring studies for systemic pesticides (coordinator: Anne Alix)

At the end of this symposium there is news about the composition of the board of the Bee Protection Group, and about the place where the next symposium over three years will be held. Finally we think it is important to mention, in order to stress the independence of this ICPBR-symposium, that this year for the first time there is no sponsoring of this symposium by industry. All costs of the symposium are born by the fees of the participants.
But as organisers we want to thank the Julius Kühn Institute in Braunschweig for publishing the proceedings in their series of the Julius-Kühn-Archive without costs to ICPBR, and to thank the Netherlands' Board for the Authorisation of PPPs and Biocides (Ctgb) and the Netherlands' Plant Protection Service (both in Wageningen NL) for their practical support in organising this symposium.

Epilogue

At the end of the symposium on November 4, 2012, Dr. Pieter Oomen announced his departure as a chairman, after participating in the group since 1985 and since taking over the chair from John Stevenson in 2002. He was thanked with a standing ovation by all participants. Also, he indicated to be willing to continue as a member of the board, as well as editor of the proceedings of this symposium.

- A new board for the ICPBR Bee Protection Group then was proposed , as follows:
- Helen Thompson, chairman (FERA, UK)
- Jens Pistorius, secretary (Julius Kühn Institute, D)
- Anne Alix, member (Dow Agrosciences, UK / Ministry Agriculture, F)
- Klaus Wallner, member (University Hohenheim, D)
- Jacoba Wassenberg, member (Ctgb, NL)
- Together with the existing members:
- Gavin Lewis, vice chairman (JSC International Ltd, UK)
- John Stevenson, member (ex chairman, UK)
- Pieter Oomen, member (ex chairman, NL)

These new and old members of the board of the Bee Protection Group were welcomed by the symposium participants with a generous applause.

As final conclusion of the 11th symposium, Prof Guy Smagghe of the University in Ghent, Belgium, invited the participants to a 12th symposium in his city and university for 2014, an invitation greatly welcomed by the new board and all participants.

Opening speech of the 11th Symposium

Wim H. van Eck
Deputy Director of the Netherlands' Food and Consumer Product Safety Authority

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Dear Mr. Chairman, ladies and gentlemen,

It is also my pleasure to welcome you to Wageningen on occasion of the eleventh symposium of the International Commission for Plant-Bee Relationship. Your agenda for this week is entirely devoted to Hazards of Pesticides to Bees. Despite the many years you work already on this topic, the issue is more relevant than ever before.

Before looking ahead, allow me to dwell for a few minutes in the past. It is now for the third time you are gathering in Wageningen, the seat of both the Plant Protection Service and the Agricultural University, nowadays called Wageningen University Research. In 1980 the late Professor Besemer, together with the national Counsel for Bee Breeding, Mr. Pettinga, organised your first meeting here in Wageningen. Mr. Besemer was a senior staff member of the Plant Protection Service and part-time professor at the university. He spent his whole professional career on pesticide research and regulations.

As the use of pesticides in agriculture was booming shortly after the second World War, already in the early fifties Mr Besemer became aware of the undesirable side effects of pesticides on nature and environment, including on bees. As attempts to solve problems with honey bees remained in vain, in 1980 he called together an international meeting of experts with the view to join forces and to learn from each other in search for solutions. Your international Commission was born.

In the years after the ICP-BR matured. Your remit was and is the harmonisation of testing methods, risk classification and risk assessment. Your strength is the fact that you bring together expertise from governments, industry, academia and bee keepers. Particularly through the latter you get feed-back on what is happening outside in the fields, urging for further improvement of risk assessment methodology.

We are now thirty years later and one proudly may say that a lot has been achieved. Harmonized and standardized testing methodology is available, endorsed by EPPO and OECD, giving your work a formal and truly international status. The same goes for the internationally agreed risk assessment approach for which you laid the foundations. Another success you may claim is the inclusion of bee protection provisions based on ICP-BR recommendations in the EU pesticide legislation. Many third countries nowadays benefit from your work. And finally, independent monitoring data shows that effective bee protection can be achieved using your risk assessment methodology.

So why then meeting again here in Wageningen? The world remains changing, at an even faster pace. Pesticides with new modes of actions and new ways of application require an ongoing review of current testing methods and risk assessment approaches. Enhanced threats of Varroa and introduction of new bee diseases urge for a rapid response. In our moderate climate the physical environment of honey bees is changing due to climate change, which might have well implications for the exposure and sensitivity of honey bees for pesticides. On top of that all of us face an enormous public and political interest. Honey bees and pesticides hit the front pages of our news papers and appear on the TV at prime time. Mr Albert Einstein is quoted. You and your work are in the spot lights.

What does this mean for the International Commission for Plant-Bee Relationship? First and foremost the Commission should retain its scientific integrity. Whatever you elaborate should be beyond any doubt. And besides that you have to work hard to keep pace with the abovementioned challenges. Testing methods and risk assessments should be adequate and up-to-date. Monitoring programs should be fit to signal new problems.

What remains is the need to continue to join forces. Your expertise and criticism is key to the success of the ICB-PR. What was valid in 1980, is still valid now: cooperation, exchange of information and experiences, learning from each other.

So many important reasons for being here at this 11th symposium now in Wageningen, And given the importance of the safety of honey bees, certainly more symposia are to come in future, wherever in Europe.

We are happy that we have been instrumental in setting up these meetings and achievements through the years. I sincerely hope – with the other Wageningen based institutions like the Board for the Authorisation of Plant Protection Products and Biocides, and the Wageningen University Research - to continue to support the endeavors of the ICB-PR in future with the view to effectively protect honey bees and other beneficial bees.

I wish you a very fruitful meeting here in Wageningen and I hope you will enjoy your stay in our city.

I thank you for your attention.

(Dr. Wim H. van Eck)

EFSA and bees

Robert Luttik

Chairman, EFSA Working Group on Bees, National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport, Bilthoven, NL

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The European Food Safety Authority EFSA

EFSA has a number of panels. The Panel on Plant Protection Products and their Residues (PPR) is about plant protection products. It deals with three different types of questions:

1. Questions from the EFSA (related to the assessment of compounds)
2. Questions from the Panel (self tasking)
3. Questions from the European Commission

About EFSA and bees

The centrepiece of the Authority's work in this area is a guidance document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees), which will be published this year 2012 (see attachment below). EFSA's advice was requested by the European Commission in 2011 after members of the European Parliament and beekeeper associations voiced concerns about the appropriateness of the current risk assessment scheme. The guidance will be preceded by another important document: an opinion on the science behind the development of a risk assessment of plant protection products on bees, which will be completed by the end of April of 2012.

Terms of reference

EFSA has received a request about the risks of plant protection products and bees. After consultation with DG SANCO it was proposed to define the term of reference as follows:

- A scientific opinion of the PPR Panel on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) and a guidance of EFSA on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)

Issues to be assessed

The assessment of the acute and chronic effects of plant protection products on bees, including colony survival and development;

- The estimation of the long term effects due to exposure to low concentrations;
- The development of a methodology to take account of cumulative and synergistic effects;
- The evaluation of the existing validated test protocols and the possible need to develop new protocols, especially to take account of exposure of bees to pesticides through nectar and pollen.

Working group members

- Gérard Arnold, National Center of Scientific Research, France.
- Jos Boesten, Alterra, The Netherlands
- James Cresswell, University of Exeter, United Kingdom
- Andy Hart, Food and Environment Research Agency, United Kingdom
- Robert Luttik, National Institute of Public health and the Environment, NL (chairman)
- Jens Pistorius, Julius Kühn Institute, Germany
- Fabio Sgolastra, University of Bologna, Italy
- Noa Simon Delso, Centre Apicole de Recherche et Information, Belgium

- Walter Steurbaut, Ghent University, Belgium
- Helen Thompson, Food and Environment Research Agency, UK

Perhaps in the future as hearing expert:

- Ann Alix, Dow Chemical, UK

More EFSA involvement in bees

EFSA's Pesticides Panel has also commissioned a literature review on topics of relevance to the revision of the guidance documents on aquatic and terrestrial ecotoxicology. In respect to bees, an overview of available scientific information on interactions between pesticides and other factors was requested and should be made available by mid-2012.

References

More information and news on: www.efsa.europa.eu and www.efsa.europa.eu/en/press/news.

At the moment of finalizing the symposium proceedings for printing, EFSA published the results of its review on its website, as cited below: Pesticides and bee health: EFSA reviews the science News Story - 23 May 2012

EFSA has published a state-of-the-art scientific review of the risks posed by pesticides to honey bees, bumble bees and solitary bees. This major piece of work will support the development of specific guidance for the assessment of possible risks to bees from the use of plant protection products. The guidance will provide up-to-date advice to those involved in the evaluation of plant protection products and their active substances, including industry and public authorities.

The opinion published today on the science that will underpin the guidance was requested by the European Commission and responds to growing concerns among MEPs and beekeeper associations about the appropriateness of the current risk assessment scheme. It is also part of EFSA's coordinated response to the decline in the numbers of honeybees, wild bees and other pollinators in Europe.

EFSA's Panel on Plant Protection Products and their Residues (PPR) started from the premise that to develop robust environmental risk assessment procedures, it is crucial to know what to protect, where and over what period. The Panel noted that bees play a valuable role as pollinators, contribute to biodiversity and provide hive products such as honey and royal jelly (for honey bees). The scientific experts then looked in detail at four key areas, as suggested by the European Commission:

- the acute and chronic effects of pesticides on bees, particularly colony survival and development
- how to estimate the long-term effects of exposure to low concentrations
- the need to take into account the cumulative and combined effects of different pesticides
- existing test protocols and possible new protocols that take account of the exposure of bees to pesticides through nectar and pollen.

The document proposes two separate assessment schemes: one for honey bees, and one for bumble bees and solitary bees. In the initial stage it is suggested to include toxicity testing that covers an exposure period of seven to ten days for adult bees and larvae. Both life stages can involve exposure of longer than one day, a risk that is not covered by standard tests.

EFSA's pesticides experts also recommend improvements to existing laboratory, semi-field (cages, tunnels and tents) and field testing procedures. Several exposure routes (intermittent and prolonged exposure of adult bees, exposure through inhalation and the exposure of larvae) are not currently evaluated in laboratory tests, and the effects of "sub-lethal" doses of pesticides are not covered fully.

In field and semi-field testing, the experts identify several weaknesses that lead to uncertainties in the actual exposure of honeybees. They highlight the need to improve the methods for detecting bee mortality, the observation of sub-lethal effects and the statistical analysis of test results.

The use of pesticides is cited widely as one of the possible contributing factors to the decline in bee numbers in some parts of the world – along with other factors such as disease, parasites, climate change and other environmental factors, and the effects of genetically modified organisms. This decline is causing concern because bees, particularly honey bees, play an important role in the pollination of a wide range of crops and wild plants. It is estimated that the production of about 80% of the 264 crop species cultivated in the European Union depends directly on insect pollinators, mostly bees, and the global annual monetary value of pollination is estimated to be in the range of billions of dollars.

Given the importance of bees in the ecosystem and the food chain and given the multiple services they provide to humans, their protection is essential. EFSA has an important role to play in ensuring the survival of bees given the Authority's mandate to improve EU food safety, safeguard animal health and welfare and ensure a high level of consumer protection.

EFSA's scientific experts are currently developing a dedicated and co-ordinated work programme related to bees in the areas of pesticides, animal and plant health, and genetically modified organisms. The Authority is also carrying out a gap analysis for risk assessment and data collection and will identify areas for further research. EFSA's pesticide experts are also preparing a statement on two articles published recently in the journal Science which suggest links between neonicotinoids and bee colony survival.

Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees).

I. Regulatory issues: honey bee risk assessment for pesticides in Europe

Bee health in Europe – Facts & figures. An Opera document

Anne Alix[1], Laurie Adams[2], Mike Brown[3], Peter Campbell[4], Ettore Capri[5], Amalia Kafka[5], Konstantinos Kasiotis[6], Kiki Machera[6], Christian Maus[7], Mark Miles[8], Petru Moraru[9], Lisa Navarro[10], Jens Pistorius[11], Helen Thompson[3], Alexandru Marchis[5],

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Abstract

Declines of managed honey bee colonies and also of some wild bee species have been reported by many countries, leading to intensive work and actions in the areas of research and regulations. Declines in pollinating insect numbers can have significant adverse effects ecologically on the diversity of plant species and economically in the productivity of crops. However, up until now, the status and relative importance of the stress factors that may affect bee populations have been relatively unclear and, in many instances, widely disputed.

In this context, OPERA1, has undertaken to produce an updated review on the issue of honey bees and pollinators in Europe, with some highlights to other continents, which would cover ecological and economical aspects related to these species in relation to agriculture.

The expert invited have gathered the latest information available on the factors influencing the health of both managed honeybees and populations of native wild bees, including solitary bees and bumble bees. The main conclusions indicate that the honey bee can cohabitate with modern agricultural practices provided necessary precautions are taken to maintain viable food resources for bees and avoiding practices that may cause adverse effects. These precautions include the design of agricultural landscapes and the implementation of practices that account for the presence of pollinators. Essential developments also concern the availability of effective and regulated veterinary compounds to help beekeepers eradicate the most important pests from apiaries. An analysis of beekeeping activity in its economical context is also provided. Finally, modern agriculture and beekeeping demands better technical knowledge and a critical lack of training and communication to better accompany the updates in science and technology to the farm and the field is identified. The case of wild bees may be considered to be very similar to that of the domesticated honey bee albeit far less well documented.

Recommendations are emitted towards all those involved in agriculture, bee keeping regulatory authorities and research, which should be communicated to all as the effectiveness of the actions will rely on their common effort to implement them.

OPERA is a young, growing independent research centre and think tank of the Università Cattolica del Sacro Cuore providing simple pragmatic solutions to support EU and national decision making, in bridging science and policy through a transparent platform to debate the right approaches for sustainable, intensive agriculture (http://www.opera-indicators.eu/eng/home.html)

Pollinators, pesticides and agriculture: developing regulatory tools for the future

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Abstract

Modern crop management practices have progressively been implemented which have allowed for an increase in cropped areas while ensuring proper control of pest populations and diseases. In order to achieve this Plant Protection Products (PPP's or pesticides) are an essential part of these management practices. Many plant species are dependent on pollinators for reproduction, and pollinators have a key role in maintaining biological diversity and ecosystem functioning, as illustrated by the estimated 450 crop species that globally depend on pollination by bees and other insects. Because of this particular attention has been given to pollinators in the regulations implemented in many countries to accompany the placing of pesticides on the market. In Europe, for example, Regulation 1107/2009/EC, requires a demonstration of both acceptable risks to human health and the environment as well as demonstration of their efficacy on the target pests to be controlled.

Recent developments in risk assessment procedures for honey bees have been possible via the input of three working groups of ICPBR on (i) semi-field and field testing, (ii) testing on larvae and (iii) risks associated with systemic products. This work has lead to the update of EPPO documents relating to pesticides testing and risk assessment for bees. The recent SETAC Pellston workshop has, in addition to a comprehensive review of knowledge, raised the question of protection of non-Apis bee pollinators and over recent years there has been an additional focus on the potential risk posed to bees due to PPP's used as seeding coatings. To address this, a dedicated workshop on treated seeds was held in Paris this year to progress the areas of risk assessment and risk management at the EU level. All these developments and initiatives feed the activities of the OECD PEIP working group who are in charge of identifying and developing testing guidelines that could be needed in future.

In addition to risk assessment, risk management measures complete the toolbox in that they aim at limiting the exposure that can be totally or partly avoided. An effective implementation of risk management measures implies that exposure conditions are appropriately described. A comprehensive characterization of exposure also allows to better design higher tier investigations, including monitoring, the relevance of which relies in the first place on their representivity of expected exposure conditions of pollinators in agricultural conditions.

This presentation aims at providing a snapshot on the regulatory tools, i.e. risk assessments performed a priori to the authorization and risk management measures implemented in the field that complement each other to achieve the protection of pollinators in the field.

Exposure of honey bees and other pollinating species to pesticides

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Abstract

Background: When considering the risk to bees a thorough understanding of the relevant routes of exposure and the magnitude of exposure is necessary.

Results: Bees forage on plants and in particular flowers to obtain food for themselves and for provisioning their young. Foliar applications during flowering will present the most extreme acute exposure situation. Bees can be exposed to direct spray and also to contaminated pollen and nectar taken back to the colony. Spray applications before flowering may lead to exposure in pollen and nectar if the substance has systemic properties and is persistent. For soil/seed treatments exposure may occur in for systemic products due to translocation from the seed or soil to the upper parts of the plant (e.g. nectar and pollen). Other possible routes for soil/seed treatments include dust-off at sowing and guttation water.

Conclusion: Risk assessment requires that relevant routes of exposure for worker bees, hive bees and young should be considered in the risk assessment for both foliar applied and seed/soil treatment pesticides. The availability of exposure models would assist in the development of honey bee and pollinator risk assessment schemes.

Keywords: honey bee, pesticide, risk assessment, exposure

1. Introduction

Plant Protection Products or PPPs (also called pesticides) are part of modern crop management practices. Prior to the placement of PPP on the market and their use an evaluation of the risks posed to the environment is mandatory worldwide requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species.

Guidance already exists and has been available for many years, which aims at characterizing the potential effects of PPPs on honey bees with the corresponding need for an assessment of risk[1,2,3]. General data requirements, risk assessment procedures for spray applied products and special considerations for products which are systemic or are larvicidal or have Insect Growth Regulator (IGR) type properties have been recognized for many years[4,5].

If in practice exposure to honey bees can occur both the hazard (toxicity) of the compound and also the potential exposure to the organism is then considered. The risk assessment usually follows a tiered approach whereby products of low toxicity and low risk are rapidly excluded; whereas products with a potential to harm honey bees are progressed to higher and more realistic tiers of evaluation. Consequently, it is usually not necessary to generate extensive and elaborate measures of toxicity and exposure in tier I risk assessments.

Exposure estimates and/or measurements need to reflect the potential route of exposure and also the level and extent of exposure for the test organism. It is not always necessary or desirable to consider all potential routes of exposure, as long as the needs for the risk assessment can be met. For example, the exposure of birds and other terrestrial vertebrates is considered to be primarily via the consumption of a dose in the animal's diet[6,7] even though other routes (e.g. dermal absorption, inhalation) are also theoretically possible. Even though there are limitations on how exposure is expressed and/or calculated a robust risk assessment is achieved at all tiers.

Bees obtain their requirements by foraging on plants and in particular from flowers to obtain food for them and for provisioning young. Nectar and other sugar sources (e.g. extra floral nectaries and aphid honey dew) are used as an energy source whereas pollen is an important protein source essential for developing young. During these activities they also provide valuable pollination services to plants.

Bees require water, and some species will also forage for nesting material (e.g. leaf cutter bees). In addition to exposure by spray applications bees and the colony can be exposed to collected and processed materials containing pesticide residues stored in the hive. Figures 1 to 4 illustrate the interaction of bees within the crop and off-crop area and the majority of scenarios for which bees can be exposed to applications of PPPs.

Tab. 1 Estimated level of residues in different matrices after the application of Plant Protection Products as spray applications

Time of application	Location of residues	Expected exposure to residues	Expected level of residues	Remarks
Pre-emergence	Soil and water from puddles formed on the soil surface following heavy rainfall close to application	Negligible to soil Dependant on puddle formation	1 kg/ha /year could result in a PEC puddle of 1.38µg/L	The occurrence of puddles depends on heavy rainfall event and soil structure. Covered by the risk assessment based on HQ calculation as an exposure to direct spray
Before flowering	Plant surface	Negligible, as not attractive to bees	Estimated through the application rate (from g/ha to kg /ha)	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed
	Guttation droplets (for systemic compounds)	Expected to be negligible due to the low attractiveness of growth stages	Peak concentrations observed in the first droplets (100-500 mg/L) down to <0.001 mg/L later on, after sprayed treatments.	The occurrence of guttation droplets depends upon systemic properties, soil and air humidity. Should be covered by the risk assessment performed for sprayed solutions
Flowering	Pollen, nectar	Importance as function of the attractiveness	Concentration may be estimated with the concentration in spray solution as a worst case. Measures range from 1.5 to 2000 µg/kg pollen (or 340 µg/kg).	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed
After flowering	Plant surface	Negligible, as not attractive to bees	Estimated through the application rate (from g to kg /ha)	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed
All	Off crop non flowering vegetation and/or non flowering crop receiving spray drift	Negligible, as not attractive to bees	Estimated drift rate as a % of application rate (2.77% to 29.2% at 1-3 meters, pending upon the spraying technique)	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed
	Off crop flowering vegetation and/or flowering crop receiving spray drift	Importance as function of the attractiveness	Concentration may be estimated with the concentration in spray solution adjusted by the drift, as a worst case. Measures range from 1.5 to 2000 µg/kg pollen (or 340µg/kg).	Covered by the risk assessment based on HQ calculation as exposure to direct spray is assumed

2. Exposure scenarios for honey bees and other pollinators

It is first necessary to consider if during the course of the use of a PPP bees will be exposed by considering the details of the product and its pattern of use. In some cases exposure of bees is not possible. For example, winter applications when bees are not flying, pre-emergence use of herbicides, wound treatments, rodenticide baits, indoor uses, use in glasshouses (where pollinators are not used) seed treatments and granules (except where there is systemic activity) and products for dipping bulbs etc. are likely to lead to negligible exposure to bees and in such cases a risk assessment is not required. A second consideration is the attractiveness of the crop plant. If the crop is not attractive to bees then again exposure will be minimal. However, other factors need to be considered such as the presence of other food sources in the treated area (e.g. flowering weeds, aphid honey dew). In general a crop is not attractive to bees when harvested before flowering, however, some crops intrinsically unattractive to bees may also be visited due to extra-floral nectaries (e.g. field beans, cotton).

These initial steps are considered in current risk assessment schemes. Under the recently published EPPO guidance separate pathways on the decision making tree are presented to cover the differences in exposure from sprayed and soil applied products. Likewise, this is covered in the US by the problem formulation stage. However, it is possible to summarize the characteristics of standard scenarios to describe the potential routes of exposure for honey bees and other pollinators. Key factors are the method of application (spray or soil/seed treatment) and whether the product contains an active substance toxic to bees with systemic activity. Tables 1 and 2 list the various scenarios where bees and other pollinators can be exposed and also gives an indication of possible residues levels present in matrices of relevance to bees[8,9,10,11,12]. Further explanations of the exposure scenarios due to spray applications and soil/seed treatment uses are given in the following sections.

2.1 Exposure to spray applications

The timing of spray applications is critical when considering the exposure of bees. Depending on the use pattern of the product applications can be made to bare soil, young seedlings, before flower, at flower or post flowering (Figures 1 to 3). Spray applications at or close to flowering pose the greatest likelihood of acute exposure for bees. This can be via direct sprays or to residues on plants, flowers and possibly in nectar and pollen. The properties of the molecule (chemical stability, presence of residues, breakdown into metabolites and mobility in plants) can also influence the route and duration of exposure.

2.1.1 Exposure to direct spray and residues on plants and flowers

Foliar applications during flowering typically lead to exposure which may be considered for the complete duration of the flowering period as a worse case. For example many tree crops which rely on pollination typically flower for a period of 2 weeks. Foragers may be exposed to direct spray and also to residues in/on plants and flowers (Figure 1). By spraying when bees are not actively foraging it is possible to reduce exposure and limit it to aged plant residues. Exposure is expected to be at its highest level at or shortly after spray and decline thereafter. Exposure will decline over time due to ageing, growth dilution and also due to visits to flowers not present or open on the day of application. Exposure in this context can be simply expressed in terms of the application rate i.e. g a.s./ha (grams of active substance per hectare).

Applications made out of the flowering period of the crop or to a crop which does not flower during the growing season significantly reduces the exposure to bees as they tend to work areas where there are adequate food sources available. However; exposure may occur on flowering weeds within the cropped (or in-field) area and due to drift to off-field areas with flowering plants such as hedgerows (Figures 2 and 3). In the case of the in-field area good weed control by the application of an herbicide or by mechanical means can be a suitable risk management measure unless this conflicts with local biodiversity objectives. For the off-field exposure to flowering plants or adjacent flowering crops, exposure can be expressed as g a.s./ha and adjusted by a suitable validated drift factor as a percentage of the field application rate.

Tab. 2 Estimated level of residues in different matrices after the application of Plant Protection Products seed coating or trunk injection

Application of the PPP	Time of application	Location of residues	Expected exposure to residues	Expected level of residues	Remarks
Seed coating	Drilling	Off crop and/or flowering crop receiving dusts, if pneumatic drillers with air pressure	Importance as function of the attractiveness	From 0.004 to 0.44 g/ha without mitigation measures to 0.002 to 0.12 g/ha when mitigation are implemented.	Emission and dispersion of dusts are very variable and specific drift values cannot be defined. Dusts should be reduced to a minimum in order to limit environmental exposure. Drift values after risk mitigation should be used in the risk assessment.
		Nectar, pollen if systemic at crop flowering	Importance as function of the attractiveness	Measures range from 1 to 6 µg/kg). A default value of 1 mg/kg is used as a tier 1 in the risk assessment scheme, based on residues measures in whole plants (EPPO, 2010).	Risk assessment scheme developed in EPPO, 2010.
		Guttation droplets if systemic	Expected to be negligible due to the low attractiveness of growth stages	Peak concentrations in the first droplets (100-500 mg/L) down to < 0,001 mg/L later on (variable pending upon crop, systemic properties and growth stage).	The occurrence of guttation droplets depends upon systemic properties, soil and air humidity. Should be covered by the risk assessment performed for sprayed solutions as an exposure to direct spray is assumed
Trunk injection	Pre flowering, flowering	Pollen, nectar	Importance as function of the attractiveness	Concentration may reach 300 µg/kg in some trees	Concentrations are based on efficient concentration in trees to control pests. Concentrations depend on the distance to injection point. Could be covered by a risk assessment considering overspray at flowering.

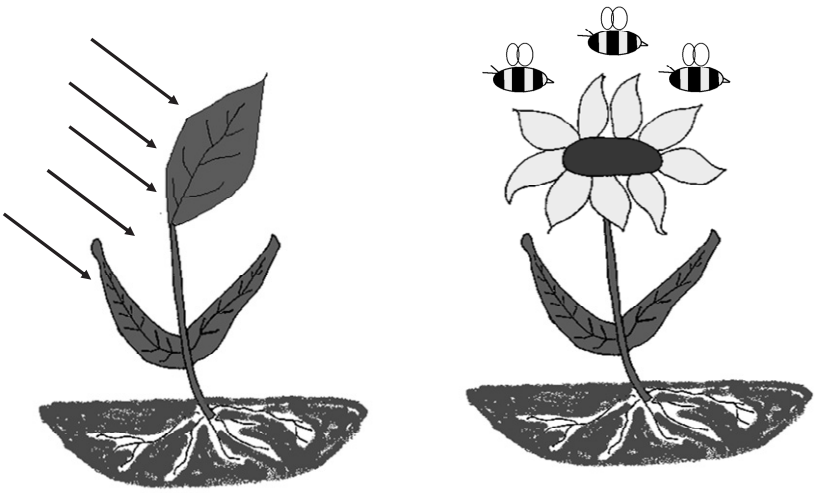

Fig. 1 Foliar spray applications before flowering. Bees are not expected to be exposed at application. However there could be a potential for exposure to residues on foliage and movement into flowers, pollen, and nectar if the plant protection product has systemic properties and is persistent. Diagonal arrows indicate spray application.

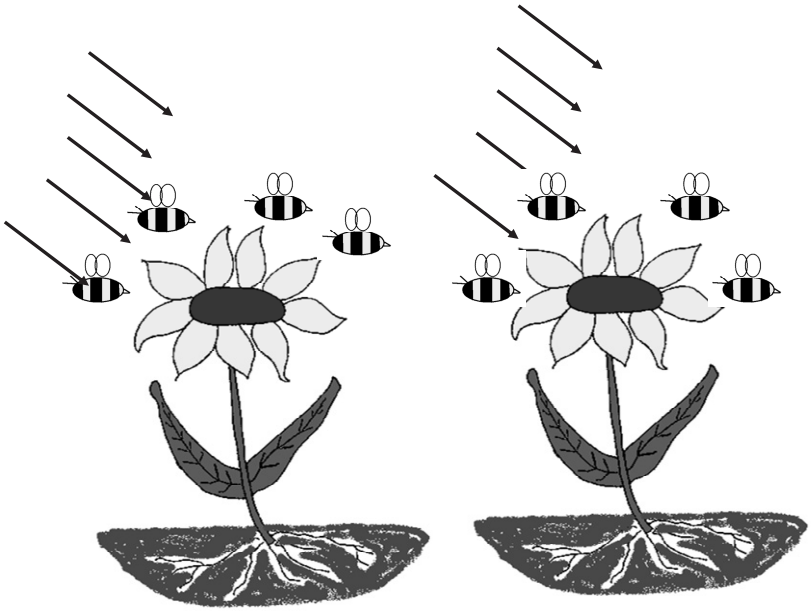

Fig. 2 Foliar spray applications during flowering. Day time applications may lead to direct spraying of bees, exposure to residues and direct contamination of pollen/nectar/flowers. If the plant protection product has systemic properties and is persistent there is the potential for movement into flowers, pollen and nectar. Applications made out of bee flight may reduce exposure. Diagonal arrows indicate spray application.

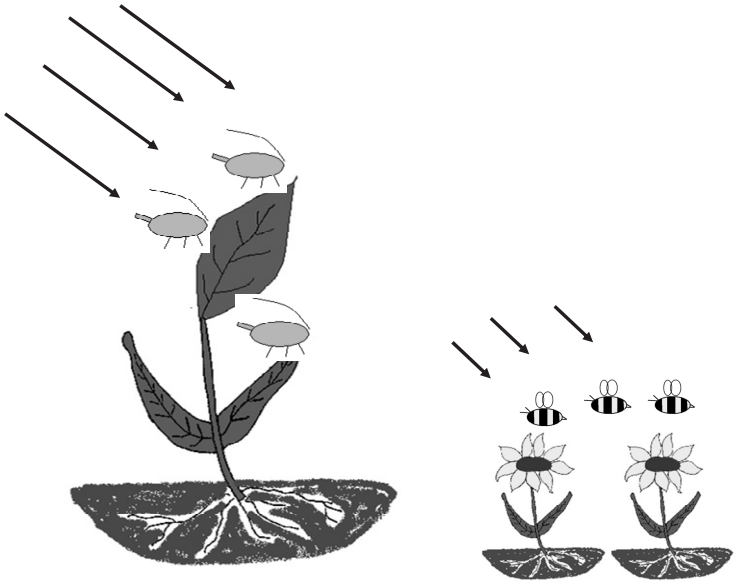

Fig. 3 Foliar spray applications after flowering or to non-flowering crop which are not highly attractive to bees. There is a potential for exposure via aphid honeydew, however if the plant protection product is an insecticide aphids may not be present. Off-crop exposure due to drift on adjacent flowering crops and off-field areas may be possible. Long diagonal arrows indicate spray application, short arrows drift.

2.1.2 Exposure to residues in nectar and pollen due to spray applications

Contamination of nectar and pollen by pesticide spray applications is possible when applications are made during flowering. The degree of exposure can depend on the architecture of the flower as open flowers (such as winter oilseed rape, apple blossom) would expect to receive a higher exposure compared to some other flowers that receive less exposure (e.g. wild blueberry flowers which hang upside down). Contamination of pollen is far more likely than that of nectar by spray applications to open flowers. In relation to exposure to foragers this is covered by exposure to direct spray and residues on plants and flowers in section 2.1.1 above. Due to the location of nectaries in many flowers, residues are likely to be low to negligible arising from spray applications, especially when compared to flowers and pollen which may receive direct spray.

Generally, applications outside of the flowering period would not lead to exposure to bees through nectar and pollen. However, in the case of products with systemic activity, pre-flowering spray applications may lead to substances and/or metabolites being present in pollen and nectar. This is because the systemic properties of molecule may allow for the material to be absorbed by the plant and translocated into flower parts (Figure 1). These residues are also subject to degradation over time, by plant metabolism, diluted by plant growth and movement within the plant, as they are not expected to concentrate in pollen or nectar[13]. Overall, the levels of residue found in pollen and nectar due to spray applications of systemic products pre-flowering will be considerably lower than those arising from direct applications to flowering crops. In the case of compounds persistent in soil, applications may lead to residues in pollen and nectar in following flowering crops. It should be possible to exclude exposure by demonstrating the absence of residues in pollen in nectar following a spray application of a systemic product.

2.2 Exposure to non-sprayed products (soil and seed treatment applications)

The exposure of bees to residues of a product applied as a soil/seed treatment may occur in the case of residue transfer (either the active substance or a degradation product) from the seed or soil to the upper parts of the plant and in particular in matrices of interest to bees (pollen, nectar and honey dew) if the crop is visited by bees. Although the risk to bees due to these types of products has been recognized for many years, it is only recently that a formal risk assessment procedure has been documented and validated[2,14].

2.2.1 Exposure to systemic products: flowering crops

Seed/soil treatment products are used in a wide range of crops. Some of these have systemic properties providing protection from pests and soil borne diseases. Flowering crops such as oilseed rape, sunflowers and many others may be treated with insecticides with systemic activity and provide targeted protection from many sap feeding pests. Residues may also transfer to pollen and nectar and also into aphid honeydew. Bees foraging on these plants can be exposed to nectar and pollen concentrations and if these are not present at lethal levels, these can be taken back to the colony. In the case of honeydew, it is clear than concentrations which do not affect aphids are unlikely also to affect bees. Some plants such as maize do not produce nectar but do produce a plentiful supply of pollen which is utilized and stored by honey bees (Figure 4).

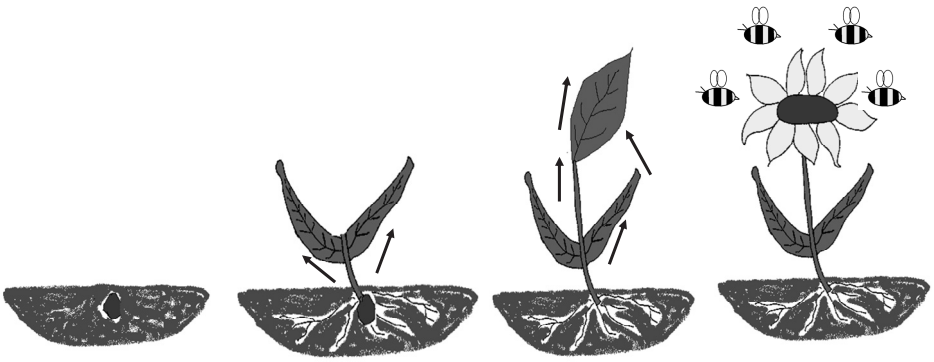

Fig. 4 Exposure to seed and soil treatment. Bees are not expected to be exposed at application (excepted in the case of abraded dust). However there could be a potential for exposure to residues in foliage, pollen, and nectar if the plant protection product has systemic properties and is persistent. At early stages of plant growth guttation fluid may be produced under certain circumstances. Upward arrows indicate movement of plant protection product within the plant.

2.2.2 Exposure to systemic products: non-flowering crops

Seed/soil treatment products are also used to protect crop plants which do not flower before harvest (e.g. many *Brassica* crops) or are unattractive to bees. In these cases the exposure for bees is much less and could be considered negligible. In these cases an assessment of risk may be necessary for a following flowering crop. In the case of systemic compounds which are persistent in soil and these residues remain in the soil to the following season they may transfer into the flowing crop. If this crop is attractive to bees then exposure may occur even if no systemic products are used during the growing season of the following flowering crop.

2.2.3 Exposure due to abraded dust at sowing

Exposure of honey bees may also occur through routes such as contact with or the consumption of dust originating from seed coated with PPPs. An example of this is the case that occurred in Germany in 2008 following the sowing of seeds coated with insecticides[15,16]. It was shown that under certain circumstances the emission of dusts at sowing may be possible resulting in bee kills. The relevance of this exposure route to bees is still subject to further consideration and work. It may be possible to reduce dust emission and dispersion through appropriate risk mitigation measures such as quality coating, equipment of sowing machines with dust drift reducing devices (deflectors) and recommendations with regard to weather (wind) conditions at sowing. The level of dust emitted and dispersed may significantly vary pending on coating and sowing conditions so that many countries have implemented strict risk management measures that will develop into good practices in this area. In France for example, an ordinance has been adopted that defines conditions for coating and drilling corresponding to the good agricultural practice, which will be the basis for further risk assessments [17].

2.2.4 Exposure due to guttation fluid

Exposure of bees may also occur due to the consumption of guttation droplets. The movement of systemic products from the treated seed/soil into the plant may result in the presence of active substance or degradation products in guttation fluid which could be used as a water source by bees. Information on the conditions and relevance of exposure are still needed for a proper risk assessment, in particular with regard to the conditions of occurrence of droplets and the concentrations of PPP in the fluid.

2.3 Routes of exposure for honey bees and wild bees

The characteristics of standard scenarios to be considered when evaluating the potential routes of exposure for honey bees and other pollinators from PPP's, are described in Tables 3 and 4. These tables present the likelihood and anticipated levels of for honey bees relative to wild bees. These differences may be important when considering the exposure of various pollinators. For a more detailed account on this matter see the publication of the OPERA bee working group[18].

Tab. 3 The relative importance of exposure of honey bees and wild bees via various exposure routes of plant protection products as spray applications.

	Honey bees		Wild bees	
Exposure	**Adult**	**Larvae**	**Adults**	**Larvae**
Direct spray	+++	-	+++	-
Spray drift	++	-	++	++
Floral residues	+++ to +	-	+++ to +	-
Nectar	- to ++	+	- to ++	+
Pollen	+ to +++	++	+ to +++	++ to +++
Foliar Residues	+	-	+ to +++	- to +++
Water	+ to ++	+	+	+
Nesting Material	+	+	+ to +++	+ to +++
Exposure to Soil	-	-	- to +++	- to +++

Tab. 4 The relative importance of exposure of honey bees and wild bees via various exposure routes of plant protection products as seed coatings, trunk injections and soil drench applications.

	Honey bees		Wild bees	
Exposure	**Adult**	**Larvae**	**Adult**	**Larvae**
Dust (off-field)	+ to +++	+	+ to +++	++
Nectar	to ++	+	to ++	+
Pollen	+ to +++	++	+ to +++	++ to +++
Foliar residues	+	-	+	+
Guttation water	+	+	+	+
Exposure to soil	to +	-	- to +	- to +

2.4 Conclusions on exposure due to spray applications and non-sprayed products

Exposure for bees and other pollinators is related to flowering as bees forage to collect food resources and due to the attractiveness of the crop. Bees can be exposed to PPPs by both spray and non-sprayed applied products. For spray applications is it typical to express exposure in terms of the application rate (g a.s./ha).

For spray applied products, treatment outside of flowering (or to crops which will not flower before harvest) will limit exposure. Honey dew may attract bees to non-flowering and unattractive crop plants and there is a risk of overspray. Likewise the presence of flowering weeds may make an area attractive. To assist with these issues it would be useful to develop a list of crop plants which are not attractive or are less attractive to bees (e.g. sugar beet, potatoes). Recent work undertaken by a working group of the French Agency on the Safety of Food (AFSSA Draft working document – Guidelines Related to Setting Maximum Residue Limits in Honey – EC Guidance document Part C4) with the aim to provide a guidance document for defining Maximum Residue Limit (MRL) for PPPs in honey has proposed a list of the melliferous plants being attractive to bees based on the presence or absence of nectar and honeydew. However, this list does not include plants such as maize, which may be attractive to bees and hence be considered in the risk assessment even if they do not produce nectar. Some recommendations on the factors to consider in assessing the level of attractiveness of a crop are also proposed, such as the presence in the foraging area of other sources of nectar/honeydew of higher/lower level of attractiveness that may influence the behaviour of bees towards the crop of interest. Similarly, the presence of bee-attractive flowering weeds or of 'secondary' crops in a non attractive crop may favour visits and lead to some exposure. A description of agricultural practices associated to the crop of concern may help in deciding if visits and exposure are expected or not.

Foliar applications of systemic products at pre-flowering crop growth stages may lead to exposure during flowering due to the transfer of residues from the foliage to flower parts in pollen and nectar. However, it are spray applications made during flowering which pose the highest potential for exposure to bees. For crops which are attractive to bees, foragers can be exposed to direct overspray, dry residues on flowers and also to contaminated pollen and nectar. Pollen and nectar can be taken to the hive if the application is not lethal to forager bees and if there is concern a risk assessment for larvae and/or brood may be triggered.

The exposure of bees to residues of a product applied as a soil/seed treatment may occur in the case of residue transfer from the seed or soil to the upper parts of the plant and in particular matrices of interest to bees (pollen, nectar and honey dew) if the crop is visited by bees. These residues in such bee relevant matrices may potentially lead to forager and colony exposure with the exposure express as mg a.s./kg. A review conducted in 2009 of residue data measured in all types of plant parts (leaves, fruit, green part, inflorescence, whole plant, grain) taken close to flowering and where available residues in nectar and pollen, showed that the majority of samples have less than 1 mg a.s. per kg matrix (95th percentile = 0.55 mg/kg, n = 62)[19]. Considering only the residues measured in pollen and nectar residues did not reach more than 0.1 mg a.s./kg. However, this dataset is limited in scope and in the absence of actual measured data it is a recommended to apply a generic worst case value of 1 mg a.s./kg to represent residues due to soil and seed treatments in plants. Under this scenario pollen and nectar can be taken to the hive if the residue is not lethal to forager bees. If there is concern a risk assessment for larvae and/or brood may be triggered.

3. Linking exposure to risk

A risk assessment scheme for sprayed products (both non-systemic and systemic pesticides applied directly to bee-attractive crops), has been in place for nearly 20 years whereas that for systemic pesticides applied as granules, seed treatments and soil drenches or as pre-flowering applications has only recently been developed[2]. An appropriate risk assessment for bees relies on the combination of suitable exposure and toxicity measurements which is beyond the scope of this paper.

Exposure due to spray applications is typically expressed in terms of the application rate in g a.s./ha. An initial screening risk assessment is presented in the EPPO guidance which uses an Hazard Quotient (HQ) validated on field observations. The HQ is calculated as the ratio of the application rate (g a.s./ha) to toxicity values for both contact and oral routes of exposure (as µg a.i./bee). Products with HQ values in excess of 50 are advanced for further evaluation of risk. In the US further evaluation is triggered for substances with contact LD50 values less than 11 µg a.i./bee. Higher tier evaluations typically involve the exposure of worker bees or colonies to spray treated flowering plants or foliage.

In the case of bees exposed to residues of a product applied as a soil/seed treatment the resulting exposure is expressed as a concentration in pollen and nectar (mg a.s./kg). This need to be converted to a dietary exposure (as µg a.s./bee) and compared to the oral toxicity values and the calculation of a toxicity exposure ratio (TER) performed which is then compared to a relevant trigger. This screening step is recommended in the most recent update of the EPPO guidance [2] and has been validated against currently available data[14].

Higher tier evaluations may focus on the refinement of toxicity values and/or measured residues in matrices attractive to bees or be based on semi-field and field tests where the test material is applied according to good agricultural practice and exposure under field conditions is closely reproduced. However there is a strong need for models to predict the exposure of bees to various application scenarios for honey bees both in the field and within the colony.

For both exposure routes (spray and soil/see treatment), evaluation under realistic field conditions in either semi-field or field tests represent the highest level of testing. Further to this, should there be unresolved uncertainty, post-registration monitoring studies are a suitable option and possible condition of registration for a product.

4. Overall conclusions on exposure

More we examine the scenarios for which there is a potential exposure for bees the more there seem to be. This is in part due to the colony structure of honey bees but also because the biology of this single domesticated species is well known. In terms of ecotoxicity, more information is probably known about this single species than any other in a regulatory scheme. It is not practical or necessary in scientifically valid risk tiered assessment schemes to consider all possible routes of exposure however models of exposure would greatly assist in the risk assessment procedure. A robust and useful risk assessment scheme should rapidly exclude products of low concern to allow efforts to be focused on high risk products. Initial risk assessments can be based on empirically derived relationships (HQ for sprays) and also on TERs for systemic exposure through pollen and nectar. In the first instance this could be achieved through the development of simple exposure models for bees. If a compound is indicated as potentially high risk then exposure may be refined via measured values in bee relevant matrices, but these need to be based on sound scientific risk assessment principles. In the case of higher tier testing and risk assessment, this can be based on exposure of bees under realistic use conditions in tunnel, cage and field tests. In these cases the effects of the direct exposure is measured without the need for complex and detailed analysis of residues and TER calculations.

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Assessing the comparative risk of plant protection products to honey bees, non-target arthropods and non-Apis bees

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Abstract

Background: In the European Union the placing of pesticides on the market requires as a prerequisite that a risk assessment demonstrates low risks to human health and the environment, among which includes pollinators. Currently risks are evaluated for honey bees and for non-target arthropods (NTA) of cultivated ecosystems. The actual protection of pollinators other than the honey bees, as for example for non-Apis bees, in relation to these risk assessments has recently been questioned and requires further investigation. We present the findings from a comparison of Hazard Quotient (HQ) value calculations to assess the risk to honey bees, non-target arthropods and to non-Apis bees (with the application of an additional safety factor of 10). Calculations were based on publicly available ecotoxicological data.

Results: The risk to NTA, honey bees and non-Apis bees, as depicted by HQ values, indicated a higher fail rate for NTA than for bees, but a similar pass / fail rate for non-Apis bees when compared to the NTA scheme. Outcome of the risk assessment for NTA using extended laboratory tests gave similar pass/fail rates compared to the screening step for honey bees.

Conclusion: A screening step for non-Apis bees could be developed based on data available on honey bees and NTAs.

Keywords: risk assessment, non-Apis bees, pollinators, pesticides, non-target arthropods.

1. Introduction

Risk assessments are conducted for plant protection products (PPP) with respect to potential impacts on non-target species.[1] These include pollinators such as the honey bee[2,3] but also other non-target arthropods (NTA), the latter covering species within the agricultural landscape and more specifically, a wide range of groups such as pollinators, herbivores, predators, parasites, fungivores and detritivores.4 In common with other areas of ecotoxicological risk assessment sentinel species are employed aiming at ensuring a high level of protection and conservatism. Tier I screening risk assessments are thus intended to rapidly exclude those substances which pose a low risk to non-target organisms and to focus resources on those for which a potential risk cannot be excluded and further studies may be undertaken to characterize the conditions and occurrence of risks.

In the case of the honey bee, the Tier I screening risk assessment is based on a Hazard Quotient (HQ) approach[3]. This HQ is calculated by dividing application rate by the LD50 (Lethal Dose for 50% of the organisms exposed in the test). Similarly for NTA, the HQ is calculated on the basis of the LR50 (Lethal Rate 50% of the organisms exposed in the test) of two indicator species (*Aphidius rhopalosiphi* and *Typhlodromus pyri*),4 in the same way as the honey bee calculation using the application rate.

In the risk assessment process as currently used in Europe, both the robustness and suitability of the NTA and honey bee HQ as a screen step for spray applied products has been validated.[4,5] In other regions such as North America, a contact toxicity trigger of 11 µg active substance/bee is currently employed as a trigger for higher tier risk assessment.[6]

The actual protection of pollinators other than honey bees, (e.g. non-Apis bees), that is achieved by these risk assessments has recently been questioned and need further investigation identified. At the Pellston workshop on Pesticide Risk assessment for Pollinators,[7] it was suggested that for risk assessment the honey bee could be a suitable surrogate species for other bee species. However, to account for potential differences in the sensitivity between the honey bee as a test organism and

other non-Apis bees a safety factor of 10 (for interspecies differences) was suggested to be applied to the trigger value used for the HQ calculated for honey bees.

To explore the effectiveness and impact of this approach, HQ values were calculated to assess the risk to honey bees and non-target arthropods. These were compared to a hypothetical HQ based on the honey bee data to cover non-Apis bees which included additional assessment factor of 10. Publically available ecotoxicological data for honey bees and NTA were used. This paper presents the outcome of this analysis and derives some recommendations in the perspective of the development of a screening step in a risk assessment scheme dedicated to non-Apis bees.

2. Data analysis

For this initial exploratory analysis Draft Assessment Reports (DAR) and ESFA conclusion reports8 were examined and LR50 values for both NTA indicator species and LD50 values for honey bees were collected into a Microsoft excel spread sheet. The good agricultural practice (GAP) i.e. application rates of active substances, disclosed in the documents was used for exposure component of the the HQ calculations.

2.1 Tier I risk assessment for honey bees

For honey bees the HQ calculated was based on the highest single application rate for each representative use (g active substance/hectare). The HQ value is calculated by dividing the LD50 derived from acute oral and contact toxicity tests[9,10] expressed as microgram of active substance/bee, by the application rate.[3]

2.2 Tier I and Tier II risk assessment for NTA

For NTA, the exposure rate was calculated using the methods of Escort-24 based on the application rate. In the case of repeated applications, a Multiple Application Factor was applied in the exposure calculation to accounted accumulated residues from preceding applications to exposure due to the final application, thus leading to the highest exposure estimate. The Tier I HQ was calculated using LR50 values for *Aphidius rhopalosiphi* and *Typhlodromus pyri* (as grams active substance/ha) derived from glass-plate tests.[3,4,11]

In the Tier II risk assessment for NTA, a 50% effect trigger is applied which is equivalent to an HQ trigger of 1 (based on LR50 values for *Aphidius rhopalosiphi* and *Typhlodromus pyri* derived from extended laboratory tests).[4,11] These tests differ from the Tier I tests in that arthropods are exposed to the product on a natural substrate (i.e. treated leaves) rather than an inert substrate (i.e. glass-plates).

2.3 Tier I risk assessment for non-Apis bees

The tier I risk assessment for non-Apis bees was performed by applying an extra safety factor of 10 to the trigger values used for the Tier I risk assessment for the honey bee.

2.4 Interpretation of the HQ values

For honey bees, if a calculated HQ value for a given product use was below the trigger value for the two exposure routes, it was concluded that the use was of low risk.

For NTA, if a calculated HQ value for a given product use was below the trigger value for the for the two species, it was concluded that the use was of low risk.

The following HQ triggers values were employed to define low risk to each ecological entity:

- Honey bees – HQ trigger = 50
- NTA – HQ trigger = 2
- Non–Apis bees – HQ trigger = 5 (i.e. 1/10th of the honey bee trigger to account for inter-species variation).

3. Results

A total of 93 product uses (for 74 active substances) were employed in the analysis. Results are presented as pass rate, i.e. the rate of products uses for which the outcome of the Tier I risk assessment calculation is below the trigger value for honey bees, NTA and non-Apis bees (Table 1).

Tab. 1 A comparison the honey bee, non-target arthropod (NTA) and the proposed non-Apis bee hazard quotient (HQ) as a screening tool for tier I for 93 product uses (for 74 active substances)

		Product uses		
% of compound uses passing at Tier I	**All**	**Herbicide**	**Fungicide**	**Insecticide/acaricide**
Honey bee HQ 50	75%	100%	91%	23%
Non-Apis HQ 5	46%	64%	53%	15%
NTA HQ 2	42%	58%	47%	15%
Total number of uses	93	33	34	26
% NTA passes at Tier II (i.e. based on LR50 from extended laboratory studies)	70%	97%	82%	8.7%

Tab. 2 List of herbicide compounds which pass and fail at Tier I for honey bees, non-target arthropods (NTA) and non-Apis bees. White cells indicate pass at Tier I, grey cells indicated further evaluation is necessary

Compound	**use**	**Honey bees**	**Non *Apis*-bees**	**NTA**
acetochlor	h		>	
aclonifen	h		>	
amidosulfuron	h			
azimsulfuron	h			
benfluralin	h		>	
bensulfuron-methyl	h			
bifenox	h			
chloridazon	h		>	
clodinafop	h			
clomazone	h			
clopyralid	h			
cycloxydim	h		>	
diclofop-methyl	h		>	
diflufenican	h			
dimethachlor	h			
fenoxaprop-P	h			
fluazifop-P	h			
flurochloridone	h		>	
metazachlor	h		>	
metosulam	h			
metribuzin	h		>	
napropamide	h			
nicosulfuron	h			
oxadiazon	h			
penoxsulam	h			
picloram	h			
prosulfocarb	h		>	
quinmerac	h			
quizalofop-P-ethyl	h			
rimsulfuron	h			
tralkoxydim	h		>	
tribenuron-methyl	h			
trisulfuron-methyl	h			

Note : > indicates that the honey bee toxicity end point used was the highest dose tested, (typically 100 µg active substance/ bee) rather than a defined LD50 value. In these cases it may be that a higher endpoint could be determined to refine the risk assessment.

A list of active substance names which pass and fail at Tier I for honey bees, NTA and non-Apis bees is presented for herbicides (Table 2), fungicides (Table 3) and insecticides/acaricides (Table 4).

Tab. 3 List of fungicide compounds which pass and fail at Tier I for honey bees, non-target arthropods (NTA) and non-Apis bees. White cells indicate pass at Tier I, grey cells indicated further evaluation is necessary

Compound	use	Honey bees	Non-*Apis* bees	NTA
azoxystrobin	f		>	
carbetamide	f			
cyflufenamid	f			
cyprodinil	f		>	
difenoconazole	f			
dimoxystrobin	f			
dithianon	f		>	
dodemorph	f		>	
dodine	f		>	
epoxiconazole	f			
fenpropimorph	f		>	
fludioxonil	f			
fluopicolide	f			
fluoxastrobin	f			
fosetyl-al	f		>	
mandipropamid	f			
metrafenone	f			
prochloraz	f			
proquinazid	f			
prothioconazole	f		>	
pyrimethanil	f		>	
tebuconazole	f			
tolylfluanid	f		>	

Note : > indicates that the honey bee toxicity end point used was the highest dose tested, (typically 100 µg active substance/ bee) rather than a defined LD50 value. In these cases it may be that a higher endpoint could be determined to refine the risk assessment.

3.1 Pass rate for honey bees (HQ < 50)

Out of the total of 93 product uses, 70 (75%) were observed to pass at Tier I using a trigger of 50, indicating that 25% of all product uses required further evaluation, testing and risk assessment. When these uses were summarised by product type (herbicide, fungicide and insecticide / acaricide), it can be seen that all 33 (100%) herbicide uses and 32 out of 34 (91%) fungicide uses pass at Tier I. For insecticide/acaricide uses, 20 out of 26 (77%) results indicated that further evaluation was required.

Of the insecticide/acaricide uses which did not indicate a risk at Tier I (n = 6) these included compounds of known low toxicity to bees, highly selective products and insect growth regulators (IGR) which are not toxic to adult stages (Table 4). Note that IGRs are known to display toxicity towards developmental stages and thus can pose a risk to larvae. Consequently for the three IGR compounds further evaluation would have been automatically triggered for larval and brood effects so overall only three uses with HQ values above 50 were considered to be low risk at Tier I. However, this would not preclude additional testing based on evidence present in other parts of the dossier.

Finally, as it can be observed in Table 4, the screening step for honey bees reliably identified the need for further investigation for all neurotoxic insecticides.

Tab. 4 List of insecticide/acaricide compounds which pass and fail at Tier I for honey bees, non-target arthropods (NTA) and non-Apis bees. White cells indicate pass at Tier I, grey cells indicated further evaluation is necessary

Compound	use	Honey bees	Non-*Apis* bees	NTA
acequinocyl	i			
acetamiprid	i			
alphacypermethrin	i			
carbaryl	i			
diazinon	i			
dimethoate	i			
etofenprox	i			
fenoxycarb	i			
formetanate	i			
imidaclopid	i			
indoxacarb	i			
malathion	i			
methomyl	i			
oxydemeton	i			
phosmet	i			
pirimicarb	i			
pyridaben	i			
spiromesifen	i			
tau-fluvalinate	i			
triflumuron	i			

3.2 Pass rate for non-target arthropods (HQ < 2)

Tier I risk assessment for NTA indicated an overall pass rate for all uses of 42% (39 out of 93). The pass rate was 58% for herbicide uses (19 out of 33), 47% for fungicide uses (16 out of 34) and 15% for insecticide/acaricide uses (4 out of 26), using an HQ trigger of 2 using both indicator species.

When findings from extended laboratory tests are considered for NTA in a Tier II risk assessment the pass rate was 97%, 82% and 8.7% for herbicides, fungicides and insecticides/acaricides respectively (Table 1).

As observed for honey bees of the four insecticide/acaricide uses which passed the screening step, one was a highly selective compound with a specialised mode of action and the remaining three products IGRs which are not toxic to adult stages. IGRs may pose a potential risk to immature arthropods and on mode of action the IGR compounds would trigger further evaluation using the appropriate tests. Consequently only a single use was considered of low risk at Tier I for NTA.

3.3 Pass rate for non-Apis bees (HQ < 5)

For non-Apis bees the pass rates were expectedly lower than for honey bees with 46% for all uses considered altogether (43 out of the total number of uses (n = 93)) (Table 1). This trend for fewer passes in the Tier I risk assessment compared to the honey bee was also seen when analysed by product type. For herbicide uses, 21 out of 33 (64%), for fungicide uses 18 out of 34 (53%) and for insecticide/acaricide uses 4 out of 26 (15%) passed at tier I using an HQ trigger of 5.

The four uses which passed the tier I risk assessment for non-Apis bees were the same as those for the NTA assessment and for the same reasons (i.e. highly selective compound with a specialised mode of action and IGRs). The three IGR compounds would have automatically triggered further evaluation of risk due to their mode of action. For the insecticide/acaricide uses which failed at Tier I for non-Apis bees these were based on actual measured LD50 values not 'greater than' values.

4. Discussion

In total 93 product uses (for 74 active substances) were employed in the analysis using publicly available data, conclusions and independent expert opinions. These represented the range of active substances evaluated between 2004 – 2001, and represent the range of product uses as herbicides, fungicides and insecticides/acaricides.

In a review by Mineau et al[12] the Tier I risk assessment for honey bees using an HQ trigger of 50 has been shown to adequately screen for spray applied products for which a risk cannot be excluded and thus warrant further investigation. The capacity of the Tier I to screen out substances of low risk from those which required further assessment was evaluated through a review of the honey bee kill incidents recorded in the United Kingdom Wildlife Incident Investigation Scheme (WIIS).[12] This analysis supported the utility and efficacy of the Tier I screening methodology, provided that special considerations on the mode of action, (as seen above for IGR and specific modes of actions and use patterns) are also considered in the risk assessment process.

The Tier I risk assessment for NTA identified far more uses for further evaluation compared to the honey bee assessment using an HQ of 50 while the Tier II risk assessment for NTA based on extended laboratory studies identified a similar number of product uses to pass to the Tier I honey bee risk assessment. As for honey bees, the Tier I risk assessment for NTA is intended to effective identify spray applied products for which a risk cannot be excluded. This is demonstrated by this analysis and has been validated and reviewed using laboratory and field data and sensitivity analysis.[13,14]

As expected, lowering the trigger value from 50 to 5 to account for non-Apis bees leads to far more product uses failing at Tier I. However, for herbicides all non-Apis bee Tier I failures were due to the endpoint being a 'greater than' value as the study did not determine a LD50 for honey bees. This is the same for all the fungicide uses which fail at Tier I (with the exception of prochloraz). For these uses studies to derive the actual LD50 may be necessary to enable a full evaluation as the real pass rate for herbicides and fungicides could be higher than predicted by the limitations of these data.

In this analysis a 10-fold safety factor to cover non-Apis bees was used only as an example and is likely to be highly conservative or even over conservative. The possible utility of value of 5 was discussed at the SETAC Pellston workshop on Pesticide Risk assessment for Pollinators workshop.[7] The selection of a definite value should however also consider available data on the relative sensitivity of the honey bee compared to other pollinating species. Recent work comparing the toxicity of dimethoate to a range of different bees indicated a very narrow toxicity range for bees, with the honey bee appearing in the middle of the range, thus indicating that the honey bee can be considered a suitable sentinel species (Figure 1).[15] It is hoped that researchers will continue to work on non-Apis bees and the database will continue to be expanded in the future.

The justification for the application of a safety factor needs to be supported by an evaluation of the uncertainties for which it is expected to account. In this analysis, the application of the 10-fold safety factor leads to the identification of a large number of herbicide and fungicide products and uses that would require further evaluation. This is in part due to mathematic biases related to the use of 'greater than' values and high application rates. However, an analysis of the uncertainties in relation to the anticipated effects of herbicides or fungicides to bees should be clearly defined when deciding on the need for a safety factor. In additional, in defining an appropriate screening step, differences in biology between bee species may be more important than differences in sensitivity, as they may affect the exposure profile.

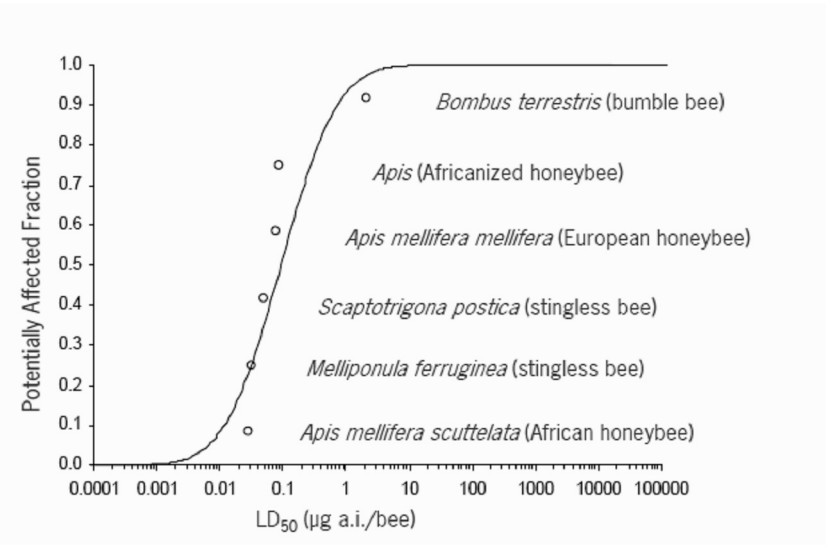

Fig. 1 Species sensitivity distribution to dimethoate for different bees species (from Roessink et al., 2011).[15]

As discussed during the SETAC Pellston workshop on Pesticide Risk assessment for Pollinators workshop,[7] some NTA species may better represent the exposure routes for non-Apis bees. Any existing data on these species should be considered when analysing the level of risks expected to non-Apis bees. As an example, adult parasitoids such as *Aphidius rhopalosiphi* feed on nectar and are thus a good representative for exposure conditions of pollinating species. Similarly, the ground-dwelling beetle *Aleochara bilineata*, (a standard species used in the European risk assessment to NTA) is tested for sensitivity to plant protection products applied to the soil and as such data may be considered informative for ground nesting bees. In cases where a refined risk assessment has been triggered for NTA, the data set developed in the European process may contain information on several different species in the laboratory and more when semi-field/field testing has been undertaken (Table 5).[4] For field tests, inventories of species identified in the tested crop may also yield useful information in evaluating whether there is a particular concern for non-Apis species which would need to be investigated further.

Tab. 5 Testing methodologies developed for the risk assessment to non-target arthropods developed in European process of evaluation of pesticides (from Candolfi et al., 2001)[4]

Testing scale	**Species (and stages tested)**
Tier I Laboratory: artificial substrate	*Aphidius rhopalosiphi* (adults + life cycle)
	Typhlodromus pyri (protonymphs + life cycle)
Tier II (extended) Laboratory : natural substrate	*Aleochara bilineata* (adults + life cycle)
	Aphidius rhopalosiphi (adults + life cycle)
	Chrysoperla carnea (larvae + life cycle)
	Coccinella septempunctata (larvae + life cycle)
	Orius laevigatus (nymphs + life cycle)
	Pardosa sp. (adults)
	Poecilus cupreus (adults)
	Trichogramma cacoeciae (adults + life cycle)
Semi-field	e.g. *Poecilus cupreus* (adults)
	Methods can be adapted for many species
Field	Arthropods (populations and communities)

In comparing the proportion of uses which passed at Tier I using the HQ trigger of 5 for non-Apis bees with the results of the NTA analysis, it was found that the numbers were very similar to the NTA analysis leading to slightly fewer Tier I passes. It has to be noted however, that the NTA analysis was conducted on data generated for the purpose of risk assessment and are not influenced by 'greater than' values. Thus the NTA Tier I risk assessment is far more conservative than the Tier I risk assessment for honey bees and similar to the non-Apis bee assessment presented in this paper. The two standard species *Aphidius rhopalosiphi* and *Typhlodromus pyri* are indeed considered as the most sensitive of the non-target arthropods tested under the European Escort scheme.4 Also due to the limitations of the non-Apis evaluation discussed above it can be concluded that based on this assessment the NTA Tier I risk assessment is more conservative than the proposed non-Apis risk assessment based on honey bee data and a HQ of 5.

Due to the sensitivity of the tested sentinel species, the range of biological traits they represent and the conservatism of the screening step as observed above, NTA may then be reliable surrogates for non-Apis bees in a screening step of risk assessment. When the outcome of HQ calculation for honey bees indicates possible concerns for pollinating insects, additional information available for NTA as laboratory or extended laboratory tests could be analysed as a screening step. As shown above, the Tier I risk assessment for NTA appeared to be the most restrictive and could then be used as a screening step for non-Apis bees. When a risk cannot be excluded on the basis of Tier I calculations, all available data on NTA including extended laboratory tests and higher tier data could be examined in order to identify any reliable information addressing the possible risks to non-Apis bees, taking into consideration biological traits, plant-insect relationship and exposure routes specific to non-Apis bees.

This initial investigation has demonstrated the potential for existing risk assessment schemes to provide a suitable screen step for non-Apis bees. However, before any such scheme can be developed additional risk assessment and further testing will probably be necessary for a number of products, and more specifically for insecticides/acaracides which may also lead to the necessity for additional testing methods and species. These issues are being dealt with within the OECD where a working group is currently identifying the requirements in terms of testing in light of risk assessment needs.

5. Conclusions

The comparison of Tier I risk assessments for honey bees, NTA and non-Apis bees based on a representative sample of 93 product uses indicated that all three are able to rapidly identify compounds and uses for which the potential for adverse effects cannot be excluded. The outcome of screening steps were consistent for insecticides/acaricides for which possible effects are expected to be the highest and those which passed the Tier I assessment would, owing to their mode of action, have triggered a dedicated risk assessment according to current guidance documents.[2,3,4] However, the Tier I screening step for NTA and non-Apis bees presented in this paper had poor discrimination for herbicide and fungicide product uses with many products potentially triggering higher tier investigations (such as extended laboratory tests for NTA). The current honey bee screen was effective at identifying substances of high risk (i.e. known insecticides and acaricides) and allowed for low risk products (most herbicides and fungicides) to pass demonstrating that the scheme was meeting screening requirements.

Taking into account sensitivity, biological and screening aspects, NTA appear promising surrogate species for screening risks to non-Apis bees. The Tier I risk assessment for NTA appeared to be the most restrictive of the three groups on the basis of available data. Thus a screening step for non-Apis bees could be developed based on the Tier I risk assessment for NTAs followed by the use of Tier II (extended laboratory data) to cover non-Apis bee risk assessment without impacting the level of protection. However, when compared to the findings of the honey bee analysis this may be an over conservative screening step. As for the honey bee, further information such as field monitoring is needed to adjust the risk assessment hypothesis. A dedicated ICPPR working group is currently working on monitoring protocols with the aim to address the issue. Then additional testing as

defined by OECD will be needed in order to fill the related gaps of laboratory and higher tier testing in the risk assessment.

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Risk assessment of pesticides on bees: evaluating risk coefficients for assessing acute and chronic toxicity

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Abstract

Background: Risk coefficients are the key in the way how a pesticide active substances or formulated products will go through the risk assessment scheme dichotomy. Defining them is thus one of the main challenges of a risk assessment scheme design. In the light of the scientific publications on the subject, the existing risk coefficients and methodologies used for the toxicity evaluation under international guidelines result questionable.

Results: LD50 values have shown to be variable. Prolonged effects following single contact can sometimes be observed when measuring the acute toxicity. The trigger value (10) of the risk coefficient results as inadequate. The toxicity derived from the exposure to substances continuously available for bees at sub-lethal doses needs to be evaluated separately, given the wide differences between acute and chronic lethal effects of pesticides.

Conclusions: The observation period of the mortality tests should be lengthened as long as mortality increases, and while control mortality remains acceptable. Whenever active substances can pollute bees' food sources, first tier tests should include laboratory tests: (1) on adult bees with: (a) acute toxicity tests; (b) chronic toxicity tests; (c) behavioural tests; and (2) larvae toxicity tests. Consequently, the decision to run higher tier tests should depend on four different risk coefficients.

Keywords: honeybees, risk assessment, acute toxicity, chronic toxicity, risk coefficient, TER

Introduction

The EPPO risk assessment scheme of pesticides on bees has recently being updated.[1] The aim was to include the evaluation of pesticides with systemic properties. In parallel, the legal framework[2] currently demands any active substance, safener or synergist to have negligible exposure to honeybees and not to show unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour under the proposed conditions of use of the product containing it.

The scheme proposed starts with a screening of the potential acute toxicity of the product under evaluation to adult bees. In case the substance can be problematic for larvae, the toxicity on brood will also be evaluated. An IGR or active substances showing toxicity to larval stages by screening or efficacy studies will be tested for brood effects. On the basis of the data obtained from this first phase, data on the LD50 of the active substance in adult bees is produced (contact and oral). When contrasting these values to the exposure potential, active substances might be further evaluated for their chronic toxicity or being classified as low risk for bees based on the comparison with a trigger value of a risk coefficient. The exposure is determined from the concentration of the pesticide in the aerial parts of the plant, considered an overestimation of the residues found in pollen or nectar (default value 1 mg/Kg).

Several publications have put in question the role of certain systemic active substances in the problem of bee decline. The observed effects range from a direct or indirect shortening of bees lifespan (leading in the long term to the collapse of the colony),[3-5] the disruption of the reproductive capacity of queen and drones,[6,7] the synergistic effect with pathologies[8,9] or the cases of acute intoxications following seeding operations.[10-12] The capacity of the EPPO scheme to discriminate between active substances that might be problematic to bees, especially in the long run, has been tested with the existing ones. Precisely, this risk assessment scheme has been calibrated to fit the

characteristics of the majority of the active substances put into question. Mainly those active substances with toxicity values in the range of ng/bee would require further testing (unless residue levels are in the range of mg/Kg). However, the capacity of the scheme to determine the impact of chronic exposure, or if other existing or future active substances could have potential acute or chronic effects on colony survival and development[2], remains uncertain.

The present article proposes a non-comprehensive analysis of the toxicity variables included into the definition of the risk coefficient categorising the risk of active substances. In order to verify if the system proposed fits the legal requirements, data extracted from a literature review were used for the analysis of the scheme.

Results and Discussion

The current risk assessment dichotomy of EPPO relies on the value of the Toxicity Exposure Ratio (TER) based on the comparison of the LD50 and the exposure (in terms of bee consumption per day). Should the TER be lower than 10, chronic toxicity studies would be run. Otherwise, the active substance would be characterised as low risk for bees.

1. Variability of LD50 and extrapolation to real conditions

It is worthwhile analysing the parameters conforming the TER. Despite the proposed standardised methods for its estimation, LD50 varies widely. The LD50 of imidacloprid, for example, has shown values between 3.7 and 40.9 ng/bee,[13] 40 and 60 ng/bee,[14] 49 and 102 ng/bee[15] and 490 ng/bee[16]. Some sources of variability are colony genetics or bee management during testing. Data have shown the variation in the measured LD50, considering different parameters or variables like temperature,[17] the age of the bees,[17,18] the bee sub-species,[19] the pattern of exposure (unique vs multiple exposure),[20,21] the exposure of the tested bees to a pesticide prior to acute testing,[21] etc. Given the diversity of the parameters mentioned in real field conditions and the different exposure to pesticides of the individuals of the colony, Belzunces in 2006,[21] suggested that LD50 values should only be used as a comparison tool among pesticides. However, this value alone should not be used to draw conclusions about the level of risk to bees in the environment.

2. Prolonged effects

OECD guidelines[22] currently recommend the daily recording of mortality at least up to 48 hours. Should the mortality rate increase between 24 and 48 hours, while control mortality remains acceptable (≤10%), an extension of the duration of the test should be to 96 hours. Certain active substances have shown increased mortality over this observation period. Suchail *et al.,* 2001,[14] showed prolonged action of imidacloprid and two of its metabolites (olefin and 5-hydroxy-imidaclopred) up to 96 hours, some of these substances showing an tendency to increase. The same is shown for fipronil sulfone, the oxidative metabolite of fipronil.[23] The toxicity evolution beyond this observation period is not known. These effects might be the result of the long-term residual effectiveness of the mother compound or the bio-activation of toxic metabolites. A longer observation period as long as there is an increase in toxicity could be envisaged, as long as the control mortality would not rise above unacceptable values. Such a modification of the methodology for the determination of LD50 would involve minimal changes in test management, but provide precious information about the toxicity kinetics of the active substances and products under evaluation. It could be argued that the existence of prolonged effects could be evaluated through tunnel tests. However, these tests are not systematically run. Furthermore, prolonged effects may result from an extended exposure to the pesticide in the tunnel.

3. Exposure - PEC

Different exposure patterns and durations can be expected depending on the use and properties of pesticide products or the behaviour and function of each of the colony members. Furthermore, a bee colony may be repeatedly exposed to the same substance in different ways at different periods of the year. Bees can get in contact with systemic active substances: (1) spread in dust (following seeding

operation of certain treated seeds)[11,24] or in the air (following spray applications); (2) in plant exudates[25-27]or superficial water;[28] (3) in pollen and nectar;[29-31] (4) present in the reserves of the colony.[32-34]

Toxic molecules suddenly distributed in the air (following spraying or seeding operation of some treated seeds) might affect foragers in an acute way. The contamination of nesting material might entail a risk to the colony members from inside the hive. The contamination of food and water sources involves, depending on the doses, either acute or chronic exposure to pollutants. The long persistence of the active substance in the environment increases the risk of chronic exposure. Indeed, residues in pollen, nectar or honey of systemic compounds can range from 0,7 µg/Kg (imidacloprid)[35] up to 94 mg/Kg (carbaryl).[36] These figures are worrying considering that honeybees should negligibly be exposed to active substances.[2]

Considering the wide range of pesticides residues found in food matrixes, the default value of 1 mg/Kg may be inadequate. A more precise approach would be the analysis of residues directly in the matrixes following the treatment.

4. Risk coefficient - TER

The trigger value established by the EPPO guidelines for the risk coefficient (10) "[…] aims at ensuring a margin of safety that is sufficient to cover the uncertainty related to longer exposure periods and possible related increased effects […]". Ideally, this safety value should cover as well the uncertainty related to the appearance of sub-lethal effects that could impact the colony and the uncertainty related to the capacity for extrapolation of the scheme to field conditions (in case such tests would not be undergone).

The value of 10 is based on unpublished results of a DEFRA study[16] showing a potential 10-fold adjustment factor between LD50 (µg/bee) and LC50 (µg/bee/day). Chronic toxicity is observed after 10 days of continuous exposure to pesticides in lab conditions.[1] The consequence of this value could be very important. Supposing the acute (oral) LD50 of a substance is 5 ng/bee, then the estimation of the chronic LC50 would be 0,5 ng/bee. Following the proposed principle and assuming a bees' exposure of 0,49 ng/bee, the TER calculation would be larger than 10 (5/0,49). Exposure to 0,49 ng/bee could be observed in numerous active substances already found in residue studies.[29-31,35] Consequently, an active substance or product would be categorized as low risk to bees, even though the bees' exposure would be almost equal to the chronic LC50.

A literature review has been carried out identifying studies done to determine the chronic toxicity of pesticides (after continuous exposure over more than 10 days).[37-41,14,16] Bearing in mind their differences in experimental set-ups, the same ratio has been calculated in order to have a notion of the magnitude of the safety factor that could be necessary. LD50/LC50 values of 31 active substances show a range from 0,51 (acetamiprid)[16] to 100.000 (imidacloprid metabolites).[14] If instead of continuous exposure, repeated exposure is considered (intermittent doses, 17 active substances), the ratio show a range from 0,05 (for emamectin)[42] to > 1.000 (various active substances).[20,42] A parallel exercise could be done with a comparison of LD50 values with doses showing sub-lethal effects without leading to mortality in the long run.

Therefore, several active substances have shown to be lethal when administered to bees at concentrations lower than those inducing acute mortality in case they are administered over a long period. The hypothesis provided to explain the differences in mortality between acute and chronic exposure are based on toxicity dynamics and kinetics: (1) existence of high and low affinity receptors, low doses activating high-affinity receptors (inducing mortality through an agonistic effect) while high doses would activate both low and high-affinity receptors (proving a compensation action);[14] (2) enzymatic bio-activation of mother compounds into toxic metabolites;[14,43] (3) detoxification capacity of bees exceeded by the daily intake of the toxic material.[41,42,44] All in all, it seems that there is no clear correlation between acute and chronic toxicity.

As a result, it seems inappropriate to use a risk coefficient (TER) based on acute terms (LD50 and an overestimation of the exposure of bees to the contaminant) to determine if chronic toxicity tests need to be run. A more suitable proposal would be, first to check the pattern of exposure of the

different colony members - exposure over short/long periods may happen. Depending on this result, either acute toxicity tests or both acute and chronic toxicity tests in adult bees should be carried out as first tier tests. These oral tests are simple, not cost intensive and provide good information about the toxicity of the active substance. Risk coefficients that would use, respectively, acute and chronic toxicity values, together with the respective exposure (to residues in spray, dust, food sources, etc.) would determine the necessity of running higher tier tests.

The trigger value of the risk coefficients using both acute and chronic terms should include a safety or uncertainty margin that would consider the variation of the toxicity parameter used.

5. Other necessary first tier tests

It needs to be noted that these toxicity tests and risk coefficients would refer only to the potential lethal effects on adult bees. Regulation (EC) 1107/2009 specifically mentions that an active substance, safener or synergist should not have unacceptable effects on honeybee larvae and honeybee behaviour under the proposed conditions of use. Brood effects should be considered separately and systematically in case pesticide exposure cannot be avoided through contamination of food sources. Therefore, toxicity testing on larvae should not be based just on the mode of action of the active substance or on screening or efficacy studies. A third risk coefficient including parameters targeting brood (toxicity and exposure) should be included into the scheme to determine if higher tier studies are needed.

Similarly, several techniques evaluating the appearance of sub-lethal effects on adult bees at very low doses are currently available. Considering the importance of behaviour and communication in social insects, it should be envisaged to include in the first tier the potential impact on bees of these sub-lethal doses. Realistic low doses of pesticides found already in bee colonies' food and water sources should be used for the tests. This is in accordance with the recommendations provided by Tasei et al., 2003.[45] The laboratory tests proposed so far (for example Proboscis Extension Reflex test) are not complicated to carry out and would complete the evaluation of pesticide active substances and products. Again, a risk coefficient including sub-lethal parameters confronted to a trigger value would determine if further studies are requested. The results of these studies may help, as well defining the observation parameters to focus while running higher tier tests.

Furthermore, the observation and systematic recording of behavioural and locomotion effects happening during mortality tests could complement these specific behavioural or developmental tests. Nevertheless, a clear and standardised scale of effects should be defined.

Conclusions

The present article argues why the EPPO risk assessment scheme is not satisfying for the evaluation of pesticides with systemic properties. As a result, several improvement proposals have been presented. The first one aims to achieving a better methodology to determine the LD50 by increasing the duration of the observation period until the mortality is stable as long as control mortality remains under acceptable levels. The second one shows the importance of reviewing the trigger values of the risk coefficients used in risk assessment. The third one presents the need to run chronic toxicity tests systematically in case food and water sources of bees can get contaminated with the active substance under evaluation. Acute and chronic exposure to contaminants entails differences in toxic dynamics and kinetics. Therefore, chronic toxicity tests should be carried out independently of the results of acute toxicity tests at first tier test in the risk assessment. Similarly, larvae toxicity tests and specific tests evaluating the impact of sublethal doses of pesticides should be included in first tier in case food sources can become contaminated. As a result, four different risk coefficients would determine if higher tier tests are needed: (1) acute toxicity/relevant exposure (oral or contact); (2) chronic toxicity/relevant exposure (oral); (3) larvae toxicity/relevant exposure (oral); (4) dose producing sublethal effects/relevant exposure (oral). Trigger values will need to be defined based on present and future studies and certitude assessment.

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Assessment of pesticides risk for bees: methods for PNEC measurements

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Abstract

Background: An individual honeybee shows a complex behavioral structure. Each bee takes part in the collective behavioral set up that ensures bee colony survival and development. Contaminants are likely to have effects on individual bees' behavior with consequences at the level of the whole colony. They also are likely to alter bees' physiology, including lifespan, fertility or fecundity, leading to colony weakness or colony collapse.

Results: Peer-reviewed scientific literature provides a wide range of methods used for testing honeybees' behavioral or physiological parameters. Apart from alterations that may appear during the conduction of acute or chronic toxicity tests, specific tests could be conducted to complement the risk assessment in order to evaluate the impact of sublethal doses of contaminants on bees. Such tests can be developed both in laboratory conditions or as part of the semi-field and field tests that are currently required as higher tier tests of risk assessment schemes.

Conclusion: The purpose of this work is to review some of these methods and discuss their relevance in the evaluation of pesticide active substances and/or products in view to propose their future inclusion in pesticides risk assessment to bees.

Keywords: honey bee, sublethal effects, risk assessment

1. Introduction

Prior to introducing pesticides on the market, these products must be assessed in compliance with European regulations (Regulation (EC) 1107/2009). This assessment is performed following the annexes of the Regulation and EPPO guidelines (EPPO PP 1/170 (4), EPPO PP 3/9). However, the guidelines and the current assessment scheme currently applied to the risk posed to bees are no longer relevant for assessing systemic pesticides that are likely to be available for bees through water, air (sowing contaminated dust), nectar or pollen (Alix and Vergnet 2007).[1] Systemic pesticides might be used both as soil or seed treatment as well as in spray. Regardless of the way of application, the potential to contaminate pollen or nectar has already been proven (Villa et al.2000, Ham et al. 2006).[2,3]

These substances raise the problem of assessing substances that bees are orally exposed to, through their food sources. Bees are faithful to their food sources (pollen and nectar). Therefore, if these matrices are contaminated, the exposure of bees will lengthen in time. The way the different castes and classes of bees will be exposed differs according to their function within the hive. Foragers may be exposed continuously along the flowering time of the crop/plant, sometimes during their whole forager life. Moreover, in the hive, pollen and honey stocks are likely to be contaminated too. As a consequence, food stocks consumption may lead to a prolonged and continuous contamination for all bee categories in the hive. Regardless the type of bee considered, systemic pesticides induce an exposure to low doses of molecules, often not able to induce acute mortality, but extended in time.

Several scientific studies have proven the impairment of bees' behavioral or physiological abilities after exposure to low doses of pesticides (Desneux et al.2007).[4] The consequences may affect the whole colony, leading to a honey production decrease, colony stress and weakness and potentially to colony death. Khoury *et al*. (2011)[5] show that a decrease in workers lifespan may conduct to the collapse of the colony. Since the exposure to lower doses of pesticides is extended in time, a relevant assessment scheme should include a careful assessment of chronic effects as well as sublethal effects.

The present article focuses on sublethal effects of pesticides on bees' physiology and behavior. It aims to expose the limits of current methods assessing sublethal effects and to make a short review of

various validated methods performed either in laboratory, in field or semi-field conditions in order to measure some bees biological parameters that are - or could be - used for assessing sublethal effects of contaminants present in water, air or food.

2. Current assessment of sublethal effects

Currently, sublethal effects are sometimes assessed in higher tier tests only through field and semi-field tests performed on the whole colony. Such an option is based on the fact that these tests are the most representative of actual field conditions. However, this evaluation structure raises some concerns because tunnel and field trials show several limitations when applied to substances having slow, indirect, chronic and/or delayed effects.

2.1. Limitations of semi-field trials

A first shortcoming concerns bee brood development assessment. Bees are disturbed in tunnels because of confinement. Confinement effects can be observed on foragers (some bees are always seen stuck on the tunnel gauze and seem to be disoriented) but it has effects on hive bees too: bee brood rearing is impaired in tunnels. The brood termination rate in tunnels is usually lower than the one of free foraging colonies (Giffard H, 2011, pers. comm.). After some weeks, brood surfaces decline in the colonies and the lack is total for some of them (see for instance the assessment dossier of the a.i. fipronil).[6] A comparison between bee brood in tested item and control allows some observations but bee brood assessment in semi-field testing has got some inconvenient limits:

- absence of difference between control and test item hives does not allow to conclude that the tested substance has no effects on bee brood success since the confinement effects could mask the substance effect
- observation of delayed or long-term effects is impossible in semi-field trials.

Hence semi-field trials are not suitable for assessing quantitatively the effects on bee brood and do not allow establishing LD50 for larvae.

A second limit is that tunnel methods are not replicable because tested product may be stored into the combs and sometimes diluted in nectar before it is consumed by larvae (Aupinel et al. 2007).[7]

A third limit is that bees' exposure in tunnels is not comparable to actual exposure in fields. Although when a product is sprayed, the way bees are exposed is the same in tunnel as in field, when applied as soil or seed treatment and available through water, nectar or pollen, the bees' exposure in tunnel cannot be considered as representative of such an exposure in field since the flower patches areas are smaller than in field. Moreover, these testing areas are unable to cover the colony needs so that the available flowers are 'over-foraged', leading to two consequences: (1) bees can show abnormal foraging behavior that are likely to mask the effects the substances can have on this behavior, and (2) the amount of nectar or pollen collected by bees cannot be considered as representative of the amount that bees usually harvest in field: bees are under-exposed in tunnels compared to actual field conditions. Moreover no toxic reference can be applied to estimate the bees' exposure.

Thus semi-field tests are not sufficient for assessing effects on bee brood, delayed and long-term effects on the colony, and some behavioral effects that can be masked by confinement effects of the tunnel.

2.2. Limitations of the field trials

Field trials are suitable since they are the most representative of the actual conditions bees will face when the substance or product will be put on the market. However such trials raise some concerns when they are applied to systemic substances used in soil or seed treatment.

The first one is concerning colonies' exposure. Even in large surface tested fields (for instance 3 ha), this surface remains well below the usual bee forage areas when the product is used at the agricultural market level. Unsurprisingly when the treated field is the only resource bees can forage, the level of this harvesting is well below normal harvesting (for instance in an assessment dossier[6] a field study on sunflower shows an increase of hive weight of 3,54 kg in the treated item, and a

decrease of 0,4 kg in the control item during exposure (12 days) when a 'normal' honey harvesting on sunflower is 60 kg on the same period (ACTA1998)[8]). On the contrary, when the harvesting level appears normal despite the restricted area of treated flowers, it usually means that bees foraged in fields outside of the study. In both cases bees are underexposed in comparison to the exposure they will be submitted to, once the product is on the market.

A second issue is the difficulty to find adequate control fields. Maize or sunflower are unable to grow normally in areas that are not allotted to such crops. The trials are thus usually performed in areas where these crops are usually grown. In such areas the pesticide use is a standard practice so that the presence of a contaminant background is practically unavoidable. When the effects that the study aims to check are sub-lethal, chronic or of a delayed-type, it is often problematic to discriminate them from the background ones.

A third limit is that field tests are statistically valid for detecting clear-cut effects only. Performing field trials is expensive (i.e. because fields treated with non-authorized products must be canceled out), therefore only a few field tests are submitted in an authorization dossier. Moreover, the high variability between hives and climatic or weather conditions needs a large sample size for detecting effects at an acceptable level. A statistical analysis of four field trials performed with imidacloprid found that two studies only were statistically able to detect a reduction in bees' performance, the first one if the reduction in honey harvesting is superior to 56% and the second one if it is superior to 33% (Cresswell et al. 2010).[9] The same study states that *the probability that all* [studies] *would fail independently to detect statistically the largest predicted field-realistic sub-lethal effect is (…) 36%, which is unacceptably high for concluding that sub-lethal effects are non-existent*.

Field tests as currently performed are thus insufficient for assessing sub-lethal effects that could lead in the long term to colony weakness and sometimes to colony collapse. For instance in Khoury *et al.* (2011),[5] the colony will collapse when the foragers lifespan is chronically reduced from 6,5 to 2,8 days, i.e. a reduction of 57% of an individual bee lifespan. The ability of current field tests to detect such lifespan reduction is uncertain.

Field trials are the only ones able to assess some parameters (for instance, for assessing quantitatively the queen's egg laying, i.e queen fertility, since the full power of egg laying is reached in large colonies only). Improving their reliability requires setting up of consistent methods to measure bees' exposure and sub-lethal effects of the tested products on bees using enlarged sample sizes in order to obtain statistically validated results. Yet it will still be difficult to ensure a bee's exposure closely representing the reality when the tested products are used in soil or seed treatment.

It appears thus necessary to adopt a more protective approach when assessing the risk of pesticides to bees, especially when sub-lethal effects may be expected. Sub-lethal effects on bees' behavior and physiology should be tested specifically, through laboratory and field studies on individuals or on the whole colony.

3. Place of sub-lethal effects evaluation in assessment scheme

The currently applied assessment scheme is based in the first tier on a hazard quotient i.e. a comparison between the acute LD50 (Lethal Dose 50, a laboratory test on individuals) and the application rate (Hazard Quotient: HQ = application rate (g/ha)/LD50 (µg/bee).

EPPO's last guidance document (Alix and Lewis 2010)[10] proposes the measurement of the acute LD50 and of the contaminant concentration bees are exposed to; at the first assessment tier it proposes to calculate a toxicity-exposure ratio, i.e. a comparison between the predicted environmental concentration (PEC) and the acute LD50 (Toxicity Exposure Ratio: TER = LD50/PEC).

Until this time, the used parameter at first tier is thus a mortality test. Both schemes consider acute toxicity only, an option that is open to criticism when applied to substances that are available in pollen or nectar the whole blossoming period long. Indeed in that case of chronic bees' exposure (continuous exposure) the resulting toxicity cannot be inferred from the acute toxicity measurements: for a lot of substances LD50 by continued exposure during 10 days is different from

LD50 by a single acute exposure, difference factor between them may reach several tens or several hundreds (Decourtye et al. 2005).[11]

Both schemes are thus not protective enough. A more protective scheme should be conceived. It should be based on a first measurement of sub-lethal effects at the individual level in order to discriminate low-risk substances from toxics that are likely to cause impairments to bees' physiology or behavior; so the overall assessment scheme would examine at first tier risks at the individual level, and at higher tier risks at the whole colony level. This option is compliant with SANCO's guidance document 10329[12] since realistic conditions involve colonies rather than individuals.

Some of the methods reviewed in the next part of this article are laboratory tests, some others field tests. Field studies can assess individual behavior alterations (for instance orientation ability) or colony behavior modifications (for instance lifespan: bees' lifetimes in laboratory or in hive are basically different since bees' survival involves relations with the nest mates in the hive as superorganism). For being compliant with the cited scheme rationale, we propose to apply the individual tests at the first assessment tier, the colony level tests at the higher tier.

All these methods could be used or adapted for establishing a PNEC (Predicted Non Observable Effect Concentration) and comparing them with PEC (Predicted Environment Concentration), allowing the establishment of a PEC/PNEC risk coefficient.

4. Existing methods for bees' physiology parameters measurements

4.1. Biomarkers

Like for human being or other vertebrates, there are biological parameters that are able to indicate toxic effects on invertebrate physiology (Hyne and Mayer 2003).[13] Acetylcholinesterase (AChE) can be used as a biomarker of neurotoxicity and exposure to deltamethrin in honeybee (Badiou *et al.* 2008).[14] Fenitrothion and cypermethrin lead to decreases in Na+/K+ ATPase and acetylcholinesterase (AChE) activities in emerging bees (Bendahou et al. 1999).[15] Due to the importance of Na+/K+ ATPase in the energetic metabolism, this can cause dysfunctions at cellular level, i.e. in the cardiac muscle (Desneux et al. 2007).[4] Imidacloprid increases the level of cytochrome oxidase in the mushroom bodies of honey bee brain; this modification is related to the impairment of the medium-term olfactory memory (Decourtye *et al.* 2004).[16]

Exposing bees to a contaminant can modify other enzyme functioning. Enzymes of the oxidant stress (superoxide dismutase, glutathione reductase, glutathione peroxidase and catalase), of the immune system at the individual or at the social level, (phenoloxidase, glucose oxidase), and others enzymes likely to be involved in detoxification mechanisms (glutathion-S-transferase, alkaline phosphatase) were successfully used for establishing toxicity profiles of four pesticides: imidacloprid, thiamethoxam, ethiprole and fipronil (Brunet *et al.* 2011).[17]

Malaspina and Da Silva-Zacarin (2006)[18] provide a mini-review of cell markers that could be useful in monitoring bees exposed to pesticides. The study focuses on stress proteins (heat shock proteins) and their relationship with histological damages in bees' midgut and Malpighian tubules.

Biomarkers analyses are cheap regarding the costs of usual trials and need only a few bees (5-6 bees for analysis of 6 biomarkers). The method needs to be developed but already appears from the first available studies to be really suitable for a first screening of the substance toxicity at the first tier of the assessment scheme as well as for monitoring honeybee status in agricultural areas where bees are chronically exposed to low levels of many environmental contaminants (Malaspina and Da Silva-Zacarin 2008).[18]

4.2. Immunity

Immunity leads us once again to biomarkers studies since they are widely based on phenoloxidase and glucose oxidase level analysis. However, they often also include a haemocyte count. Using these three parameters, Alaux *et al.* (2010)[19] showed a synergic interaction between imidacloprid and Nosema microspores.

4.3. Reproduction, fertility and fecundity

4.3.1. Brood development

Dai *et al*. (2010)[20] have studied the effects of deltamethrin and bifenthrin at sub-lethal dose on fecundity, growth, and development of honeybees. They observed the fate of eggs mapped on a transparency and measured daily fecundity, egg weight, larva weight, hatching rate, capping rate, emergence rate, success rate of development, egg stage, unsealed brood stage, sealed brood stage and immature stage. They found that both pesticides affect these parameters. Particularly the global period before emergence was longer in colonies fed with contaminated syrup than in control. This is of significant importance since post-capping times have an effect on mite population growth (Wilkinson and Smith 2002).[21] Aupinel *et al.* (2005)[22] proposes a standardized method for assessing bee brood development *in vitro*. This method was used for testing the effects of dimethoate and fenoxycarb contaminations; it yielded NOAEC (Non Observable Adverse Effect Concentration) measurements.[11] It is already validated at the French national level and could be shortly validated at the European level. It should be performed at the first tier of the assessment scheme as soon as the tested substance is likely to contaminate pollen, alongside the LD50 measurement on adult bees because the toxicity to larvae can differ widely (being higher or lower) from the toxicity to adults and cannot be drawn from the chemical family or from the mode of action of the concerned pesticide (Alix and Vergnet 2007).[1]

4.3.2. Queen rearing.

Studying the effects of pesticides on queen rearing is of particular relevance since beekeepers often complain of re-queening failures. Such failures are often associated to other problems (among others colony losses) where the influence of pesticides is suspected (personal observation). Contacts with contaminants are actually able to cause queening problems: for instance an increased rejection of grafted larvae is observed when the cups wax is contaminated by coumaphos (Pettis *et al.* 2004).[23]

4.3.3. Drones fertility.

Pesticide effects on drone fertility were never specifically studied from a toxicological point of view at this time to our knowledge; nevertheless, useful methods exist in other studies. Motility of drone spermatozoa and its evolution were measured in a study of intra- and heterospecific insemination (Phiancharoen *et al.* 2004).[24]

4.4. Lifespan

Lifespan can be measured in a laboratory test and results can be submitted to a statistical treatment that evaluates lifespan estimation of bees (Dechaume-Montcharmont *et al.* 2003).[25]

Tagging bees cohorts and controlling their daily survival at the hive entrance can be used to test lifespan in field. Such methods have been developed in studies focusing on biological issues, for instance the dependence of lifespan upon flight performance (Neukirsch 1982)[26] or upon brood rearing (Amdam *et al.* 2009).[27]

It is of first importance to assess the contaminants effects on bees' lifespan since a reduction of this parameter can lead to the collapse of the colony (Khoury *et al.* 2011).[5] The first kind of tests (laboratory measurements) could be used at the first tier and the second ones at higher tiers to confirm lifespan decrease at the colony level if such effects were detected in laboratory tests on individuals.

5. Existing methods for bees' behavior parameters measurements

5.1. PER (Proboscis Extension Reflex) trial

PER trial is a well-known method used in various purposes since the reflex was discovered by Kuwabara in 1957 (for a general discussion, see Giurfa and Malun 2004).[28] It is used by a lot of research laboratories for assessing bee's memory and of its susceptibility to various conditions, for

instance sleep deprivation (Hussaini *et al.* 2009)[29], protease inhibitors (Pham-Delègue *et al.* 2000)[30] or pesticides contamination (Guez *et al.* 2001, Decourtye *et al.* 2005, El Hassani AK *et al.* 2005).[31,11,32]

Effects on the conditioned learning are now proved for several pesticides, among others pyrethinoids (Decourtye *et al.* 2004, Decourtye *et al.* 2005),[33,11] neonicotinoids (Decourtye *et al.* 2003, Decourtye *et al.* 2004, Decourtye *et al.* 2005, Guez *et al.* 2003)[34,33,11,35] and phenylpyrazoles (Decourtye *et al.* 2005, El Hassani A.K. *et al.* 2009)[11,36]. However, the correlation between decrease of the response obtained in laboratory tests and foraging performance at the colony level remains uncertain at this time (Pham-Delègue *et al.* 2002).[37] Nevertheless, since PER is the appetitive reflex of the bee, it clearly plays an important role in bees foraging performance.

This test appears now robust and provides a first approach of bee brain central nervous system integrity. For these reasons it appears to be a really suitable trial at the first tier of the assessment scheme.

5.2. Homing flight trials

Homing flight trials test bees' ability for orientation during inbound flight. This ability is of first importance since bees' disorientation results in their disappearance and further in the whole colony collapse. Indeed, foragers produce a pheromone (ethyloleate) acting as an inhibitory factor delaying the onset of foraging behavior (e.g. nurse bees remain nurses for a longer period before becoming forager bees) (Leoncini et al. 2004).[38] Foragers' disorientation and disappearance results in a lack of this pheromone: hive bees early become foragers and the number of nurses decreases. It results in a reduction of brood area; if the forager disappearance goes on, the hive collapses and dies.

There are a lot of methods for assessing bees' orientation ability. The simplest one was used in France by Cerutti *et al.* (unpublished data; INRA Avignon), in order to evaluate influence of thiametoxam (a neonicotinoid pesticide) on homing flight. Bees were captured at the hive entrance when flying out; they were tagged and put in an incubator where they were fed with contaminated or non-contaminated syrup. They were then released 240 m away from the hive. Homing flight time was measured.

This laboratory/field test is suitable at the first tier because it is simple, cheap and checks various abilities of the bees, including orientation, locomotion and memory.

Many other methods exist, including semi-field tests (Vandame *et al.* 1995).[39] RFID microchips were used for assessing pesticides effects (Decourtye *et al.* 2011).[40] This method allows recording the whole flight pattern. It could be used at higher tier for precising toxicity observed in the first tier trials (for instance an increased duration of the inbound flight).

5.3. Maze tests.

A lot of tests involving the orientation ability exist. Some of them are simple. For instance Medrzycki *et al.* (2003)[41] used a simple box for recording bees' movements on a single comb; bees' behaviors were classified and compared between treated and control samples. Such a trial allows assessing bee locomotion and mobility.

Other maze tests are more complex and allow assessing complex abilities like visual learning and matching-to-sample ability. Han *et al.* (2010)[42] successfully uses a complex T-maze with colored marks for assessing sublethal effects of GMO contaminated pollen on bees: Cry1Ac + CpTI shows a non-significant effect compared with negative and positive (imidacloprid) controls.

Various decision-boxes with marked holes were used in a several studies, for assessing pesticides toxicity (Decourtye *et al.* 2009)[43] or for studying honeybee visual cognition (for a review see Benard *et al.* 2006). [44]

5.4. Thermoregulation.

Honey bees colony's thermoregulation involves different behaviors (among others heat production by shivering, i.e. titanic contraction of bee's flight muscles, and ventilation). It radically differs in winter from spring and summer.

As soon as the colony rears brood, the temperature range must be 33-36°C in the nest. Bees raised below this temperature show decreased performance levels (Tautz et *al.* 2003),[45] and *Ascosphera apis* spores germinate when the nest temperature falls below 32°C for more than two hours (Wilson-Rich *et al.* 2009).[46]

In broodless colonies, bees of the cluster core produce heat for ensuring the cluster survival. The core's temperature increases when the exterior temperature decreases for maintaining bees that form the mantle edge at least at 7°C (Fahrenholz *et al.* 1989);[47] below this temperature bees collapse and fall at the bottom of the hive. Winter cluster thermoregulation is an accurate and complex mechanism, aiming to maintain the minimum temperature that allows bees' survival, avoiding energetic waste that would lead to stocks overconsumption and to precocious aging of the cluster bees. Indeed high energy consumptions cause a decrease in winter bees' lifespan Neukirch 1982),[26] a process that can lead to colony collapse if it continues over long periods.

Thermoregulation accuracy and efficiency are thus necessary for ensuring colony development in spring and cluster survival during winter. It involves various abilities, among others temperature sensitivity and shivering thermogenesis. It is thus of first interest to assess thermoregulation abilities since its impairment is likely to bring about serious colony disturbances including the cluster death in winter.

Thermoregulation assays are achieved with infrared cameras. This tool was used for investigating synergistic action between a pyrethroid (deltamethrin) and azole fungicides (Vandame *et al.* 1998).[48] Such measurements were performed in other studies investigating the cluster regulation mechanisms (Stanbentheiner *et al.* 2003)[49] or brooding mechanisms (Bujok *et al.* 2002).[50]

Thermographic studies of individual bees are a matter for the first assessment tier. Such studies can be performed at the colony level as well; they then should be part of the higher tier assessment and achieved when effects are suspected based on the first tier tests.

5.5. Foraging behavior

Foraging behavior impairment can lead to colony decline (Desneux 2007)[4] since brood rearing is linked to harvesting. As a consequence, beekeepers will face important economic losses.

Foraging behavior is often assessed in semi-field or field tests (higher tier tests). Counting bees on flower patches is not sufficient: for being useful, the assessment needs to be based on an observation framework that lists signs of abnormal behaviors such as, for instance, motionless bees on flowers or abnormal cleaning behaviors (Giffard and Manet 2009).[51]

However, when the product is a systemic pesticide applied in soil or seed treatment, field and semi-field tests do not allow measuring the effect intensity related to particular concentrations. Such measurements can be achieved by using an artificial feeding device (Yang *et al.* 2008, Borlotti *et al.* 2003),[52,53] which also allows establishing repellent concentrations.

5.6. Other topics

A lot of bee behaviors are now well known but were never used in pesticide assessment tests to our knowledge. For instance the communicative behavior pattern of honeybees is highly complex. It involves dances (Dyer 2002)[54] as well as sounds (Kirchner 1993)[55] or vibrations signals (Schneider 2004)[56] and plays a crucial role in the colony dynamic as a super-organism. For instance, colony survival and development implies that bees choose the most profitable nectar source; therefore, the recruitment dance behavior is of major importance (Seeley *et al.* 1991).[57] It also implies that the colony regulates its activity level to the resource abundance, a regulation bees achieve with the shaking signal (Seeley *et al.* 1998).[58]

Fundamental scientific articles provide many methods for investigating such behaviors but methods for assessing them quantitatively or qualitatively often do not exist for the moment.

6. Conclusions

Assessing sublethal effects of plant protection substances and products is a major issue of pesticide regulation. Following (EC)1107/2009 Regulation, a pesticide can be put on the market if *it may be expected that it shall have no unacceptable effects on the environment, having particular regard to (…) its impact to non-target species* (including honey bees). Development of new plant protection substances such as pesticide coated seeds has led to a new exposure mode of honey bees to pesticides: substances are less concentrated on the plants but are present in all plant organs including pollen and nectar or in exudation water droplets in small amounts that can be brought back to the hive and induce sub-lethal intoxication of bees at all stages (from larva to foragers) and castes (worker, drone or queen). Sub-lethal effects on individual bees can lead to unacceptable effects at the colony level, including colony death. For this reason, the assessment at the first tier of acute toxicity only is no longer sufficient for substances that are likely to contaminate pollen, nectar and water consumed by bees and thus to poison them by chronic exposure day after day.

Methods for measuring physiological or behavioral parameters provide thus important tools not only for higher tiers of the assessment scheme, but for the first tier as well, since a protective approach of honey bee toxicology should take sub-lethal effects into account from the beginning of the assessment. We hope this mini-review can help to choose and develop methods assessing representative parameters of the honey bee health status for a better protection of our colonies.

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Towards the comparative ecotoxicology of bees: the response-response relationship

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Abstract

Background: When an ecological system is exposed to an anthropogenic toxin, each species has an idiosyncratic sensitivity, but it is reasonable to expect some generality in response, especially among related species such as bees. If two species are similarly sensitive to a toxin their dose-response relationships will be similar. We propose a method to facilitate comparison between dose-response relationships, namely the response-response relationship, which can be applied to any biomarkers whose responses to the same pollutant are measured across a similar range of doses. We apply the method to bumble bees (*Bombus terrestris*) and honey bees (*Apis mellifera*) exposed to a dietary pesticide, imidacloprid, and we investigate both lethal and sublethal biomarkers.

Results: We found cross-species similarity in dose-dependent responses, but only in certain sublethal biomarkers. In honey bees, sublethal biomarkers were more sensitive than mortality. In bumble bees, fecundity was the most sensitive biomarker.

Conclusion: Our results provisionally suggest the existence of cross-species generalities. The greater sensitivity of sublethal biomarkers than mortality suggests that testing protocols which are overly focussed on mortality may underestimate the ecological impacts of toxic pollutants.

Keywords: *Apis mellifera*, *Bombus*, dose-response, imidacloprid, neonicotinoid

1. Introduction

Ecological systems are often richly complex, but scientists are constrained logistically to study only a few of their facets. When an ecological system is exposed to an anthropogenic toxin, each species has an idiosyncratic sensitivity to the pollutant. However, it is reasonable to expect that some generalities about the impacts of pollutants can be made and transferred from those organisms that have been studied to those that have not[1]. In the case of bees, for example, it will be valuable to know whether the impacts of pollutants on non-*Apis* bees can be inferred from the results of studies on honey bees (*Apis mellifera* L.), which are the focal species of current toxicological regulatory testing[2]. Based on overall toxicity, a previous comparative study[3] indicated broad similarity in the sensitivity of honey bees, bumble bees and solitary bees to a wide range of pesticide pollutants. Here, we investigate the detailed dose-dependence of various biomarkers, including sublethal endpoints.

The sensitivity of a species to a toxicological stressor is evident in the dose-response relationship, or exposure-response relationship[4]. Conventionally, the dose-response relationship is a simple graph that relates the magnitude of the stressor (e.g. the dietary concentration or the ingested amount of the pollutant) to the organism's response, which is quantified by a change in a specified biomarker, or endpoint (e.g. a physiological or behavioural variable, reproductive success, or mortality). If two species are similarly sensitive to a toxin, their dose-response relationships will be similar. Consequently, the recognition of toxicological generalities relies in part on our ability to recognize similarities among dose-response relationships. Here, we propose a method to aid comparison.

It is possible to compare two dose-response relationships simply by overlaying the plots on the same pair of axes. This is feasible when we compare two biomarkers whose responses are measured across a similar range of doses of the same pollutant. However, a new plot based on the same data facilitates the rapid evaluation of the differences between the two relationships. The plot is a response-response relationship (see Methods) and its shape is diagnostic for the relative sensitivity of the two responding biomarkers.

We need not be limited to comparing the sensitivity of different species. It is also valuable to compare the sensitivity of different biomarkers in the same species. For example, mortality is typically the focal biomarker for toxicological studies in bees and its cardinal value is the LD_{50}, the dosage required to kill half of the exposed individuals. The LD_{50} is conventionally used to compare potency among pollutants. However, if we are interested in ecologically important impacts, then we should also inquire about a toxin's effects on reproduction, because population dynamics are influenced by birth rates as well as death rates[5]. It is therefore valuable to know whether fecundity is a more or less sensitive biomarker than mortality, because this determines whether the overall demographic toxicity of the pollutant is correctly indicated by its LD_{50}. We can establish the relative sensitivity of these demographically important variables from a suitably arranged response-response relationship.

2. Experimental methods

Consider two conventional dose-response relationships, *A* and *B*, that are curves over the same range of dosages of the same toxin. Each point on relationship *A* is denoted (D_A,R_A) and (D_B,R_B) denotes points on relationship *B*. Each point on the response-response relationship is given by (R_A,R_B) when $D_A = D_B$.

If the two dose-response relationships, *A* and *B*, exhibit identical levels of sensitivity, then $R_A = R_B$ for any $D_A = D_B$ and the response-response relationship will be the line of equivalence, i.e. $y = x$. If instead one biomarker is more sensitive, the response-response relationship will deviate away from the line of equivalence and towards the axis that denotes the response of the more sensitive biomarker.

2.1 Case study 1: performance in honey bees vs. feeding in bumble bees exposed to imidacloprid

We compare the sensitivity of honey bees and bumble bees to dietary residues of the neonicotinoid pesticide, imidacloprid. Imidacloprid is a widely-used neonicotinoid pesticide whose residues appear in the nectar and pollen of treated crops[6]. It disrupts the insect nervous system by acting on nicotinic acetylcholine receptors[7] and it causes both mortality and various sublethal effects[8].

The dose-response relationship for honey bees was obtained from a meta-analysis of experiments testing the effects of dietary imidacloprid on honey bees[9], which established a dose-response relationship whose biomarker was average performance across a variety of sublethal biomarkers, which included learning ability of individuals[10], flight activity at the hive[11], and brood production[12,13]. Honey bee performance was defined relative to the biomarker's magnitude in undosed bees and the consensus dose-response relationship was described by: *relative performance*(%) = 100*[1 – 0.06*exp*(0.478*ln*(*dose*))], where *dose* has units of µg imidacloprid L^{-1} feeder syrup[9].

The dose-response relationship in bumble bees was established in laboratory experiments on individually caged bumble bees (Cresswell, *unpublished*) that were fed *ad libitum* on syrup (50% inverted sucrose; Attracker, Koppert B.V., Berkel en Rodenrijs, NL) containing imidacloprid at a range of ten dosages. The bees (*Bombus terrestris* L.) were obtained as domesticated colonies from a commercial supplier (Natupol Beehive, Koppert B.V., Berkel en Rodenrijs, NL). Bees were maintained in a controlled environment room (temperature 25°C, 40% relative humidity, 12:12 hours of light:darkness). In order to quantify their intrinsic variation in feeding rate due to variation in size, bumble bees were maintained on a control diet of syrup for three days before dosing began. Once dosing began, each cage was provided with a syrup feeder containing either control syrup or a syrup with one of the following nine doses of imidacloprid in units of µg imidacloprid L^{-1}: 125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; 0.08. Imidacloprid was obtained as a solution in acetonitrile (Dr. Ehrenstorfer GmbH, Augsburg, Germany) and the acetonitrile was removed by evaporation with a vacuum dryer (ScanVac MaxiVac Beta, Labogene, Lynge, Denmark) and the imidacloprid was suspended in water before being mixed into feeder syrup.

2.2 Case study 2: lethal vs. sublethal biomarkers in honey bees exposed to imidacloprid

Both dose-response relationships were obtained from a meta-analysis of experiments testing the effects of dietary imidacloprid on honey bees[9]. The dose-response relationship for sublethal effects uses relative performance as the biomarker and it is as described in the first case study. The dose-response relationship for lethal effects[9] is given by: *mortality*(%) = 10.2 + [(75.3 – 10.2)/(1 + *exp*(0.567 (0.194 – *ln*(*dose*))))].

2.3 Case study 3: feeding vs. fecundity in worker bumble bees exposed to imidacloprid

Queenless microcolonies[14] of four or five worker bumble bees were established from queenright colonies of *B. terrestris*, (Natupol Beehive; Koppert B.V., Berkel en Rodenrijs, Netherlands) in softwood boxes (internal dimensions: 120 × 120 × 45 mm). Each microcolony fed on syrups prepared as described above (section 2.1) and at the same range of doses. Each microcolony contained a pollen ball that was prepared by grinding pollen pellets collected from honey bee hives (Werner Seip Bioprodukte, Butzbach, Germany) into a powder and mixing the mass with water to form dough. Brood production was quantified after 14 days of exposure to dosed syrups.

3. Results

The daily feeding rates of bumble bees and the sublethal performance of honey bees averaged across various biomarkers are equally sensitive to dietary imidacloprid up to 125 μg L^{-1} (Figure 1). In honey bees, sublethal performance biomarkers are more sensitive than mortality to dietary imidacloprid up to 125 μg L^{-1} (Fig 2). Performance is predicted to decrease by over 50% at a dosage of dietary imidaclopid equivalent to 0.1 LD_{50} (Fig 2). In bumble bees, the fecundity of adult workers in queenless microcolonies is more sensitive than the daily feeding rate of individuals to dietary imidacloprid up to 125 μg L^{-1} (Fig 3).

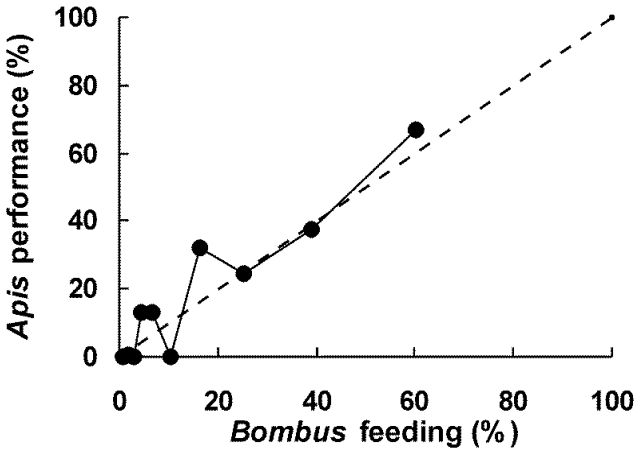

Fig. 1 Response-response relationship showing percentage reduction in the performance of honey bees (*Apis mellifera*) averaged across various sublethal biomarkers (*y*-axis) versus the percentage reduction in daily feeding rate of individual bumble bees (*Bombus terrestris*; *x*-axis) when exposed in laboratory trials to dietary imidacloprid in feeder syrups at nine doses in units of μg imidacloprid L^{-1}: 125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; 0.08. Points are interpolated for ease of inspection only.

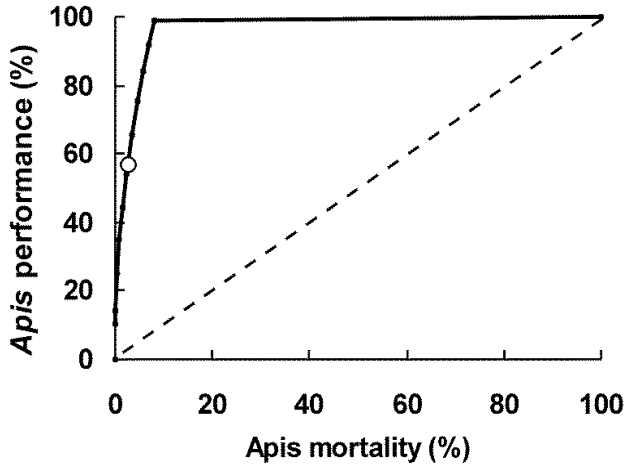

Fig. 2 Response-response relationship showing percentage reduction in the performance of honey bees (*Apis mellifera*) averaged across various sublethal biomarkers (*y*-axis) versus the percentage increase in mortality rate of honey bees averaged across various studies (*x*-axis) when individuals are exposed in laboratory trials to dietary imidacloprid in feed syrup. The abrupt inflection in the response-response relation occurs at a dosage of 350 µg imidacloprid L^{-1}. The open circular symbol indicates the point on the relationship that corresponds to $x = LD_{50}/10$, or 5% mortality.

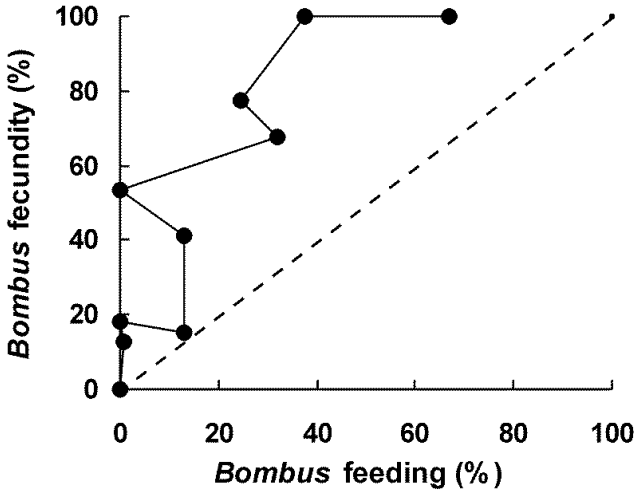

Fig. 3 Response-response relationship showing percentage reduction in the fecundity of worker bumble bees (*Bombus terrestris*) in microcolonies (*y*-axis) versus the percentage reduction in daily feeding rate of individual bumble bees (*x*-axis) when exposed in laboratory trials to dietary imidacloprid in feeder syrups at nine doses in units of µg imidacloprid L^{-1}: 125.00; 50.00; 20.00; 8.00; 3.20; 1.28; 0.51; 0.20; 0.08. Points are interpolated for ease of inspection only.

4. Discussion and conclusions

The proposition that we can find cross-species generality in toxicological sensitivity in bees receives some support from the similarity in dose-dependent responses to dietary imidacloprid between the feeding rate of bumble bees and sublethal performance biomarkers in honey bees. Arguably, this similarity emerges because the overall health of an individual is equally affected by dietary imidacloprid in both species. An individual's feeding rate probably reflects its levels of metabolic and locomotory activity. In honey bees, the sublethal performance biomarkers, such as colony activity and individual learning performance, are similarly indicative of overall health. This apparent similarity in sensitivity between honey bees and bumble bees contrasts with the substantive disparity between their LD50s for imidacloprid. However, we show below that biomarkers can differ substantively in sensitivity even within species, and this is so particularly when comparing sublethal markers and mortality. We therefore argue that the similarity between honey bees and bumble bees in sensitivity in biomarkers of overall health begins to suggest the existence of a generality, but this conclusion is highly provisional and it must be properly established by more comparisons across species and genera.

In honey bees, the greater sensitivity of sublethal biomarkers than mortality potentially has implications for the security of the conventional testing protocols used by regulators in pesticide approval, which typically focus on mortality. In Europe, regulators may give attention to the toxicity-exposure ratio, TER[15], which is calculated as: TER = LD50 / exposure. A compound is declared sufficiently non-toxic to bees if its TER $\geq$ 10, which means that, in theory, a compound could be approved if the exposure of bees is one tenth of the LD_{50}. Our findings (Fig 2) suggest that it is theoretically possible that substantive sublethal effects could emerge at an exposure of 0.1 LD_{50}. However, we note that we have demonstrated this only for an insecticidal compound, imidacloprid, whose toxicity necessarily yields TER <10. It will be valuable to investigate whether any compound that just satisfies the initial screening criterion, i.e. TER = 10, has biologically significant sublethal effects at the upper threshold exposure of 0.1 LD_{50}.

For bumble bees, fecundity was the most sensitive biomarker among those investigated here, whereas mortality was the least, if we judge by the relative magnitude of the oral LD_{50} for imidacloprid[16], which is about twenty five times greater in bumble bees than in honey bees (approximately 200 $\mu g\ kg^{-1}$ *vs*. 8 $\mu g\ kg^{-1}$). The high degree of sensitivity of bumble bee fecundity to dietary imidacloprid occurs despite the relative insensitivity of mortality as a dose-dependent biomarker, which suggests that the magnitude of the LD_{50} can mislead about the potential of a compound to have an ecological impact through demographic toxicity. A pollutant's demographic toxicity describes its impact on a target organism's population dynamics[17], which is determined by effects on both birth rates and death rates. If fecundity is more sensitive than mortality, the LD_{50} underestimates demographic toxicity.

The biological basis of the differential sensitivity of fecundity and mortality in bumble bees is largely obscure. Imidacloprid, the compound considered here, is neurotoxic to bees. It is understood that the bee is a highly integrated physiological unit with many processes under nervous control[18], but the pronounced sensitivity of fecundity relative to feeding rate, for example, is nevertheless not readily explained. We hope that future research into the mechanistic basis of these toxicological effects will pay dividends both by increasing our fundamental understanding and also by improving the prospects for the development of pesticides with low impacts on bees.

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Neonicotinoids and bees: an overview on concentrations, side effects and risk assessment

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Abstract

The concern about bee mortalities, honey bee colony losses and CCD includes pesticides as potentially contributing factor. Especially systemic insecticides, in particular the neonicotinoids and fipronil, are pointed at. We give an overview of the literature about neonicotinoids and bees from the introduction of imidacloprid in 1991 to date.

The systemic nature, together with their relative specificity, determine the ways of use (spray, soil application, seed coating) as well as the potential routes by which honey bees may be exposed: directly (topically through sprays, dust), and orally through residues in surface water, guttation water, pollen and nectar, as well as honey dew.

Residues of neonicotinoids as well as their metabolites have been found in the above mentioned matrices, generally in the low µg/kg range but for guttation water in the mg/L range. Residues have also been found in bees, in bees wax, honey and bee bread (pollen stores) in the hive. The data available in the open, peer-reviewed literature is limited, especially for nectar, and confined to only few plant (crop) species.

The toxicity for bees has been assessed in many laboratory and (semi-) field tests: acute and chronic lethal concentrations as well as sub-lethal effect concentrations for effects on behaviour, reproduction, disease resistance and overwintering. Calculation of 'worst case' exposures based on residues found in pollen and nectar and the probable food consumption point to serious risks in some cases.

(Semi-) field tests using field-realistic concentrations did not reveal measurable lethal and sub-lethal effects on bees and bee colonies. Similarly no conclusive evidence for the involvement of neonicotinoids in colony mortality was obtained in bee monitoring studies, despite the found residues.

The proposed risk assessment scheme of Alix et al. (2009, JK Archive Ch. 10) is adequate and applicable for the risk assessment of neonicotinoid insecticides. Nevertheless, the lack of data on residues in nectar and pollen should be covered by future research (or by publication of already carried-out field experiments). Field experiments should be done with field-realistic concentrations, at a reliable scale (number of colonies, power analyses) and duration (including wintering and spring development).

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Effects of neonicotinoid dust from maize seed-dressing on honeybees

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Abstract

In the last years bee and colony losses have been reported in numerous countries worldwide and many factors were taken into account to explain these phenomena. However, time-space differentiation of bee mortality factors needs to be considered. In Northern Italy since 2000 to 2008, the spring bee mortality was clearly linked to the maize seed dressing. In fact, it was shown that pesticides may be dispersed from the pneumatic drilling machine during sowing and bees may enter in contact with these contaminated dusts in several ways: by direct contact (when bees fly through the toxic cloud in the sown field), by indirect contact (when bees walk on contaminated leaves of the vegetation surrounding the sown field) or by ingestion (when bees collect nectar or dew from the vegetation contaminated with the dispersed dusts).

The pesticides used for maize seed dressing are extremely toxic for bees with lethal and sublethal effects depending on the level of exposure. In Italy, the high bee mortality during the sowing of coated seeds resulted in the suspension of use of the active ingredients imidacloprid, clothianidin, thiamethoxam and fipronil for seed coating (Ministerial Decree 17/09/2008). At the same time a research project "APENET monitoring and research in apiculture" was financed in order to establish the causes (external and internal to the hive) of bee mortality and the possible ways of mitigation. In our studies we investigated the effects of clothianidin derived from corn seed dressing on honey bees in laboratory (indirect contact) and semi-field conditions (ingestion, direct and indirect contact) in order to evaluate possible effects at individual and at colony level. In laboratory test, the effects of the dust emitted by the clothianidin-based product Poncho®, were compared to those of a liquid formulation of the same active substance (trade name: Dantop®). To do so, forager bees (10 bees per cage) were allowed to walk for 3 h on treated apple leaves, placed on the bottom of plastic cages. The tested quantities of active ingredient corresponded to the amount deposited on the ground during sowing at 5 m distance from the edge of the field . Talc was used as a dispersing agent for the dust of Poncho formulation. In the control, leaves were treated with talc only.

Our results showed that, up to the 24th hour, mortality induced by the two products was comparable, with both products proving to be 'slightly toxic'. During the subsequent hours, instead, the number of dead bees increased more in the Poncho dust treatment than in the Dantop spray treatment. In the semi-field test, the flowering oilseed rape was contaminated with the same concentration of a.i. as the one applied in the laboratory. The effects of the clothianidin-dust treatment in comparison to the control (plant treated with talc only) were evaluated by introducing bee nuclei into tunnels cultivated with oilseed rape (1 nucleus per tunnel of 40 m^2). A total of 6 tunnels were used, three for each treatment. In each tunnel we assessed the following parameters one week before and two weeks after treatment: bee mortality, colony strength, flight activity, foraging behavior, socio-physiological parameters linked to the colony vitality (temperature and humidity inside the hive, capacity in the construction of a honeycomb).

During the first two days after dust application bee mortality was significantly higher in treated than in control tunnels, while at a colony level no effects were observed, even eight months after treatment. Our results showed that contaminated dusts dispersed during sowing operations have negative effects at individual level, but no effects seem to exist at the colony level in the our experimental conditions.

Risk mitigation measures for seed treatments using neonicotinoids

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Abstract

Background: In 2008 the poisoning of about 12000 bee colonies was reported in Germany. These poisonings were caused by the drift of dust particles containing the insecticidal substance clothianidin following the seeding of maize, treated with the insecticide Poncho Pro. Dust abrasion from coated seeds occurred because of inadequate seed dressing quality, resulting in high quantities of dust emitted into the environment. In order to cover this specific risk, Regulation (EC) No. 1107/2009 provides for special arrangements for the placing on the market of treated seeds. In addition, the Commission Directive 2010/21/EU lays down specific provisions relating to certain neonicotinoids and fipronil for seed coating and seeding.

Results: According to these provisions the German authorities applied risk mitigation measures in the form of specific labels for certain products and seed bags of treated seeds.

Conclusions: Dust within seed bags and drift from seeding actions is a common phenomenon for a number of crops. However, the quantity within the seed bags and the emission of dust can be reduced significantly by technical means (e.g. treatment recipe, facility equipment, deflector technique) and by additional mitigation measures (e.g. max. wind speed). These can be established within the authorization procedure by the Member States.

Keywords: honeybee, poisoning, seed treatment, dust, guttation, risk mitigation

1. Introduction

Over the last decade, honeybee poisonings were reported with a close correlation of spring mortality of bees and the sowing of maize seeds dressed with neonicotinoids, e.g. from Austria, Germany, Italy, Slovenia. In 2008 severe poisonings occurred in Germany, with approx. 12000 colonies being affected. These were attributable to high quantities of contaminated dusts from maize seeds, emitted onto flowering plants (e.g. OSR, fruits, weeds) esp. by vacuum-pneumatic seeders.[1,2,3,4,5] The findings of the Julius Kühn-Institut (JKI) (Heimbach U and Stähler M, 2011, unpublished) indicated that seed bags of different crops may contain significant total quantities of contaminated dust. These data indicate that total quantities of dust within the seed bags as well as the emission of dusts need to be regulated. Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market provides special regulations for the placing on the market of treated seeds in Article 49:

4. Member States shall not prohibit placing on the market and use of seeds treated with plant protection products authorised (…) in at least one member state.
5. Where there are substantial concerns that treated seeds (…) are likely to constitute a serious risk to human or animal health or to the environment and that such risk cannot be contained satisfactorily by means of measures taken by the Member State(s) concerned, measures to restrict or prohibit the use and/or sale of such treated seeds shall be taken immediately (…).
6. (…).
7. (…) the label and documents accompanying the treated seeds shall include the name of the plant protection product with which the seeds were treated, the name(s) of the active substance(s) in that product, standard phrases for safety precautions as provided for in Directive 1999/45/EC and risk mitigation measures set out in the authorisation for that PPP (…)[6].

In 2008 authorizations of neonicotinoids for treatment auf maize seeds were suspended by the German Federal Office of Consumer Protection and Food Safety[7], the suspensions still being in force.

To meet the political requirement of a free market for treated seeds within the EU, a harmonized approach for risk assessment and risk reduction measures is a condition precedent. Due to the honeybee poisonings attributable to the sowing of neonicotinoid treated maize seeds reported over the last decade, the Commission Directive 2010/21/EU laid down the following general recommendations for mitigating risk arising from the emission of dusts:

8. 1 The seed coating shall only be performed in professional seed treatment facilities. Those facilities must apply the best available techniques in order to ensure that the release of dust (…) can be minimised.
9. 2 Adequate seed drilling equipment shall be used to ensure a high degree of incorporation in soil, minimisation of spillage and minimisation of dust emission.
10. 3 The label of the treated seed includes the indication that the seeds were treated with the specific active and sets out the risk mitigation measures provided for in the authorisation.
11. 4 The conditions of the authorisation, (…), include, where appropriate, risk mitigation measures to protect honeybees. [8]

2. Results

According to the provisions established by the European Commission the German authorities decided to apply the following risk mitigation measures (RMM) via labelling of neonicotinoid products (PPP) or seed bags of treated seeds:

2.1 RMM on the PPP for the application in professional facilities

The following labelling is issued as part of the authorization procedure:

- The seed treatment shall only be performed in professional seed treatment facilities, which are registered in the index of "Seed Treatment Facilities with Quality Assurance Systems to Minimise Dust" of the Julius Kühn-Institut (visit the homepage of the Julius Kühn-Institut <http://www.jki.bund.de)/>).

This restriction currently applies for all uses as seed treatments of neonicotinoids and will be extended to all crops and other substances toxic to honeybees and other non-target organisms if found necessary based on a risk assessment on a case by case basis. This is because the findings of the JKI indicated that seed bags of different crops may contain significant total quantities of contaminated dust (Heimbach U, 2011, Heimbach U et al., 2011).[9,10] Cereals contained more dust than maize, OSR or sugar-beet, if normalized for a field size of one hectare. The quantity of fine-grained dust in barley seed bags showed more than 300 times higher amounts than fine-grained dust in sugar-beet seed bags, if normalized for one hectare (Table 1).

Tab. 1 Amount of free dust from seed bags of several crops (Heimbach U and Stähler M, 2011, unpublished)

CROP/Year of treatment	Target drilling rate of seeds [a] (kg or No. ha^{-1})	Fine-grained dust [b]< 0.5 mm (g ha^{-1})	Coarse-grained dust [b]> 0.5 mm (g ha^{-1})	N
Cereals 2009				
- Barley	180	11.3 (31)	46.0 (116)	30
- Wheat	250	9.5 (28)	6.7 (19.2)	31
- Rye	150	5.1 (24)	6.6 (32.9)	23
Maize	100000			
- 2008		4.5 (25.6)	6.1 (47.3)	82
- 2009		1.99 (5.8)	3.5 (12.1)	45
OSR	700000			
- 2007		0.81 (4.72)	-	22
- 2008		0.27 (0.88)	-	24
Sugar-beet	100000	0.035 (0.125)	-	22
- 2008				

[a] Cereals given in kg seed rate ha^{-1}; [b] Amounts given in mean (max) g normalized for one ha

Furthermore, the findings of the JKI (Heimbach U et al., 2011, unpublished) showed that concentrations of the active substances may vary between treatment facilities, supposedly depending on the individual treatment procedures, recipes (esp. additives, stickers) and the implementation of effective dedusting equipment.

According to the JKI the resistance of treated seeds to abrasion can be considerably improved by implementing a quality assurance system (Heimbach U et al., 2011, unpublished) (Table 2).

Tab. 2 Resistance of treated maize seeds to abrasion using the Heubach-Dustmeter (Heimbach, U. et al., 2011, unpublished)

CROP/Year of treatment	Target drilling rate of seeds (No. ha^{-1})	Heubach-value[a] (g ha^{-1})	N
Maize			
2008	100000	1.11 (4.15)	53
2009		0.42 (0.91)	81
2010		0.33 (0.66)	43
2011		0.18 (0.4)	34

[a] Amounts given in mean (max) g ha^{-1} normalized for target drilling rates of 1 ha

In preparation for a quality improvement initiative of the German professional treatment facilities for maize, the resistance of the treated seeds to abrasion was significantly improved. While the seeds treated in the year 2008 showed mean normalized Heubach-values of 1.11 g ha^{-1}, the resistance to abrasion was improved to 0.18 g ha^{-1} in 2011. This optimization is also reflected in the maximum normalized Heubach-values for maize seeds that were reduced by about 90 % from 4.15 g ha^{-1} in 2008 to 0.4 g ha^{-1} in 2011.

Further investigations of the JKI using the Heubach-Dustmeter revealed that the resistance of treated seeds to abrasion can be regarded as a key factor for the amount of dust potentially being contained in the seed packages. Sugar-beet turned out to show the best resistance to abrasion, followed by OSR, maize and cereals. For maize seeds it was demonstrated that the overall emission of contaminated dusts can be reduced by about 90 % by improving the seed coating quality of seeds in terms of resistance to abrasion.[3] Therefore, in order to guarantee for a high technical standard of resistance of the seeds to abrasion and low amounts of dust in the seed bags, the use of neonicotinoids for seed treatment has been restricted to those facilities, which have adopted a quality control system (QS). This QS includes e.g. the training of staff members, the improvement of treatment recipes and procedures, the compliance with maximum permissible values for dust (e.g. Heubach-values: OSR < 0.5 g ha^{-1}; sugar beet < 0.25 g ha^{-1}), a technical check and where applicable a reconstruction of the technical equipment (incl. dedusting techniques, packaging, storage of pesticides and treated seeds, disposal of waste). Finally the compliance with the QS is inspected, verified and certified by an independent service. Only those facilities that have received recognition by the independent service are listed by the JKI. However, because a QS has not yet been established for the treatment of maize seeds, the suspension of neonicotinoid PPP as well as the prohibition of the sowing of maize seeds treated with neonicotinoids is still in force in Germany.

2.2 RMM on the seed package for the use of pneumatic seeding machines

The following label must be printed on the seed package:

- Treated seeds may only be sown by using a pneumatic seeding machine which operate with negative pressure, if this machine is registered in the "List of drift reducing sowing equipment" of the Julius Kühn-Institut (this can be seen on the Julius Kühn-Institut's website at <http://www.jki.bund.de/geraete)/>).

In order to reduce dust emission, the use of vacuum-pneumatic seeders for sowing of seeds treated with neonicotinoids is allowed only, if the emission of dust is reduced by a tested reconstruction of the vents, in order to guarantee for a high technical standard of reduction of dust drift. It has been

established for pneumatic (vacuum) maize seeding machines, that the emission of contaminated dusts can be reduced by about 90 % by reconstructing the vents (Rautmann D, 2011, unpublished) (Figure 1).

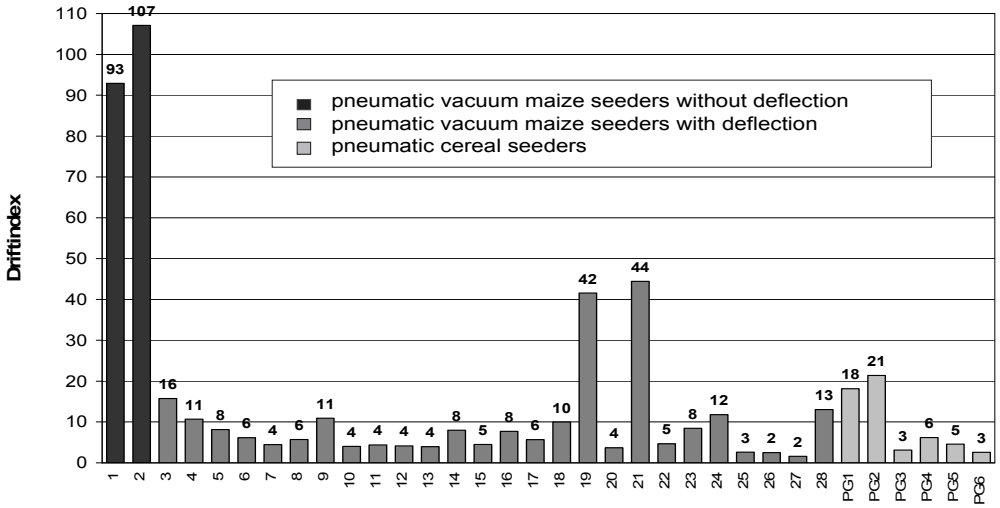

Fig. 1 The relative emission of dust by vacuum-pneumatic seeders for maize without (1, 2) and with deflection technique (3 to 28) and pneumatic cereal seeders (PG1-PG6) (Rautmann D, 2011, unpublished).

Only those seeders or deflector techniques that fulfill the requirements of 90 % reduction of emission compared to without deflector have received recognition by the JKI and are allowed to be used for seeding operations of seeds treated with certain insecticides.

2.3 RMM on the seed package to avoid dispersion of dusts and the spillage of treated seeds

To avoid the dispersion of dusts the following label must be printed on the seed package:

- Do not sow treated seeds at wind speeds of more than 5 m s^{-1}.

In order to minimise drift of dust particles, the sowing of seeds treated with neonicotinoids is allowed only, if the maximum wind speed does not exceed 5 m/s. This regulation is based on the findings of a literature study prepared at the University of Essen (Höke S and Burghardt W, 1997, unpublished). Obviously drift of soilborne particles of different nature into adjacent areas increased, if wind speed exceeded approx. 5 m s^{-1}. Furthermore the size and shape of particles affect the potential of drift with respect to distance and duration of sedimentation. However, further research and development activities should be initiated. Currently there is a lack of knowledge about the particular size distribution of dust particles from treated seeds and especially the transportation of particles smaller than approx. 70 microns. This fraction is subject to the mid and long distance transport and may contain particles of high pesticide concentration (Heimbach et al., 2011, unpublished).

To avoid the spillage of treated seeds the following label must be printed on the seed package:

- The treated seeds, including any dust they contain, or dust which is produced during the sowing process, has to be incorporated completely into the soil.

The spillage of treated seeds has been regularly reported in Germany and drift of dust from seeding actions is considered a common phenomenon for a number of crops. As honeybees collect water from different sources, e.g. puddles on or beside fields, they are likely to be exposed to contaminated water, creating a very high potential of risk, at least if neonicotinoids are concerned. In order to

reduce this risk, treated seeds and dust must be incorporated completely into the soil, when sowing seeds treated with neonicotinoids.

2.4 RMM on the seed package to protect honeybees

In order to make sure, that colonies are not located under unfavourable conditions, for instance directly adjacent to fields that were planted with treated seeds, beekeepers must be informed prior to the sowing of seeds treated with neonicotinoids.

The following label must be printed on the seed package:

- The farm manager is obligated to notify the area designated for the sowing of the treated seeds to beekeepers, whose bee hives are located within a radius of 60 m to the sowing area, at least 48 hours prior to sowing.

Bees usually find and collect water close to their colonies. Further to that, honeybees do not seem to prefer guttation water of treated plants but seem to use any other available water source near to their colony. However, current studies clearly showed that under certain conditions honeybees may forage on guttation drops of plants (Pistorius J et al., 2011, unpublished) near-by to their colony. Because concentrations of neonicotinoids in guttation drops of field crops may be very high for up to about 8 to 9 weeks (showing highest concentrations in maize of approx. 100 µl l^{-1}), these drops create a high potential of risk. Under certain situations it seems therefore advisable for beekeepers to place their colonies in a safe distance to the field or to provide appropriate water sources. In addition, the direct exposure of honeybee colonies to dusts from sowing can be omitted in order to further reduce the risk for honeybees.

3. Discussion and conclusions

In general, from the data available, it can be concluded that contaminated dust within the bags of treated seeds is commonly occurring and highly dependant on the type of crop and the treatment procedure. Usually, bags of cereals and maize contain higher quantities of dust compared to OSR and sugar beet. Dust particles once emitted by seeders deposit on soil and on plant surfaces. The drift of dust particles highly depends on the size and shape of particles, the type of seeder and surrounding conditions (e.g. wind speed, soil humidity). In fact the findings indicate that for seeding operations of some crops (e.g. cereals, maize and OSR), treated with compounds highly toxic for honeybees, best seed treatment techniques (i.e. reducing free dust within the seed bags as well as the abrasion of dusts) together with the best seeding techniques (i.e. reducing the dust emission e.g. by effective deflectors for vacuum-pneumatic seeders) need to be mandatory. For example, the total emission of dust occurring at maize seeding could be reduced by about 99 % compared to 2008 levels, if the treatment of seeds and the outlet air pipe of the seeders are improved.

So, in principle, the serious risks posed by some insecticides to honeybees may be contained satisfactorily by risk mitigation measures, as required in Article 49 of Regulation (EC) No. 1107/2009. However, in order to implement these legal conditions, Member States will need harmonized quality criteria for seed treatment and seeding technique, a harmonized approach to Risk Assessment as well as an agreed and open data base of relevant data for Risk Assessment and Risk Management. The appropriate risk mitigation measures as well as appropriate label phrases still need to be agreed. All aspects should be covered by the relevant Guidance Document which is currently being prepared lead-managed by the Netherlands.

Finally, further research and development activities should be considered, e.g. covering the occurrence, dispersal and toxicity of different fractions of dust from treated seeds.

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About the recent re-evaluation of neonicotinoids regarding bee risks in the Netherlands

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Abstract

Background: This paper describes the re-evaluation of the risk to bees of four insecticidal substances authorised in the Netherlands, performed in 2011 at the request of the Dutch government.

Results/conclusion: All products retained their authorisation but some label revisions were necessary. Several risk mitigation issues were highlighted during the evaluation process.

Keywords: neonicotinoids, bees, pesticides, risk assessment

Introduction

The decline of bees is a worrying phenomenon in many areas of the world. Bees are nowadays getting a lot of publicity in the Netherlands as well as in many other countries. In the media, an often heard cause for the decline is the use of pesticides, notably neonicotinoids. In early 2011, stimulated by public pressure, the Dutch Government decided that a re-evaluation of the risk of neonicotinoids to bees should be performed by the competent authority, the Board for the Authorisation of Plant Protection Products and Biocides.

Methods

This re-evaluation deviated from normal re-registration procedures in which individual products are considered according to fixed timelines prescribed legally. Instead, all products containing the active substances in question were assessed together. Furthermore, industry was requested to immediately submit all studies relevant for the risk to bees which had not been submitted yet in the normal application processes. This yielded a large amount of new information as many of the products were up for re-evaluation at short notice and a lot of new studies had been already prepared for this.

Four systemic insecticidal substances were selected for the re-evaluation: the neonicotinoids imidacloprid, thiamethoxam and clothianidin, and the pyrazole fipronil. Other neonicotinoids allowed on the Dutch market are less acutely toxic to honeybees and were not included in the project. The re-evaluation included both plant protection products (spray applications and seed treatments) and biocides and concerned a total of 55 products. The risk was assessed in accordance with the most recent guidance, the EPPO guidelines of 2010[1,2]. Exposure routes considered in the evaluation were: direct in- and off-field exposure, from spray drift but also from dust from treated seeds; indirect exposure from uptake in flowering organs of the crop itself, weeds and succeeding crops, via honeydew and guttation.

Next to the protected dossiers submitted by industry, public literature was also considered. A number of meetings with bee researchers, industry and the Food and Consumer Product Safety Authority were held during the evaluation process. The newly submitted studies, information from public literature and new options for risk mitigation were discussed in these meetings. Label mitigation measures were ensured to be enforceable and manageable in practice.

Results/discussion

Of the 55 products, none was eventually taken off the market. The label of 14 products was revised. It should be noted that many products already contained restrictions for risk mitigation for bees before the re-evaluation.

During the project, risk mitigation issues were identified which needed further consideration:

- The term 'flowering crop' needs to be defined for bee relevance to enable enforcement, both for one flower and for the percentage of flowers in a field.
- A list with crops attractive to bees needs to be determined and made easily available online.
- Risk mitigation for non-professional users may be used but should be formulated in a simple way.
- The risks to bees from succeeding crops and risks from re-sowing after crop failure for persistent substances were assessed in the Netherlands for the first time. In some cases, a minimum waiting period for bee-attractive succeeding crops may be necessary.
- It should be ensured that use restrictions are seen by the right person. Restrictions are mentioned on the product label, but for seed treatments they need to be taken to the seed bags; furthermore, for e.g. cabbages, seedlings are grown indoors and sold to a third party, so restrictions then may need to be taken to the buyer of the seedlings. If waiting periods are necessary, it should be considered that for some crops the user of the land may change from year to year.
- In the Netherlands there is a specific use as dipping treatment of flower bulbs. However, since the residue level in pollen and/or nectar in the flower and thus the exposure to bees after this type of treatment is unknown, it should be avoided that treated bulbs would flower.
- Spray or dust drift restrictions to protect bees in the off-field area may be necessary.

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Guttation and the risk for honey bee colonies (*Apis mellifera* L.): a worst case semi-field scenario in maize with special consideration of impact on bee brood and brood development

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Abstract

Background: The possible risk of guttation for bees was investigated in two semi-field studies with maize treated with clothianidin. In a worst-case scenario set-up the effects on adult and brood mortality of bees with special consideration of the brood development of the bees were assessed.

Results: Due to the weather conditions in the first experiment guttation occurred only once, which caused a high mortality and a brood-termination rate of up to 100 % in the worst-case scenario without additional water supply but no clear increase of mortality or brood termination was observed when water was supplied. In the second experiment guttation in maize occurred on 5 of 10 days. The mortality in treated variants with water supply and control variants with untreated seeds was on a similar level and within normal range. The brood-termination rate was in the control below 16 %, in the treatment from 16 to 43 %.

Conclusion: In the first experiment in the variant with treated maize and no additional water supply, an artificial and extreme situation a high impact on mortality and also on the brood development was observed, indicating the sensitivity of the test system but representing an unrealistic worst case scenario.

In variants with treated maize and additional water supply no clear effects on adult mortality and brood were observed in the first and the second experiment.

Keywords: honey bees, guttation, pesticides, clothianidin, seed treatments, brood development

Introduction

Like other organisms, a bee colony needs water to maintain its vital functions. Except availability of water in the beehive and excessive water from collected fresh nectar, the remaining water demand must be collected outside the beehive. Frequent water sources are dew, rain, streams, lakes and also guttation drops.

The use of guttation drops as water source may be a potential route of exposure for bees to systemic active substances used for seed treatment of different crops (1). Residues of clothianidin in guttation drops of small maize plants (treated with Poncho Pro®) may reach up to about 100 mg/l directly after emergence (2). The residue levels detected in guttation drops of maize may result in high mortality if consumed by bees (3). Exposure in the laboratory by adding sugar water to guttation water does not represent realistic exposure for water foraging bees and hive bees, to which these liquid may be passed on. Therefore in a semi-field study it was investigated whether guttation drops may be used as a water source and may pose a potential relevant route of exposure. In two experiments with clothanidin treated maize the effects on adult bees and bee brood with or without access to alternative, uncontaminated water sources were investigated.

Experimental methods

Two succeeding experiments were performed under semi-field conditions in Lucklum (Braunschweig, Germany), in seed treated (a.s. clothianidin, Poncho Pro®, 0.5 mg/kernel) and untreated maize crops. Four tents (96 m^2, 16 x 6 m) were set up on the treated, 2 tents on the control plot, covered with a gauze permeable for wind and rain but impenetrable for bees. The study was repeated twice, in the

first experiment (BBCH 13-15) with two treatment variants, one with and one without artificial water source containing uncontaminated tap water and one control for each variant. In the second experiment (BBCH 15-19) at the same field location, all 4 colonies in the treatment and 2 in control had an additional uncontaminated water source.

Bee colonies used were of similar size with approximately 10.000 bees, (one-room, 'Zander'), had an oviparous one year old queen bee and contained sufficient honey and pollen stores in the hives. The bees were allowed to forage on additional sugar feeding paste and pollen sources provided *ad libitum* in the tents.

The mortality of bees was assessed daily in dead bee traps and on linen sheets in the crop. The flight activity and behaviour of bees at the hive entrance and in three flight squares were determined once daily. The observation period in the tents was 11 (1st run) and 10 (2nd run) days. An observation of brood development of 100 individual brood cells per hive was conducted for about four weeks following the protocol of Schur et al., 2003 (4), using a digital brood assessment system. The evaluation of the cells was done with a test version of digital image processing software (RIFCON GmbH). Over the whole study the occurrence of guttation was documented and guttation droplets were sampled daily for residue analyses. After the exposure period the bees were relocated for observation of further brood development and placed at the Julius Kühn-Institut near the city of Braunschweig with very few agriculture in the surrounding.

Results

In the first experiment the study was performed in six flight tents in maize (BBCH 13-15) with one bee hive per tent. Two treatment tents with and two without artificial water source containing uncontaminated tap water, one control for each variant in the untreated field side.

Due to the weather conditions in the first experiment guttation of maize occurred only once, after small amount of precipitation (<1mm) occurred in the night from the 27th to very early morning of the 28th of May. Guttation droplets were immediately used by the bees which had no access to any water outside the hive for 3 days. The use of guttation droplets as water source caused a high adult bee mortality of more than 1000 bees/hive and day (colony 1: 1900, colony 2: 160) in the waterless variant (Fig.1). The mortality was clearly increased for several days. In colonies set up in tents in treated maize, which had access to guttation droplets containing residues and to an uncontaminated, alternative water source no increase in mortality compared to the control was observed.

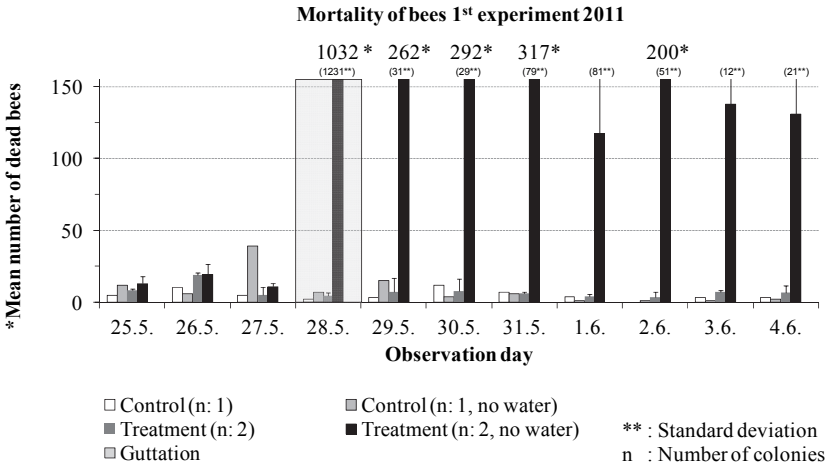

Fig. 1 Effects on adult mortality in the different variants of the 1st experiment. One treatment and control without an additional water source. Guttation took place only on the 28th.

It seems unlikely that all 1900 bees of colony 1 were actively collecting guttation droplets directly, thus it can be assumed that water was passed on rapidly also to other hive bees. The residues in sampled guttation droplets were between 0.010 and 0.018 mg/l clothianidin in the control and 7.953 to 46.55 mg/l clothianidin in treated variants. The residues detected in dead bees were between 0.021 to 0.079 mg/kg in variants without water source and below limit of detection in the control. Colony 2 had a lower but still clearly treatment related highly increased mortality. The difference between the two colonies may be assigned due to individual water use and water need of the colonies. In dead bees, high residues even higher than the oral LD_{50} were detected (Table 1).

Tab. 1 Residues detected in dead bees in the first experiment in control and treated variants without artificial water source

		Residues of clothianidin	
Date	**Variation**	**(mg/kg)**	**ng/bee**
28.05.2011	Treatment hive 1, no water	0.037	3.79
	Treatment hive 2, no water	0.079	7.80
29.05.2011	Treatment hive 1, no water	0.022	2.37
	Treatment hive 2, no water	0.021	2.02
29/30.05.2011	Control hive 1+2	Not detected	Not detected

The condition of the colonies and the amount of adult bees, eggs, young larvae, capped brood, nectar and pollen was assessed one day before set up in the tents and a second time after the exposure period, 16 days after first colony assessment. At the start of the study the colonies showed an equal distribution of the different brood stages and the total covered area was also on a similar level in the six colonies. At second colony assessment in the first experiment the area of capped brood cells of the waterless variant (treatment) was decreased compared to the other variants.

As the brood termination rate ranged up to 100 % in the waterless variant and also in both control variants of the first experiment, the results are of limited value. The confined semi-field conditions may have caused stress for the colonies, which might have been the reason for a high or total termination rate. Nevertheless, it can neither be concluded nor be fully excluded that some treatment related brood mortality occurred in this trial.

The second experiment of the study was performed at later BBCH (15-19) development of maize with four treatment and two control tents (in the untreated field side), all with an additional artificial water source containing uncontaminated tap water. Bee colonies were of similar size (adult bees and bee brood) like in the first run.

In the second experiment guttation of the maize occurred on 5 of 10 days. The mortality in treated and control variants was on 3 days with guttation at a similar level and within normal range and on 2 days slightly increased in the treatment (Fig. 2). The residues of guttation droplets sampled in tents were between 0.006 to 0.008 mg/l clothianidin in the control and 0.205 to 1.710 mg/l clothianidin in treatment.

In second experiment the condition of the colonies and the amount of adult bees, eggs, young larvae, capped brood, nectar and pollen was assessed two days before set up in the tents, and a second time after the exposure period, 11 days after first colony assessment. The colony strength in the control and treated variants were at a similar level and in a normal range.

In all variants of the second run, most marked eggs reached the expected stages up to the last BFD and hatched successfully (Fig.3). A termination rate under 30% is considered to be in the normal range in confined semi-field conditions. In one of the 4 treated variants a moderate increase of the termination rate was observed between BFD +5 and BFD +11 (29th of June).

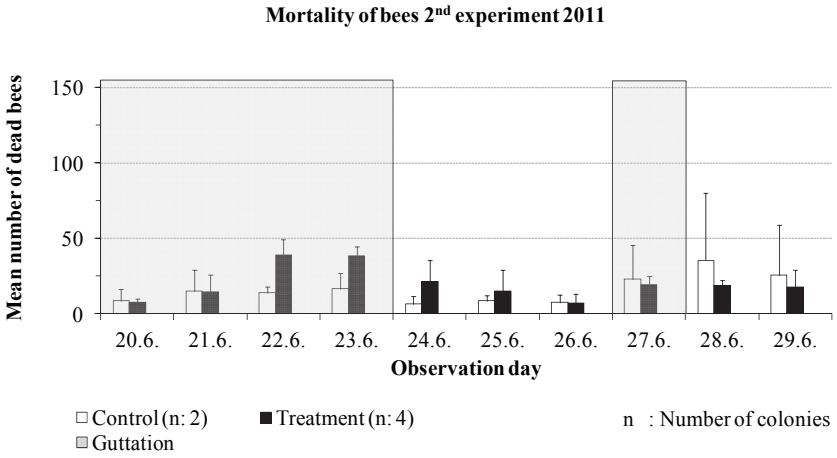

Fig. 2 Adult mortality in the treatment and control variants of the 2nd experiment. All variants with an additional uncontaminated water source. Guttation was present from 20th to 23rd and on the 27th of June.

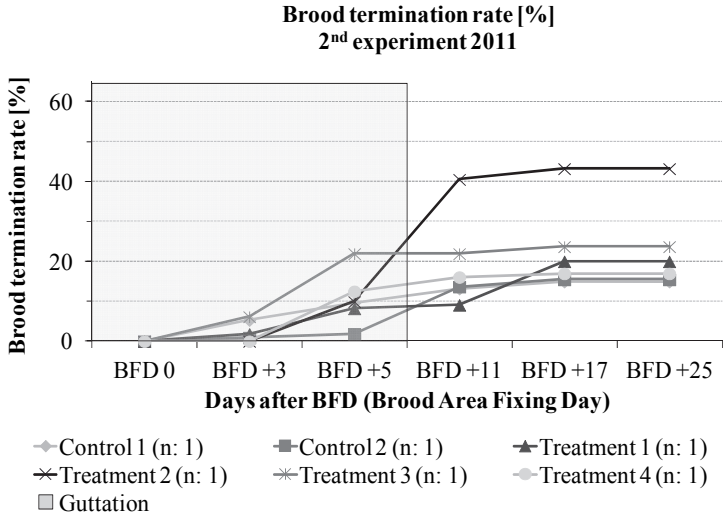

Fig. 3 The brood assessment of the 2nd experiment reflected the termination rate between BFD 0 at the beginning and BFD +25 at the end of the trial. Guttation was present from 20th to 23rd (BFD+5) and on the 27th of June.

Conclusions

In the first experiment, bees suffered lack of water in the artificial extreme situation without any additional water supplies for three days before first occurrence of guttation. As guttation droplets, which contained high residues of the active substance used as seed treatment, first occurred they were immediately used by bees as a water source. A high impact on adult mortality was observed especially in one of the two colonies of this treatment group, which can be clearly linked to the uptake of guttation water from maize plants. It can also be concluded that some water foraging bees collected guttation drops and managed to pass these on to other hive bees and potentially also to

bee brood. A very high termination rate was observed in the two waterless variants by the assessment of 100 individual brood cells per hive. But the reason for this keeps unclear, because of a high termination rate also in the control variants. In the colony assessment of the first experiment the area of capped brood cells of the waterless variant (treatment) was decreased compared to the other variants.

The first experiment demonstrated the sensitivity of the test system but represents a unrealistic worst case scenario. In variants with treated maize and additional water supply, no effects on adult mortality and brood mortality and brood development were observed. In the second experiment, no such high increase of mortality was observed as all variants had an additional water source. A moderate increase of brood termination rate was observed in one out of four colonies. The potential exposure to clothianidin (residue in guttation) was clearly lower in the second experiment, though still being present at toxic levels which would result in lethal effects on adults after consumption of only a few microlitres of guttation fluid per bee.

To assess the potential risk for bee colonies as used by beekeepers, further field studies should be conducted to investigate if an increase of mortality and effects on bee brood development may occur in realistic field conditions caused by systemic and bee toxic substances in guttation.

Acknowledgements

RIFCON GmbH for supplying a test version of digital brood evaluation software.

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Guttation and risk for honey bee colonies (*Apis mellifera* L.): Use of guttation drops by honey bees after migration of colonies - a field study

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Abstract

Background: The aim of this experiment was to investigate whether honeybees from colonies that are not familiar with their surrounding landscape, due to short-distance migration to a new location, are more at risk by guttation drops from seed-treated plants than bee colonies which are already familiar with alternative water sources in the surrounding of their apiary.

Results: The mean mortality of bees, which occurred after moving beehives to a new location, increased only slightly from 6 bees/day (-1 day before moving) up to a maximum of 21 bees/day (1 day after moving). No significant differences in the mean number of dead bees between bee colonies that were familiar with all sites of water sources in the surrounding area and bee colonies that were only recently moved to the field were observed.

Conclusion: There was no indication that honey bee colonies which were not familiar with the surrounding landscape are more at risk by guttation drops from seed-treated plants than bee colonies which are already familiar with the alternative water sources in their surrounding landscape.

Keywords: guttation, seed treatments, transport beehives, pesticides, honey bee, water foragers

Introduction

Ensuring a good nectar flow is essential for good honey yields and successful beekeeping. However, sometimes flowering crops providing nectar are out of reach for the bees. Therefore, the migration of beehives to flowering crops is a well established procedure in beekeeping[1]. Short-distance transport for example to widely grown crops like oilseed rape mainly occurs to enhance the honey or pollen yield of the bee hive, but also long-distance transports for example to obtain special types of honey or for pollination services are possible. Regardless of the travelled distance, relocated bee colonies are facing similar problems, they are not familiar with their new surrounding and therefore have to reorient themselves[2]. In addition, due to the transport, the food supply to the beehive which consists of nectar, pollen and water is interrupted. However, contrary to nectar and pollen, water is not stored in the bee hive and therefore has to be actively collected by the bees whenever needed[3]. During the time of transport bees have no access to any water sources from outside. Therefore, there may be a shortage of water in the beehive after the transport. Depending on the new location, various water sources may be available for the bee colonies. These sources can be permanent ones such as lakes or not permanent available water sources like dew, rain or guttation drops. However, little is known about the water collecting behaviour of honeybees particularly after a transport to new locations. Due to energetic reasons it is assumed that honeybees usually collect water near and around their hive[4, 5]. This could particularly apply to bees which are not familiar with their surrounding landscape. In addition it can be assumed that after the migration of bee hives the bees use the easiest accessible water source in their proximity and if these colonies are placed next to seed treated crops, they may be exposed to residues in guttation droplets.

Since 2009[6, 7], it has been subject of discussion whether guttation drops of crops grown from seeds treated with systemic insecticides may constitute a relevant route of exposure. To address this question it is necessary to gain more information about the water-collecting behaviour of bees. Therefore, the present study was focused on the water collecting behaviour of honey bee colonies in the first days after transport to a new location. However, the hypotheses was that bees which are not

familiar with their surrounding landscape after short-distance transportation, are more at risk by toxic guttation drops from seed-treated plants than honey bees which are already familiar with every site of water sources in their surroundings.

Experimental methods

The experiment was conducted from the 18th of May until 28th of June 2011. Overall, four locations in the surrounding of Brunswick (Lower Saxony/Germany) were used for placing colonies. The main experimental field was located in Lucklum and consisted of one plot planted with seed-treated maize (a.s. clothianidin, Poncho Pro®, 1.25 mg/kernel) and one plot with untreated maize. The environment of the remaining locations was various (grassland, forest etc.). All these sites were at least 6 km away from the experimental field. The essential part of the trial – placing of bees unfamiliar with the surrounding and potential exposure to guttation droplets containing insecticidal residues - took place on the maize field. It started on a day with guttation events in both plots (16th June), approximately 4 weeks after emergence of the plants when high residues are expected in guttation droplets of seed treated plants[8]. During this period the climatic conditions (relative air humidity, air and soil temperature) and the presence of guttation or dew drops were recorded. In addition, on several days guttation drops were collected for subsequent chemical analysis including all seed treatment ingredients.

In total, 18 identical bee hives (approximately 10.000 bees/hive) were used with three bee hives for each of three variants at each of the two different plots of the experimental field. The bee hives in the first and second variant (V1, V2) were moved to the field border of the two experimental fields before starting of the main experiment and had the chance to get familiar with the field and its surrounding landscape (including all sites of water sources). The first variant was located permanently in the experimental field. The second variant was set up for six days in the experimental field and then moved to the three various locations in the surrounding of Brunswick, at least 6 km far from the experimental field. They were moved back to the experimental field at the beginning of the main study (absence from experimental fields < 9 days). The third variant (V3) was located at the same three various locations as the second variant at the start of the whole experiment and moved for the first time in the experimental field at the start of the main experiment. The beehives were moved to new locations to get more information on the normal bee mortality occurring after transport. To guarantee a period of less than 9 days of absence of the bees of V2 from the experimental fields these bees were placed for another period of 6 days at the experimental field and moved away 8 days before the main study started. This was necessary because guttation occurred only rarely in 2011. In the main study all bee colonies were placed directly at the field border with the hive entrance pointing towards the maize crop. The population development of the beehives was assessed three times by using the Liebefelder method[9] and the bee mortality was observed daily using modified Gary beetraps[10]. Starting a few days before the main experiment of this study two semi-field studies were carried out in the same experimental field (Frommberger et al.[11]) on guttation and the risk for honey bees.

Results

The mean mortality of bees, which occurs by moving beehives to nearby locations only slightly increased from 6 bees/day one day before moving up to a maximum of 21 bees/day one day after moving (Fig.1).

During the main study guttation occurs only five times before and six times during the main study. As expected, especially in the early development stages of maize high residues were found (Fig. 2). In addition, also in the untreated maize plot low residues of active ingredients were found in guttation drops.

In the main study no significant differences in the mean number of dead bees between bee colonies that were familiar with all sites of water sources in the surrounding or bee colonies that were recently moved to the field were observed (Fig. 3).

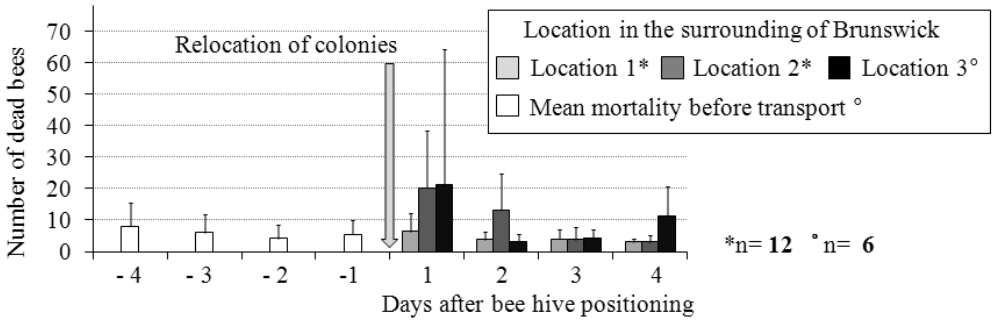

Fig. 1 Bee mortality four days before and after moving beehive to a new location.

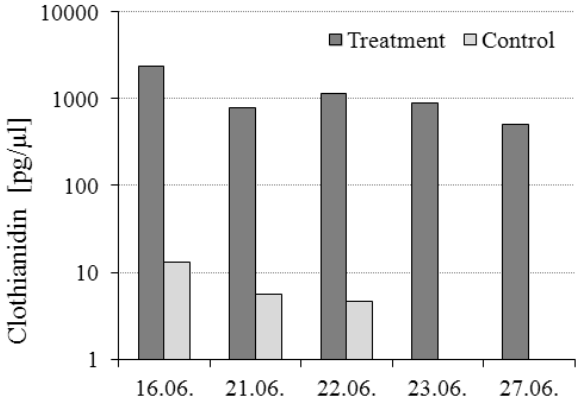

Fig. 2 Range of residues in the guttation drops of treated and untreated maize (BBCH 15-19).

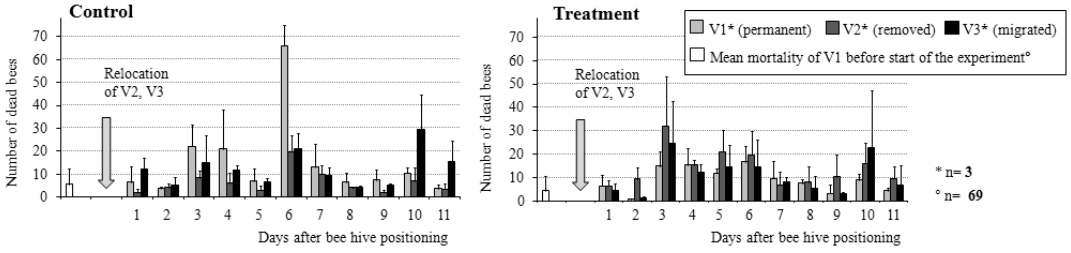

Fig. 3 Bee mortality after moving hives to clothianidin treated and untreated maize (Guttation took place on day 0, 4, 5, 6, 7 and 11)

In addition no adverse effects on the development of all bee colonies were detected.

Conclusions

The hypotheses was that bees not familiar with their surrounding landscape after short-distance transport may be more at risk to collect toxic guttation drops from seed-treated plants than honey bees which are already familiar with every site of water sources in their surroundings. Although several bees were seen scanning the leaf surface of maize plants, high residues were present in the guttation droplets no adverse effects on the development of the beehives or the bee mortality were observed. Frommberger et al.[11] showed at the same time in worst case semi-field experiments (on the same field as the field experiment reported here) without additional water supply in the tents a high impact on adult mortality and also on the brood development which was not detected any more if water was provided within the tents. Also in this study reported here, no effects on adult mortality were detected in the realistic field exposure scenario of this experiment.

Acknowledgements

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Thiamethoxam in the cultivation of hop – does it pose a threat to honey bees?

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Abstract

One serious problem in the growing of hop is the feeding damage caused by different soil insects (e.g. *Curculionidae, Alticinae*) during springtime. In 2010 the grower of hop in the Hallertau, the largest hop growing area in Europe, tested a new agent, thiamethoxam (Actara®), that belongs to the group of neonicotinoids. The application process in hop is a drench application with 200 ml solution (50 g a. i. / ha) around the growing plant.

To find out if there is any exposure of the bees to this agent, various investigations were undertaken. 24 Beehives were set up in groups of 8 at three different places with different distances to the hop fields. From April to July, twice a week homing bees were caught at the hive entrance in the early morning and were deep frozen. Dead bees were collected from dead bee traps three times weekly and also the population development and the honey production were measured. In the hop garden the occurrence of guttation of the hop was observed in regular intervals. Guttation of the grass and the plants in between hop rows was collected. Additionally, further samples of the soil, plants and puddles were taken. From the intercepted bees the honey sac was dissected and prepared for further examinations. Also the pollen loads were analysed for residues.

The used agent and the known metabolite clothianidin were detectable (LOQ 0,001 mg/kg) neither in the pollen loads from single bees (n=26), nor in the honey sacs (n=2000), nor in bee bread samples (n=9) nor in harvested honey (n=9). The population development and the honey production were similar to the control group. Results of the dead bee traps showed no noticeable effects on the colonies.

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Effects on honey bee colonies following a granular application of Santana® containing the active ingredient clothianidin in maize in 2010 and 2011

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Abstract

Wireworms, which are the larvae of click beetles (family *Elateridae*) have become a serious pest of corn. Thus, the application of Santana®, a granulate with the active ingredient clothianidin, was allowed in 2010 and 2011 under strict regulations in Germany. The granules are deposited with the grain of seed in the soil during sowing. Clothianidin belongs to the group of neonicotinoids and is toxic for bees. An exposition of honeybees to clothianidin by dust during sowing as well as by guttation liquid might be possible. If guttation liquids collected with the beginning of guttation were mixed with sugar and fed to caged bees high mortality was observed; bees fed with this mixture died within one hour. Thus, the effect of guttation under realistic field conditions was observed.

In 2010 and 2011 honey bee colonies were placed at fields before sowing of corn and Santana®. All colonies were equipped with dead bee traps in front of the hives to estimate the mortality of honey bees in the hive. In both years during sowing and the following days no increased mortality was recorded. During the guttation period the mortality in the bee traps increased marginally on a few days. In some samples clothianidin was detected.

Neither in 2010 nor in 2011 negative effects on colony development were recorded.

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Water collection by honey bees – How far will foragers fly to use water sources like guttation drops? A first distance trial using cereals and oilseed rape

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Abstract

Background: Depending on the location, various water sources may be available for bee colonies. These sources can be permanent, such as ponds, or incidental like dew or guttation droplets. The aim of the experiment was to investigate whether bees prefer guttation drops as a water source compared to dew or rain drops. Furthermore it was analysed if bees use these water sources up to a distance of 50m from their hives.

Results: During the experiment 147 bees were observed scanning the surface of the plants without landing, 13 bees took up guttation fluid and 36 bees collected dew or rain drops. Few bees were observed collecting guttation fluid at 50m from their hives but most in close proximity of the hives. Furthermore, in some dead bees residues of the seed treatment were detected (imazalil: 0.0011 µg/bee – 0.329 µg/bee; LD50= 35.1 µg/bee).

Conclusion: In the majority of observations, bees were spotted scanning the leaf area of the plants. Only single bees were observed that actually took up water from plant leaves. It seems these bees did not distinguish between dew, rain or guttation droplets. The majority of water collecting or bees resting on plants were observed in the close proximity of their hives.

Keywords: guttation fluid, pesticides, seed treatment, honeybee, water source, distance

Introduction

The water demand of a bee hive is highly variable throughout the year[1]. In spring, the collected water is required primarily to dilute the stored honey whereas in summer it is necessary for temperature and humidity regulation[2]. Throughout the whole year water is required for the preparation of larval food and supply of minerals[3]. Contrary to nectar and pollen, water is not stored in the bee hive and therefore has to be collected when needed[4]. In most cases the colony´s water need is met by collection of fresh nectar or water condensed within the beehive[5]. If needed specialized bees, the water foragers, collect water from various water sources like ponds and ditches or from the surface of plants[6]. Experiments in a desert have shown that water foragers were able to fly up to 2 km to find water sources[7] but in general long distance flights are avoided for energetic reasons[8, 9]. Therefore, one of the potential water sources used by honey bees could be guttation drops from plants in the surrounding of their hives. Guttation describes an event at which xylem fluid is excreted as droplets along the edges or tips of plant leaves[10]. The guttation fluid predominantly contains inorganic substances in lower concentrations compared to plant fluids[11]. Recent studies on several seed treated crops showed that also systemic active substances from the seed coating can be found in the droplets[12]. While most seed treatments contain only fungicides that are not toxic to bees, many insecticidal active ingredients like e.g. neonicotinoids are highly toxic for bees. Insecticidal seed coatings, on the other hand, have been considered as harmless for bees up to now because no direct contact and no relevant exposure of bees to the active substance were assumed[13]. However, it was first shown in some experiments conducted in Germany[14] and Italy[15] in 2009 that systemic substances from the seed treatment were excreted via the guttation fluid in concentrations relevant for honey bees. Considering these results and the fact that most crops grown in Germany have the ability to show guttation[16], the question arises whether guttation drops of insecticidal seed treated crops can

constitute a relevant route of exposure for bees. To address the potential risk from guttation in realistic field conditions, several factors determining the potential exposure have to be considered. Therefore, the aim of this experiment was to obtain more information on water collecting behaviour of honeybees and to investigate if and at which distance guttation drops may used as a water source for honey bees.

Experimental methods

The experiment was conducted from 10th of April until 09th of May 2010 and was set up on one organically and one conventionally managed field near Ahlum (Lower Saxony, Germany). The distance between both fields was 300m. The experimental fields consisted of two plots; one planted with cereals and one adjacent plot with oilseed rape (fig.1) . In the organically managed variant the seeds were untreated. In the conventional variant the cereal seeds were treated with a fungicide (Zardex G®: 20 g/l imazalil, 5 g/l cyproconazol), the winter oilseed rape seed was treated with an insecticide (Elado®: 10 g/kg clothianidin). In both variants the bee hives were placed in the cereal plot with a distance of 0m (field border), 10m, 20m, 30m and 50m to the adjacent oilseed rape field and a distance of 50m from each other. On each field a total of three small bee colonies (one-room, 'Zander') and two *full size colonies* (two-rooms, 'Zander') were set up, the full sized at 0 and 50m and the small sized at 10, 20 and 30m. The hive entrance of all bee colonies pointed towards the oilseed rape plot. All colonies had an oviparous one year old queen.

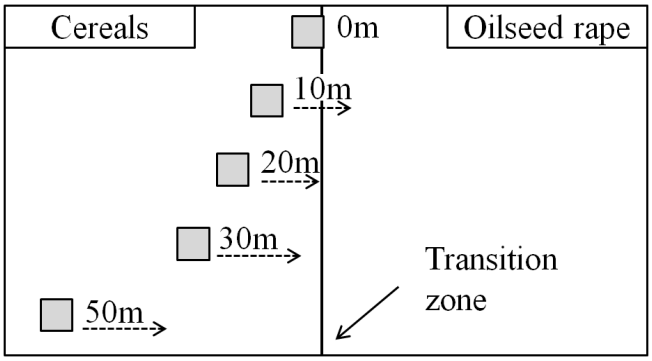

Fig. 1 Experimental field

The observations were conducted several times daily until no more guttation droplets were visible. If no guttation was present, the observation was conducted only once.

During every assessment the climatic conditions (relative air humidity, air and soil temperature), the growth stage of the crop plants using the BBCH monograph[17] and the presence of guttation, rain or dew drops was recorded. With beginning of daily bee flight, the flight activity at the entrance of the hive and behaviour observations on honey bees interacting with plants started. The observations of behaviour were conducted at two fixed observation lines and one observation point. Two of them were located at 0 - 5m distance from the bee hives at the entrance and at the back side. The third observation point was about 1 m^2 large, located at the field border and half grown with cereals and oilseed rape (transition zone). The distance of this point to the hives was depending on the location of the hives 0 to 50m. Each observation of behaviour lasted five minutes. In addition, four times in the course of the study the population development of the beehives was assessed by using the Liebefelder method[18] and the bee mortality every day using modified Gary-traps[19]. After completion of the field experiment residue analysis of dead bees from the Gary-traps was conducted.

Results

The development stage of the cereals at the beginning of the experiment was BBCH 29 (end of tillering), that of oilseed rape was BBCH 53 (inflorescence emergence). The experiment was terminated at flowering of the oilseed rape crop (BBCH 65). During this period both crops often showed guttation drops. In the cereals, guttation was observed more frequently than in oil seed rape (fig. 2).

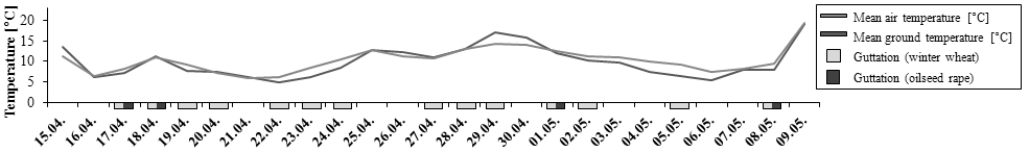

Fig. 2 Course of temperature and guttation events in the experimental fields.

A total of 38 bees were observed searching or collecting nectar within the assessment areas. These were not considered in the following figures and evaluations. The majority of bees, interacting with plants, were observed adjacent to the bee hive (fig. 3, left). In the transition zone between the cereal and oil seed rape a smaller number, approximately about 15 % of the total number was observed. With increasing distance from the bee hives, fewer bees were observed (fig. 3, right).

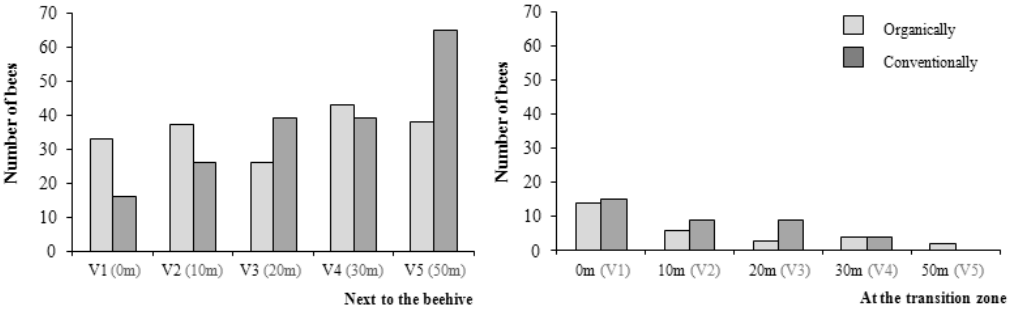

Fig. 3 Number of bees at the observation points (left part = two 1 - 5m lines next the beehive, right part = 1 m^2 point at the transition zone with 0m, 10m, 20m, 30m or 50m distance to the beehive).

In the majority of cases, bees were spotted resting or scanning the leaf area (fig. 4). There was no significant difference in the number of bees that took up guttation, dew or rain drops. At a distance of 50 m from the bee hive in the transition zone of the plots only two bees were observed that took up guttation fluid.

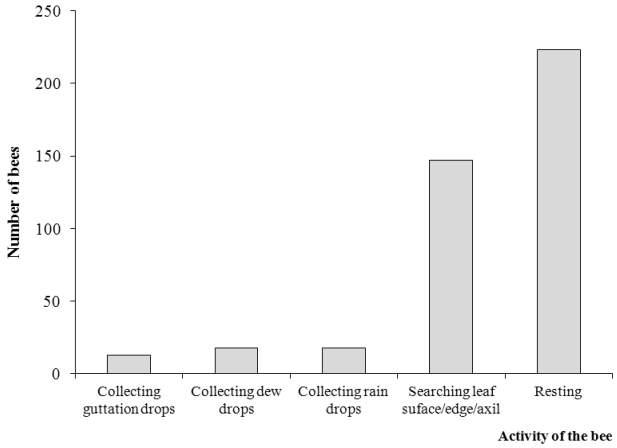

Fig. 4 Overview of the activity of all observed bees irrespective of the observation point.

No adverse effect on the development of bee colonies was detected. The development on both fields was at a similar level. Also the mortality of both the organically and the conventionally managed fields was at a comparable level (fig. 5).

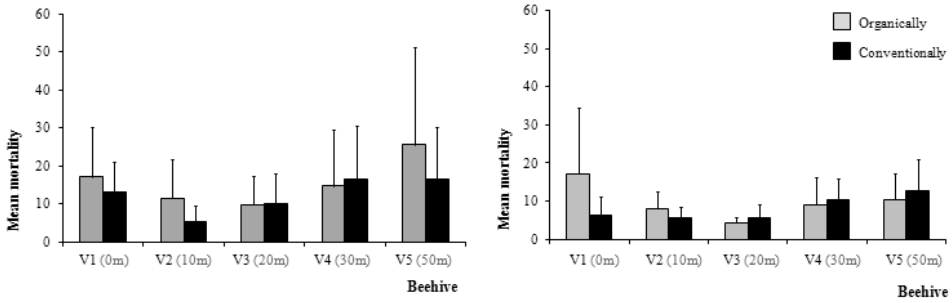

Fig. 5 Mean bee mortality of days without (left part, n= 14) and with (right part, n = 9) guttation in any crop.

No residues of clothianidin, which may have originated from the treated oil seed rape in the conventional variant, were found in dead bees. However, in some samples, residues of the fungicide imazalil (0,0011 µg/bee - 0,329 µg/bee, LD50= 35,1 µg/bee) from the seeds of the conventional cereal plot were detected.

Conclusions

Bees used water from the surface of plants, the edge of leaves and also the leaf axial water as a water source. They did not distinguish between dew, rain and guttation droplets but were often observed scanning the leaf edges. Therefore, it seems that bees are capable of learning where guttation water droplets can be found. The findings of the fungicide in some dead bees from the conventionally managed variant indicated that they took up guttation fluid. With increasing distance from the bee hives less bees interacting with plants were observed. Although most bees were observed adjacent to the bee hive, some were found collecting guttation fluid up to a distance of 50m. In this experiment

no adverse effects on the development of the bee colonies was observed. The development and mortality of all bee colonies independent of the seed treatment used was at a comparable level.

Acknowledgements

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Frequency and intensity of guttation events in different crops in Germany

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Abstract

Background: In crops of economic relevance and some common weeds in Germany, several glasshouse and field trials were conducted at the JKI and cooperating research partners (DWD, IfZ, BDP and UFOP) from 2009 to 2011. Aim of the investigations was to analyze the guttation frequency of these crops, to document climatic conditions that trigger guttation and to describe the quality and quantity of guttation events (e.g. size/number of drops).

Results: Guttation occurred in all crops mainly at young growth stages. Only maize, cucumber, oilseed rape and potato produced guttation droplets until emergence of inflorescence. The excreted amount of fluid was comparable for maize and oilseed rape but considerably lower for sugar beet and barley. In field situation guttation occurred frequently in more than one plant species in parallel when it was not dry or too windy or frosty.

Conclusion: Monocotyledonous crops and weeds showed a higher guttation frequency than most dicotyledonous crops. Maize showed large guttation drops even under low relative air humidity, whereas guttation drops in sugar beets were much smaller and only observed under very high humidity conditions (>90%). Other dicotyledonous crops, such as oilseed rape and potato, showed a much higher guttation frequency than sugar beets.

Keywords: guttation, honey bees, pesticides, crops, weeds

Introduction

Guttation describes an event at which xylem fluid is excreted as droplets along the edges or tips of plant leaves[1] and was presumably first described in 1672 by Abraham Munting and in 1887 mentioned as guttation in the literature by Burgerstein[2]. Since the beginning of the 20th century, it was reported in a wide range of plants e.g. 1925 by Lippmann[3] and therefore considered as a general phenomenon. For a deeper understanding of guttation it is important to recall that plants need to maintain a certain level of water and nutrient transport between the roots and the leaves. This is done by passive mechanisms acting at a declining gradient such as transpiration. However, under climatic conditions that are not suitable for transpiration, e.g. under a relative humidity over 75%[4] close to the leaf surface, guttation may occur. These climatic prerequisites often occur at night or in the early morning. They are the same also for triggering the formation of dew drops[5]. The guttation fluid is excreted via hydathodes. They vary in their anatomical set up. Plants display two basic forms of hydathodes: active and passive. Active hydathodes operate with special epithem cells, whereas in passive hydathodes guttation is a result of root pressure[6]. The composition of the guttation fluid may vary slightly depending on its excretion way (passive and/or active hydathodes) and the growth stage of the plant or leaf[7,8]. Even though the general occurrence of guttation has been well described in literature, no data are available which compare the occurrence, frequency and intensity (size/number of guttation drops, number of guttating plants) of guttation between crops of economic relevance in Germany. To address this question several greenhouse and field trials were conducted by the Julius Kühn-Institut, in cooperation with research partners (DWD, IfZ, BDP and UFOP) from 2009 to 2012.

Experimental methods

A total of eleven important widely-grown crops (e.g. oilseed rape, maize, sugar beet) and twenty-one common weeds like e.g. *Poa annua* were examined in greenhouse or field trials (tab.1). The frequency and intensity of guttation of different crops and weeds in greenhouse trials were compared under the same climatic conditions. In field situation, several areas within the field border (covered with weedy plants) adjacent to the crop field or in neighbouring field crops (preferably cereals) were investigated and compared to the observed field in parallel. The observations started at early plant emergence and ended at the growth stage when guttation ceased. In the glasshouse daily assessments were conducted. Assessments of guttation frequency and intensity in the field trials were carried out daily or in some cases only under climatic conditions suitable for guttation on pre-selected days (e.g. high air humidity, low wind speed, occurrence of dew). At each assessment the climatic conditions (relative air humidity, air and soil temperature), the growth stage of the crop plants using the BBCH scale[9] and the presence of guttation or dew drops were recorded. However, in the field trials, additional climatic information like sky cover, soil humidity was assessed. The size of guttation drops was determined only in glasshouse trials. For this the guttation drops of each plant were counted and balanced on a filter paper.

Tab. 1 List of all investigated widely-grown crops and common weeds.

			Assessment	
Crop	**Greenhouse**	**Field**	**Daily**	**Pre-selected**
Maize	x	x	x	x
Barley	x	x	x	x
Wheat	x	x	x	x
Oilseed rape	x	x	x	x
Cucumber	x	x	x	
Potato	x	x	x	x
Sunflower	x		x	
Sugar beet	x	x	x	x
Onion	x	x	x	
Carrot	x	x	x	
Pea		x	x	
Weeds*	x	x	x	x

**Alopecurus myosuroides, Apera spica-venti, Avena fatua, Chenopodium album, Cirsium arvense, Echinochloa crus-galli, Elytrigia repens, Fallopia convolvulus, Fumaria officinalis, Galium aparine, Lamium amplexicaule, Lamium purpureum, Matricaria recutita, Mercurialis annua, Poa annua, Polygonum aviculare, Solanum nigrum, Stellaria media, Thlaspi arvense, Tripleurospermum perforatum, Viola arvensis*

Results

The different crops required different relative air humidity conditions before any guttation occurred in the greenhouse. Maize started to guttate at a relative humidity of 80%, the other crop species needed a humidity of at least 90% before guttation started. Above 90% humidity maize and cereals showed guttation in greenhouse (90-100% of observation days) nearly daily, whereas at the same conditions oilseed rape and potato guttated often (~60% of observation days) and sugar beet only rarely (20-30%). Similar results were recorded in field situation. In a field situation with several crops and weeds guttation occurred frequently when it was not dry or too windy or frosty, most of the time in more than one plant species in parallel (fig. 1). However, in the field in contrast to greenhouse even under climatic conditions suitable for guttation, guttation was observed on at most 50 % of

preselected observation days for any crop (tab. 1). For weeds similarly as for crops guttation was mainly more frequent for monocotyledon species compared to dicotyledonous ones.

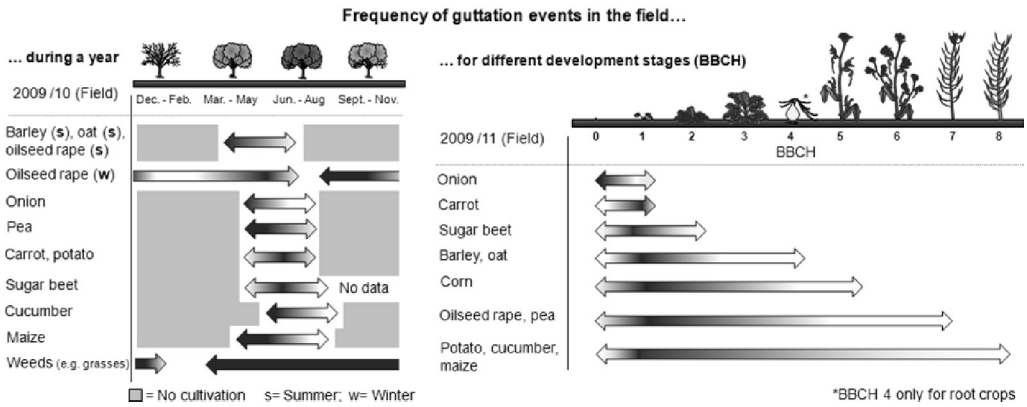

Fig. 1 Frequency of guttation events in the field based on several field studies.

For the majority of crops, guttation can be observed in greenhouse and field studies from the emergence of the seedling until the end of the leave development. Only maize, cucumber, oilseed rape and potato produced guttation droplets until the emergence of inflorescence (fig. 1). During this period the number of guttation drops increased depending on the number of leafs. Maize showed large guttation drops even under low relative air humidity, whereas guttation drops in sugar beets were much smaller and only observed under very high humidity conditions (>90%). However, oilseed rape plants produce very many small drops at a medium to high frequency and in summary more guttation liquid than maize plants with few large drops at a high frequency (tab. 2).

Tab. 2 Amount of guttation fluid of various crops in greenhouse studies, average values of all assessments (BBCH 10-19).

Crop		Oilseed rape	Potato	Maize	Barley	Sugar beet
Number of drops/plant	Mean	37	15,9	3,7	7,4	3,5
	SD	15,6	10,6	0,8	2,6	1,4
Weight of one drop [g]	Mean	0,0022	0,0042	0,0215	0,0021	0,0038
	SD	0,0008	0,0027	0,0077	0,0008	0,0066
Amount of liquid [g]	Mean	0,0814	0,06678	0,07955	0,01554	0,0133
	SD	0,01248	0,02862	0,00616	0,00208	0,00924

Taking the greenhouse results on the amount of excreted guttation fluid and the greenhouse and field studies on the occurrence of guttation into account, a classification of the intensity of guttation was created for different crops (fig. 2).

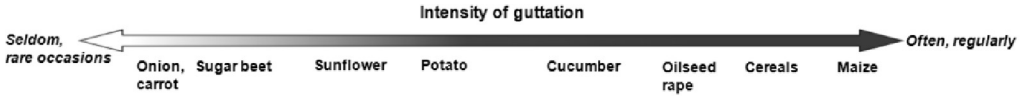

Fig. 2 Classification of the intensity of guttation of various plants based on several greenhouse and field studies.

Conclusions

A valid prognosis of the climatic conditions triggering guttation for a specific widely-grown crop is not possible yet. Even under climatic conditions suitable for guttation, guttation was only observed on 50 % of preselected observation days in investigated fields. However, during most of the year guttation occurs frequently in several crops or weeds and then usually also in many individual plants in parallel. The frequency of guttation however is particularly high in early growth stages of the crops and some plants show guttation more frequently than others. In general, monocotyledonous crops such as maize and cereals showed a higher guttation frequency than dicotyledonous crops such as sugar beets. However, some dicotyledonous crops such as oilseed rape and potato guttate more frequently. Similar results were recorded for some weeds.

Acknowledgements

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Review on activities in Germany to assess the occurrence, residues and possible risk of guttation for honey bees

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Abstract

Findings of high concentrations of bee-toxic compounds in guttation fluid from young crop plants that had been seed-treated with systemic insecticides gave recently rise to concerns about a potential risk to honeybee colonies posed by exposure to guttation of seed-treated crops.

Measurements of high residue levels of some intrinsically highly toxic, systemic insecticides in guttation droplets were reported by different researchers. Consequently, in the past 3 years, a large number of different approaches have been conducted by industry and public research institutes to assess the possible risk of guttation in treated crops to bees. Different approaches of studies with bees in lower and higher tier tests were set up to gain clarification if and how this concern would need to be specifically addressed in the risk assessment for bees. A large number of studies were conducted on the environmental conditions and factors favoring guttation, the occurrence of guttation in different crops, residues in guttation droplets in different crops with different active ingredients. Studies with honey bees were conducted under laboratory conditions as well as semi-field trials, field trials and monitoring with honey bee colonies in various crops and different active ingredients. The review on available studies on guttation describes which experiments were done in different research facilities in Germany.

Orientating experiments on guttation fluid of seed treated maize (*Zea mays* L.) in relation to the water collecting behaviour of honey bees (*Apis mellifera* L.)

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Abstract

The major task of this study was to examine the honey bee's (*Apis mellifera* L.) water collection behaviour in relation to the process of guttation in a corn plot treated with clothianidin. As shown in experiments 2009 under field conditions, guttation fluid of seed coated maize contains concentrations up to 8000 ng ml^{-1}. Based on these results, further investigations were conducted to examine the honey bee's water collection behaviour in relation to designated water collection stations and varying water quality under tent conditions. In the 'corn tent' a high number of maize seed coated with clothianidin (Poncho and Poncho Pro, Bayer CropScience) was sown to create an extreme situation for the bees. Beside the guttation no alternative water sources were offered. In the 'mesh tent' the soil was covered with texture to exclude natural water sources so defined water sources were placed at varying distances and with varying qualities of water. Pollen and sugar dough were offered on feeding stations Observations on bees drinking at the water sources, their behaviour and reaction on water quality were induced in both tents.

The instances of death in the experimental bee colonies were regularly noted, and the dead bees collected.

These tent experiments showed, that bees, which collected guttation droplets in seed dressed corn or clothianidin-spiked water at the artificial water source return to the colony and get damaged after a certain time with the known symptoms. Dead bees can be found in the colony as well as in front of the hive. The number of affected bees in the colony is limited but under the chosen conditions the consumption of contaminated water led to a reduced colony development.

II. Bee health issues - country specific experiences including Varroa and varroacides

Efficacy of selected acaricides on *Varroa destructor* and evaluation of their environmental risks on *Apis mellifera*

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Abstract

The present study examined the effects of two synthetic (Bayvarol and Apivar) and two natural acaricides (Apiguard and ApiLife Var) on *Varroa destructor* by controlling the level of mite infestation and on the honeybee *Apis mellifera intermissa* by measuring the amounts of protein, carbohydrates and lipids in the whole body and hemolymph and acetylcholinesterase (AChE) and glutathione S-transferases (GSTs) activities in the adult stages. The results showed that all acaricides significantly reduced the levels of varroa infestation on adult honeybees and worker brood, but the efficacy was higher for natural acaricides (93–98 %) compared to synthetic acaricides (82–90 %). The amounts of the principal components (protein, carbohydrates and lipids) were significantly different between honeybees treated with acaricides and the control honeybees. All acaricides have no significant effect on AChE activity but led to increase GST activity as compared to controls.

The biochemical components are affected and bees are exposed to toxic stress when acaricides, especially synthetic ones, are used as treatments in hives. Acaricides, especially synthetic ones, affect bees in controlling mites. For these reasons, beekeepers should take into consideration of timing and doses when using acaricides.

Kewords*: Apis mellifera intermissa, Varroa destructor,* acaricides, efficacy, secondary effects.

III. Test methodology - laboratory, semi field, field, etc.

Assessment of brood development and index calculations

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Abstract

According to European requirements, regulatory testing and risk assessment on honeybee brood is required in cases where colonies are exposed to treatments that could induce forager bees to bring back residues to the hive in nectar or pollen. The impact of chemicals to honeybee brood can be assessed both in laboratory and field conditions.

The existing brood testing methods are available only for laboratory conditions (Aupinel and al., 2007) and for semi-field conditions (OECD n°75, 2007). However, the impact on honeybee brood is easily been assessed in a field test, combining recommendations of EPPO n°170 or French CEB n°230 for the field part and the OECD 75 for the brood assessment part.

Currently brood tests under laboratory conditions are managed according to the OECD document n°75 'Guidance document on the honey bee (*Apis mellifera* L.) brood test under semi-field conditions'. According to this guideline, data on the brood development and growth stages in at least 100 marked cells over 22 days are collected.

In the evaluation the test results allow calculations of indices:

- the brood termination rate (failure of the brood development),
- the brood index (measure of the larval development)
- the compensation index (indicator for recovery).

However results from field conditions and from semi-field conditions may differ because of the colony behavior or because of external conditions such as climate and enclosure under tunnels. Although all results are valid, in some cases the control and the reference item provide unexpected results. Such data with mortality in the control are usual in beekeeping practice but not suitable for calculation to the indices. This occurs mainly under semi-field conditions. Hopefully the OECD document n° 75 indicates "*Specific statistical analysis… are still under development*". It is so reasonable to provide information for deciding how to use the results.

The eggs laid by the queen develop into larvae, then pupae and then honeybee, emerging in a precise time sequence. Individuals deviating too much from the normal time sequence are disqualified. For evaluating the different brood stages of single marked cells, the recorded growth stages are recalculated into values from 0 to 5.

At least 100 cells containing an egg are selected on a dedicated area on a first observation timing (the Brood Fixing Day = BFD)

If a cell does not contain the expected brood stage during the period from 5 days to 16 days after the Bood Fixing Day (BFD+5 to BFD+16), the cell has to be counted as 0 at the assessment day and also on the following days. Most of the time, when the brood development is abnormal this justification will eliminate the cell from the further calculation. However, in a limited number of situations, it is possible that the brood development is quite normal although the expected stage is not attained. Beekeepers observations confirm that honeybees may deviate from the theoretical time sequence. In such a case index calculations are not adapted to the colony development.

Adapted formulas allow considering this normal brood development when conditions require. In this way raw data (brood development) need to be transformed to adapted data (complementary data = to be used for the index calculation).

For instance an *Excel* formula takes into account the early stage of capped cells:

- By considering the initial conditions of eggs then larvae into cells (IF/OR in the software) and the expected development (AND/OR in the software) with the realized development in each individual cell (Brood evolution data /Complementary data).
- In order to increase the data significance we think it is reasonable to use adapted formulas from a bee keeper point of view. Brood tests in-field or semi-field cannot easily be replicated; therefore we find it necessary to increase the significance of collected data in single studies.

Keywords: brood, OECD guidance document

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Comparison between EPPO and CEB field methodologies

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Introduction

In France, to protect honeybees and pollinators, spray applications of insecticides and miticides are banned during the flowering period and the honeydew production [1]. This bee ban can be waived provided that laboratory and semi-field data (tunnel test according to CEB[2]) show an acceptable risk for honeybees.

The French CEB procedure of tunnel tests was revised in 2003 to include the toxic reference dimethoate and to account for a mandatory mitigation measure which is to spray the product out of the presence of bees, during early morning or late evening, on the entire crop area. These conditions are clearly worst-case, more than the previous ones where sprayings were conducted on half of the crop area in the presence of bees. However, applications out of the presence of bees are now considered. This revised procedure is in line with the recommendations of the EPPO guideline n°170 which was recently revised [6].

The semi-field test was the preferred option since test conditions are homogeneous regarding soil, crop, climatic exposure and bee colonies settled in similar insect-proof tunnels, whereas field studies imply greater heterogeneity of conditions.

However, field trials are the last step of the testing scheme developed by the EPPO guideline n°170 which is the reference frame for regulatory risk assessment for honeybees in Europe [3, 7]. Since assessment for the bee ban waiver in France could not be separated from a full risk assessment according to the EPPO risk assessment scheme, a procedure for field trials was necessary to complete the CEB methods.

Therefore, the French Bee Working Group was commissioned to write a field methodology suitable for the assessment of impact to honeybees when a bee ban waiver is required.

Keywords: field, French CEB, EPPO

Development

The French working group worked during two years under official supervision on the text of a methodology adapted to field studies with honeybees. This working group is composed of members from academia, industry, beekeeping and consultancy. This group presented the result of this work in March 2011 to the Ministry of Agriculture. This field methodology became an official guideline under CEB recognition, compliant with the EPPO guideline[3, 6].

Although field studies were not recommended in France for a long time because of poor confidence in their reliability to represent worst-case conditions, they became of interest for post-registration monitoring of plant protection products and to complete the testing procedures to apply for a bee ban waiver in France. By extension they became of high interest to specific studies such as the impact of plant protection products on the honeybee brood in field conditions.

The working group focuses at simple parameters in order to manage field studies with honeybees that could also be used for registration at the European level. The objective of this field methodology is to assess the effect of foliar application of plant protection products on honeybee colonies.

Compared to semi-field studies, characteristic of the open field tests are the representative conditions of agriculture, with sufficient surfaces for the foraging activity of honeybees. In these agricultural conditions honeybee colonies are settled in hives suitable for a professional use. They are strong enough to forage large surfaces, comparable to those used by professional beekeepers. By the strength of the colony and the normal bee activity of forager bees there is no experimental stress in

the swarming behavior. These field studies allow mid-term observations and quantitative samples of different matrices for residue analysis (of worker honeybees, brood, flowers, honey and pollen).

Although EPPO guideline n°170 and French CEB n°230 are being harmonized about field studies, there are still some significant differences on the following points:

- According to beekeepers recommendations the French methodology requires surfaces of at least 2 ha per test unit in order to provide sufficient crop plots to forager bees while presenting realistic conditions of agricultural practices. EPPO recommends plots of at least 2500 m^2 of *Phacelia* or about 1 ha for mustard or rapeseed.
- In both methodologies the number of hives required is adapted to provide enough data. Four hives per test unit are recommended in the EPPO guideline when the French CEB proposes 3 hives per hectare that makes a minimum of 6 hives per test unit. In both cases it is mandatory to use dead bee traps and recommended to use pollen traps (for further analysis). These numbered hives are used to limit the variability of bee keeping conditions.
- The timing of applications is also different. For regulatory reasons applications in France have to be realized during flowering and after the flight bee activity, close to or during the night. However the European guideline recommends maximizing the bee exposure with product application during the foraging activity. In this way it is considered as a worst case for the impact to bees. It is therefore possible to add a test modality during bee flight in the French schedule, but this could require a previous authorization for trial if the study treatment is under development or known to have adverse effects to bees.
- Similarly two test modalities only are supposed to be tested in this French guideline (study treatment and water control) whereas it is possible to add a toxic reference in some cases when using the EPPO guideline. The use of a toxic reference such as dimethoate is strictly forbidden during flowering in field trials in the CEB requirements, not only because of the impact to bees but also and mainly because of the effects to all beneficials in the surrounding.
- Mortality and foraging observations are collected with similar timing within both methodologies and bee keeper's visits cover at least one brood cycle (up to 28 days).

Discussion and conclusion

Over the experimental phase in fields with treated and untreated modalities, collected data are supposed to bring a useful tool in the assessment of impact to honeybees. Despite limits to field trials (field surfaces, homogeneity of tested and control plots, only high effects on adult mortality are detectable, impossible statistical validation without replicates)[4] we think it is reasonable to consider the harmonization of both French CEB and EPPO field trials to honeybees.

For the validation of the field study the French Bee Working Group proposes that mortality levels and foraging activity should be similar among treatments before foliar applications. Then the control mortality should normally not increase more than 50% after application, unless it is demonstrated that the increase is not biologically significant. On the other hand the EPPO bee guideline suggests to repeat the test if mortality is too high in the control or too low in the reference modality. The criteria for deciding whether a mortality rate is too low in a toxic reference or too high in a control are currently under discussion by a working group of ICPBR.

Both guidelines require the use of statistical analysis when appropriate. However sufficient available and reliable data are necessary for a significant analysis.[5]

This French CEB guideline for field studies intends to bring an improvement to the EPPO guideline, considering more realistic conditions for the validation of field trials with honeybees. Therefore the Bee Working Group of the French CEB proposes ICPBR to look at potential improvements in the parameters for conducting field trials, in the revision of the field guidance to the EPPO 170 guideline.

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Scanning & analysing individual bee (*Apis mellifera* L.) behavior using RFID

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Abstract

A complete system to make real-time flight entrance observations possible is described. The system consists of:

a. **RFID reader** covering the flight entrance of a colony in a standard hive (25 cm wide). Each passing tagged bee is detected. ID, day, time is stored in the database and shown via the web-interface.
b. **ApiScan** covering the same entrance width; giving in and out flight-activity on an individual basis.
c. **Camera** allowing a permanent remote observation and registration of behavior at the flight entrance.
d. **Weather station** registering air temperature, air humidity, wind velocity and direction, rain. The equipment and the data storage allows an in-depth analysis of the flight behavior of the worker bees during the measuring period or the lifespan of a worker bee.

Keywords: honeybee, RFID tags, foraging behavior, automatic monitoring

Need for individually marking honey bees

The tunnel and field tests are the higher tier risk assessment to the honeybee colony for plant protection products (PPP's) based on Directive 91/414/EEC criteria. Those tests have to disclose "through an appropriate risk assessment that under field conditions there are no unacceptable effects on honeybee larvae, honeybee behavior, or colony survival and development after use of the plant protection product according to the proposed conditions of use" [1] .

The only natural way for a PPP to contaminate the matrix honeybee-colony is to be carried into the hive by gathering honeybees.

This very first step - flying back from a contaminated source into the hive - has been used to find effects of field relevant sublethal doses of different insecticides for the honeybee: for deltamethrin[2], imidacloprid[3,4,5,6,9], fipronil[5,7,8] and clothianidin[9].

Instead of direct visual observation of individually paint marked bees, Decourtye[8] and Schneider[9] used modern RFID-techniques to register behavior of foragers in their experimental setup. The passive RFID-chips tagged on the thorax of a honeybee weigh 3 mg, adding about 3% to her bodyweight.

The chip-size is comparable to the classical numbered tags used for marking worker and queen honeybees. For this small-size passive chip a distance from the reader of max. 5 mm guarantees a secure identification of the marked honeybee.

Up till now the existing research set-ups with this technique [8,9] use small entrance holes (tunnel diameter <= 7 mm), which restricts the number of bees under observation during each experimental session.

Also the classical automated observation tool on flight entrance activity uses small entrance-holes to allow each bee to individually pass an infrared counter (the BEESCAN BEECOUNTER ®from Lowlands electronics). This equipment is judged as unreliable. At high flight activities the equipment hampers the natural behavior of the colonies under observation and the data body produced is inconsistent.

Alix et al.[10] postulate: "Possible effects on adult survival and foraging behavior and on bee colonies should be checked". This urgently asks for an automated observation of the flight entrance on the

basis of individually marked bees. The equipment used should not limit the passing through the flight entrance.

RFID reader and tracking software

Co-author Egbert Touw, as a social responsibility project in the light of the high colony losses in the Netherlands, managed to initiate his employer - Nspyre - to develop a RFID reader design covering the full width of the flight entrance of a standard hive (25 cm). As partner in the cooperation Microsensys GmbH in Erfurt (D) developed a reader using 16 overlapping antenna areas, fit to detect and read-out the passages of their MIC-3 tags .

A number of students of Fontys Hogeschool Eindhoven co-developed the tracking system e.g. the software behind the row of reader antennae. The whole setup integrates the data from a RFID-scanner, a video observation, a weather station, an APISCAN counter. All these data are collected and stored in real time and can be linked to the internet.

Demonstration

At the Floriade Horticultural World Expo in Venlo NL (5th April -7th October 2012) a complete system is active and all these data are shown in real-time together on a web interface (46" monitor) in the Bijenpaviljoen (Fig. 1).

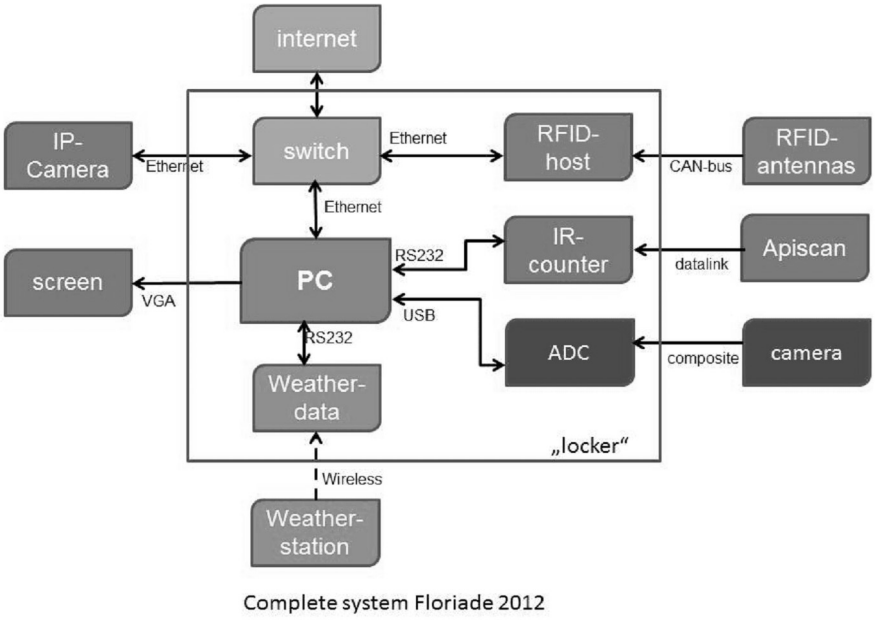

Fig. 1 Dataflow-schedule of the complete system as shown on *Floriade*.

The equipment and the data storage allow an in-depth analysis of the flight behavior of the worker bees during the measuring period or the life span of a worker bee. Changes in behavior eventually due to the use of a PPP can be easily detected and traced thanks to the real-time registration and logging of all the relevant parameters. The system allows the tracing of individual honeybees in there natural environment as part of a whole, normal sized colony (up to 50.000 individuals).

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Digital image analysis tool to improve the assessment and evaluation of brood development in higher tier honey bee studies

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Abstract

The potential impact of plant protection products on honeybee brood development is of increasing concern. Therefore, regulatory authorities request the studies to be monitored for potential adverse effects on honeybee brood development (Guidance document OECD 75). Current methods have a number of inherent technical limitations which we solved by computerizing the analysis. The computer-aided digital image analysis and evaluation method of brood development in honeybee combs which we developed allows to systematically evaluate brood development on the basis of high definition pictures of brood frames taken during semi-field or field honeybees trials. The computer-aided method enables the *post-hoc* analysis of virtually any number of cells in the comb, overcoming the issue of the low cell number, usually monitored with the acetate-sheet method as well as the traceability and verification of the data.

The recording method and software have been designed and compiled with the intention to provide a tool for a 100% traceable analysis of bee brood studies which is gap free and systematically documented. This is of utmost importance when working under Good Laboratory Conditions (GLP). The method minimizes adverse impact on bee brood by reducing the out-of-hive time and hence is likely to increase the success rate of studies. The availability of digital images allows the *post hoc* analysis of any number of cells. The automated tracing of the cells under investigation, together with the automated classification of the data excludes manual data transcription errors which are possible when the acetate-sheet technique is used. As a result, data reliability, quality and statistical power have been significantly improved. For more details please see Jeker *et al.* (2012).

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Introduction to the BEEBOOK, a manual of honeybee research methods

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Abstract

COLOSS (Prevention of Colony LOSSes, www.coloss.org) is a large international effort with a goal of investigating the potential causes of honey bee colony losses globally. The group is composed of more than 250 scientists from over 40 member countries and is financed by the EU Cost Action.

The BEEBOOK is an effort of COLOSS members who seek to produce a manual that outlines standard honey bee research methodologies. Recognition of and adherence to these methodologies will make data more uniform and comparable across laboratories globally. The BEEBOOK concept is similar to that developed by the *Drosophila* community ('*Drosophila*, a Practical Approach' edited by D.B. Roberts).

Broadly, the BEEBOOK will include methods for rearing queens, equalizing colonies, measuring colony health parameters, conducting toxicological and molecular investigations on honey bees, etc. The BEEBOOK will exist online so that the chapters can evolve as new methods are developed. However, in 2012 a three member editorial team will collect all chapters into a single volume that will be published as a hardcopy book.

The current contribution presents the history of the BEEBOOK idea, its structure and a brief description of contents, with particular attention given to the section on toxicological protocols.

Influence of some experimental conditions on the results of laboratory toxicological tests on honeybees

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Abstract

The official guidelines for the assessment of the risk of pesticides on honeybees are based on specific protocols. They contain the procedures that must be applied in order to make the results usable for the pesticide registration process. The described test conditions must be respected to make the result valid.

Often for some of these parameters a broad range of values are acceptable. For example the EPPO guidelines allow to run laboratory toxicity tests at the temperature of 25±2°C.

In our studies we have noticed that the LD50 value may vary significantly within this temperature range. Thus the current guidelines allow to the subject interested in pesticide registration to run toxicity tests at such a temperature level that produces less effects.

The present contribution is aimed to discuss some of the test parameters (like temperature, alimentation, sanitary conditions, bee sampling method) that may significantly influence the results of toxicity tests.

Proposals for improvement of official risk assessment guidelines are also provided.

A new experimental method for studying trophallaxis as an additional determining factor in the effects of chemicals on foraging bees (*Apis mellifera*)

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Abstract

Foraging bees perform daily 10 trips on average, lasting 30-80 min each. Thus, the nectar can be stored in the honey-bag even for about one hour before the return to the hive. In the presence of contaminated food, this is a quite long time of exposure. In fact, if the honey-bag's wall has chemical affinity for some or all pesticides, then direct active ingredient's absorption will occur in addition to the quantity ingested by the bee for its own requirements. Therefore, the foraging bees should be much more exposed to pesticides than their sisters in the beehive.

To test this hypothesis, a new experimental method was created, in order to simulate the foraging activity in the laboratory. In a hoarding cage, two groups of bees are divided by a membrane that allows only trophallaxis between the two groups. Only one group (donors) can access the feeders, collect the food (sucrose solution) and transfer it to the other group (receivers). In a separate cage, the bees of a third group (autonomous) have to provide only for their own feeding. Donors and autonomous bees must be necessarily foragers, while receivers are younger bees. Finally, the mortalities of donors and autonomous bees are compared to determine the impact of foraging activity on the intoxication of foragers.

This method was first applied in some demonstrations, in which the two pesticides clothianidin and fipronil were tested at sublethal doses. On one hand, the optimal exchange of food between donors and receivers was verified, confirming the technical validity of the method: it can function properly for at least 72 hours. On the other hand, the results show a significant difference between the mortalities of donors (higher) and autonomous bees (lower) fed with the two active ingredients.

Role of food quality in bee susceptibility to fipronil and clothianidin

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Abstract

Pollen is the honey bee's main protein supply. Newly emerged bees need pollen alimentation to guarantee correct development of physiological conditions and breeding potential. To ensure functional and efficient adult bees not only the quantity but also the quality of pollen is important: pollen of different plant species vary in nutritional quality for honey bees. Previous studies showed that a protein alimentation with pollen mixture is more adequate for bees than monofloral pollen. The kind of pollen collected by bees is in close relationship with the vegetation spectrum of the hive surrounding area. Thus, if the bee colony is surrounded by areas characterised by intensive agriculture - e.g. the Po Valley in Italy - it may mainly collect monofloral pollen. In these areas, use of pesticides is generally widespread.

The present study is based on the hypothesis that the quality of pollen available for the honey bee colony may influence the bee susceptibility to the intoxication by pesticides. For this reason, the same pesticide treatment could cause negligible or significant damages in relation to the availability of high-quality pollen (high amino acid diversity and high protein content).

In the experimental apiary, in order to obtain newly emerged bees of the same age, the queen bee was isolated on a comb in a queen-excluding cage for about 30 hours. Subsequently the queen bee was removed from the cage and the comb was left isolated for another three days, to avoid further egg laying. The comb was incubated inside the beehive for 20 days in order to guarantee the most natural conditions, then it was moved to an emerging cage and kept at 34,5°C. The bees were incubated at this temperature until the end of the test. At the beginning of the emergence the bees were fed *ad libitum* with water, organic *Robinia* honey and a kind of pollen in relation to the thesis: *Zea mays*, *Papaver*, *Cruciferae*, *Trifolium*, *Taraxacum* or a mixture of pollen. Thus groups of bees provided with different pollen diets were obtained.

On the 9th day, the bees were divided into groups of 20 individuals in small test cages in which clothianidin or fipronil - pesticides considered as one of the possible causes of the recent colony losses - in sucrose syrup (50%) were administered. Once the bees consumed completely the test solution, sucrose syrup (50%) was supplied *ad libitum*. Bee mortality was recorded and the LD50 of the active ingredients was calculated in relationship to the alimentation quality.

In conclusion, the influence of the quality of protein nourishment provided during the first days of adult life on the response to intoxication by clothianidin and fipronil was shown as evidence. Bees fed with low-quality pollen (low amino acid diversity and low protein content) seem to be more susceptible to pesticide intoxication and other stress factors than bees fed with high-quality pollen.

Effects of spinosad on honey bees (*Apis mellifera*): Findings from over ten years of testing and commercial use

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Abstract

Background: Spinosad is widely used as an insecticide in crop protection against thysanopteran, lepidopteran and dipteran species. As such it is intrinsically toxic to insects and among them to the honey bee (*Apis mellifera*). An updated risk assessment is presented in the context of the regulatory evaluation of spinosad products and is in accordance with the latest recommendation of regulatory guidance documents.

Results: The intrinsic toxicity to the honey bee as observed in laboratory conditions through oral and contact tests on adults does not appear to impair honey bee colonies when exposed to treated attractive crops in tunnel conditions. Reasons for this could include reduced availability of residues of the product on plant surface compared to laboratory conditions, together with a fast dissipation from treated plants and the absence of active degradation products.

Conclusions: Spinosad products present a negligible impact on honey bees when used under the current label recommendations and conditions of agricultural use. This conclusion deduced from data available for the regulatory risk assessment has been confirmed by the feedback of surveys on incidents, which address the potential impact of spinosad products under realistic conditions of exposure, including other environmental and chemical factors that are common in cropped areas.

Keywords: honey bee, pesticide, risk assessment, risk management, spinosad

1. Introduction

Spinosad is an insect control agent derived by fermentation of the Actinomycete bacterium, *Saccharopolyspora spinosa*. The active ingredient is composed of two metabolites, spinosyn A and spinosyn D. Spinosyns (such as spinosad and spinetoram) have a novel mechanism of activity on nicotinic acetylcholine receptors which is identified as the primary cause of death.[1] The action of spinosyns on nicotinic receptors is unique in comparison with other insecticides and is at a different site from that of nicotine and imidacloprid.

In common with other insecticides spinosad is intrinsically toxic to honey bees. In laboratory tests worker honey bees were exposed orally (in sugar water diet) or to doses topically applied. LD_{50} values for technical material of 0.057 and 0.0036 µg a.s. bee^{-1} for oral and contact routes of administration respectively were recorded. Similar levels of toxicity were exhibited for a 480 g a.s. L^{-1} SC formulation containing spinosad with LD_{50} values of 0.049 and 0.050 µg a.s. bee^{-1} for oral and contact routes of administration respectively.[2]

Spinosad achieved its first registration 1998 in the US for control of bollworms in cotton. Since then it has been registered globally on over 150 crops in North America, Latin America, Asia, Europe and Australasia under a variety of trademarks (such as Tracer, SpinTor, Entrust, and Success; registered trademarks of Dow AgroSciences LLC.). Spinosad is used in vegetables, fruit trees, turf, viticulture and ornamental cultivation to control lepidopteran larvae, thysanopteran some dipteran, coleopteran and hymenoptera pest species.

It is important that plant protection products (PPPs) are authorised for use only in ways that do not pose an unacceptable risk of harm to honeybees. Data should be obtained to enable the safety to be evaluated. An evaluation of the risks to honey bees has thus been undertaken in every country where the product is authorized based on the data available at this time.[3,4] Rules for risk evaluation have been updated since, in Europe as well as in the United States, which require that potential effects on the development of larvae and the behaviour of adults are considered at earlier steps of the risk

assessment process.[5,6,7] Beside the regulatory risk assessment, it is useful to look at the feedback from public research and from field surveys of apiaries in the countries where the product is used.[8,9]

This paper summarises the effects of spinosad to the honeybee (*Apis mellifera*) and updates the current knowledge on this substance. Data come from a range of Dow AgroSciences reports conducted to meet regulatory requirements. This dataset has been complemented with a survey of literature data and feedback from surveys on incidents, if any, involving honey bees in the countries where the product is authorized from the past 10 years of use.

2. Experimental methods

Semi-field studies were re-analyzed with regard to mortality data with the aim to characterize and address uncertainties on this parameter as a function of the application rate. Ten trials performed in France, UK and Germany were used. These studies (tunnel + cages) were conducted according to CEB method n°129 and EPPO guideline 170.[10,11] Application rates were ranging from 10 to 540 g a.s./ha, depending on the pest, sprayed onto flowering crops. The crops (*Phacelia*, oilseed rape, wheat with sugar solution) were selected to maximize foraging and exposure. These ten trials were analyzed for mortality rates at the key use rate of 96 g a.s./ha. These studies are summarized in table 1.

Tab. 1 Description of the semi-field trials used for the analysis of mortality rates at 96 g a.s./ha.

Year	Site type	Country	Crop	Application type	Rate (g a.s./ha)	Number bees/hive	Replicates
2004	Tunnel	France	*Phacelia*	During bee activity	144 96	10,000	1 2
2002	Tunnel	France	Winter wheat with sugar	During bee activity	96 144	10,000	2 1
2003	Tunnel	France	*Phacelia*	During bee activity Evening	144 144	10,000	1
2006	Tunnel	France	Rape seed	During bee activity During bee activity During bee activity During bee activity Evening	96 50 10 20 96	10,000-15,000	1
2006	Tunnel	France	Rape seed	Evening	96	10,000-15,000	1
2010	Tunnel	Germany	*Phacelia*	Evening	96 76	3,500-4,000	3
2010	Tunnel	Germany	*Phacelia*	During bee activity Evening During bee activity Evening	76 96 76 96	3,500-4,000 3,500-4,000	3 3
2010	Tunnel	Germany	*Phacelia*	Evening	76 96	3,500-4,000	3
2002	Tunnel	France	*Phacelia*	During bee activity	96 144	10,000	2 1
2000	Tunnel	United Kingdom	*Phacelia*	Morning	144 540	3,500-4,000	1 1

To allow for a comparison across the studies a mortality index was calculated according to the CEB method:
tox = Ma/Mb x Cb/Ca.

With Ma: mortality in test substance after treatment, Mb: mortality in test substance before treatment, Ca: mortality in control after treatment, Cb: mortality in control before treatment.

The Itox index was analyzed over time, per day and up to one week after treatment as well as the influence of the time of application *i.e.* when bees are present and when bees are not present (early in the morning or late in the evening). A toxic reference treatment (dimethoate) and a water treated control were included.

New tunnel tests were analyzed as well, with the aim to further describe effects of spinosad applied at 76 and 96 g a.s./ha. These studies were conducted according to EPPO guideline 170 and OECD.[10,12] Parameters relating to these studies are summarized in table 2.

Tab. 2 Description of the semi-field trials used for the analysis of effects on bees at 96 g and 76 g a.s./ha.

Test description		**Test 1**	**Test 2**	**Test 3**
Application rate		**76 and 96 g a.s./ha**	**76 and 96 g a.s./ha**	**76 and 96 g a.s./ha**
Application time	During bee activity	✓		
	Out of bee activity	✓	✓	✓
Assessment	Mortality	✓	✓	✓
	Foraging	✓	✓	✓
	Brood development	✓	✓	✓
	Behavior	✓	✓	✓
	Colony strength at 28 days		✓	✓
	Colony strength at 60 days	✓		

3. Results

Results of the semi-field studies are presented in figures 1 to 5. Mortality records expressed as Itox index 1 day after treatment (1DAT) as a function of the application rate and the activity of honey bees at the time of treatment were calculated. Itox index values were comparable (mean ranging from 1.10 to 2.41) in honey bees for treatments performed out of foraging activity, for all application rates i.e. from 76 g a.s./ha to 540 a.s./ha. For sprays during foraging activity, Itox index ranged from 1.25 to 3.12 (mean values) for application rates ranging from 10.08 g a.s./ha to 76 g a.s./ha. Spray at 96 g a.s./ha lead to a higher mean Itox index value (4.01) but the median of 11 values was slightly below 2. Both the mean (6.26) and median Itox value for honey bees exposed to a spray with 144 g a.s./ha were ca 6. When applied out of bee activity at 96 g a.s./ha Itox values less than 2.0 were observed daily from one to seven days after exposure. In contrast, dimethoate (400 – 600 g a.s./ha) applied during bee flight resulting in an Itox value of 20, by one day after application (figure 1).

Mortality in honey bees exposed to a spray application spinosad performed out of bee flight are reproduced in figure 2, and expressed as the mean number of dead bees after a spray of 76 or 96 g a.s./ha. Mortality records were comparable to the water treatment control before and after treatment for both application rates. Peaks of mortality were recorded in bees exposed to the acute toxic standard (Perfekthion: dimethoate) on the day of 1 day after treatment, and mean mortality was significantly higher than in the control up to 3 days after treatment.

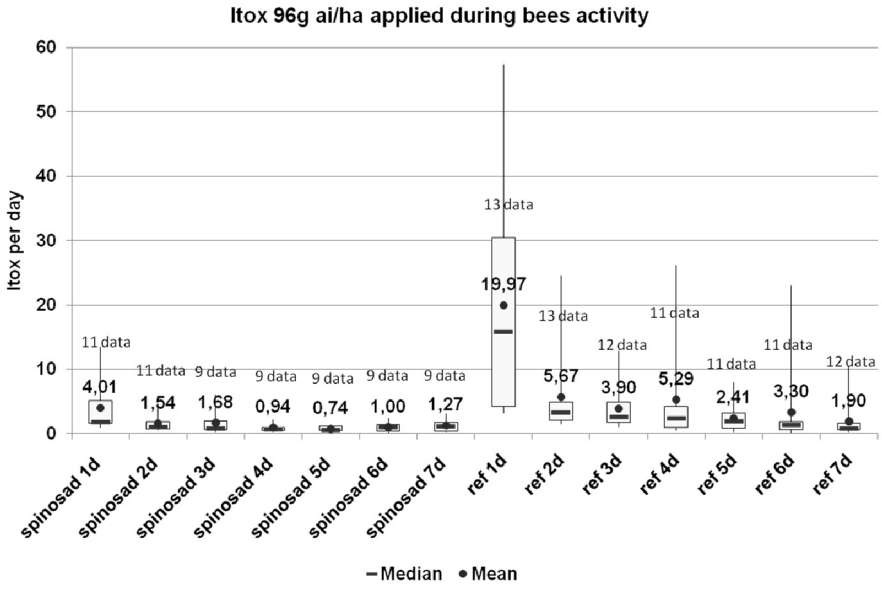

Fig. 1 Effects of spinosad on honey bees in tunnel tests when applied out of bee flight. Daily mortality expressed as a toxicity index (Itox). The ref(erence) is dimethoate. See text for explanation and calculation.

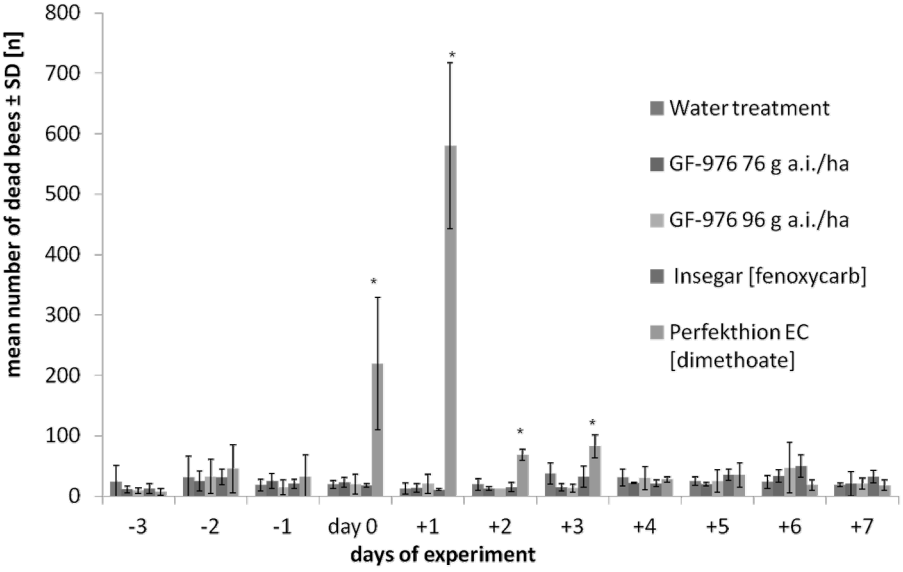

Fig. 2 Mortality to honey bees after exposure to applications of spinosad (GF-976) at 76 and 96 g a.s./ha applied out of bee flight under tunnel test conditions.

Foraging activity in honey bees exposed in the trials described in table 2 are reproduced in figure 3, and expressed as the mean number of bees /m² flowers. All records followed a similar trend with an increase in bee presence on flowers from day 3 pre-treatment to day 3 after treatment.

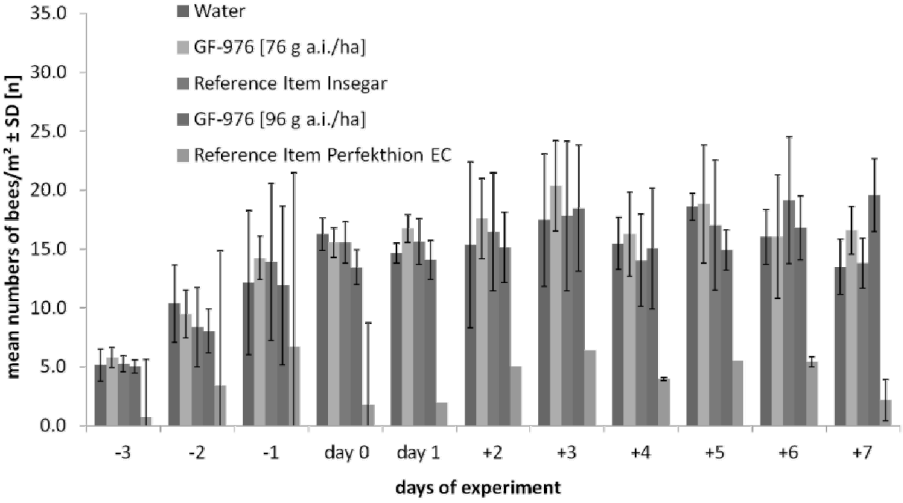

Fig.3 Foraging activity of honey bees after exposure to applications of spinosad (GF-976) at 76 and 96 g a.s./ha applied out of bee flight under tunnel test conditions.

Effects on brood were expressed as brood compensation index from day 6 to day 21 post brood fixing date. Results are reproduced on figure 4. The brood compensation index in honey bees exposed to a spray of spinosad at 76 or 96 g a.s./ha was comparable to the brood compensation index in honey bees of the water control, and as for the control it slightly increased within time. Both reference items Insegar and Perfekthion lead to a reduced brood compensation index at all assessment dates.

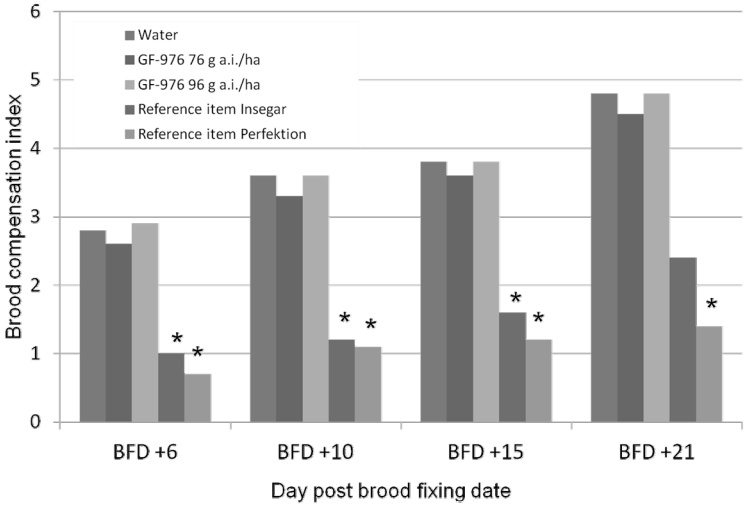

Fig. 4 Impact on brood of honey bees after exposure to applications of spinosad (GF-976) at 76 and 96 g a.s./ha applied out of bee flight under tunnel test conditions.

Finally, honey bee colony strength was measured as the mean number of bees/colony throughout the study, *i.e.* from day 6 before treatment to day 60 post treatment. Results are represented in figure 5. Colony strength was comparable in all treatments, with ca 3,000 bees/colony.

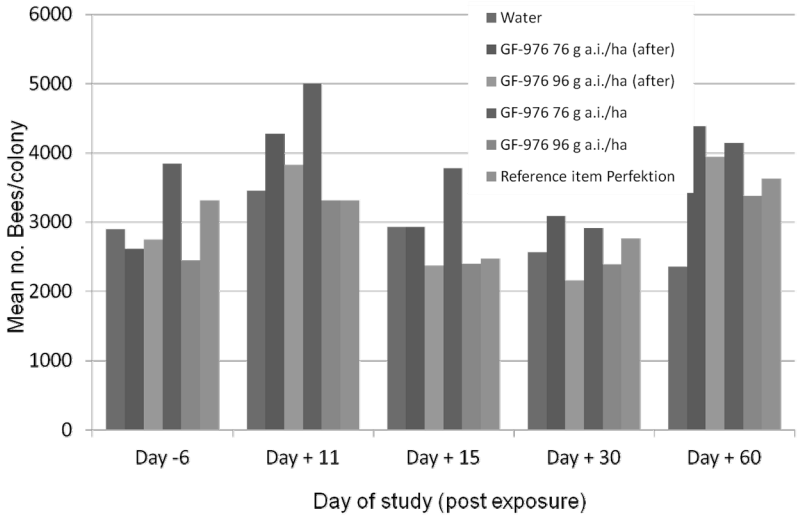

Fig. 5 Colony development and strength of honey bees after exposure to applications of spinosad (GF-976) at 76 and 96 g a.s./ha applied out of bee flight under tunnel test conditions.

4. Discussion

As previously stated, spinosad is intrinsically toxic to honey bees when tested in standard laboratory studies where worker honey bees are exposed orally (in sugar water diet) or topically to the test material. These laboratory tests are used in the screening step which is performed as an entry point in most risk assessment processes, as for example in the U.S or in Europe.[5,6] In the European risk assessment process, the screening step necessitates the calculation of Hazard Quotients (HQ), that compare toxicity to the application rate (HQ = application rate / LD_{50}). HQ values above the trigger value of 50 indicate a need for a refined risk assessment, which could involve higher tier testing (e.g. semi-field or field tests). In the case of spinosad, an application rate of 96 g a.s./ha results in HQ values ranging from 1,920 (oral HQ) to 1,950 (contact HQ) and thus indicate, in accordance with European regulation, a potential risk to honey bees "*unless it is clearly established through an appropriate risk assessment that under field conditions there are no unacceptable effects on honeybee larvae, honeybee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use*".[13]

The studies undertaken in tunnels on various crops, application periods and application rates aim at investigating the level of effects that are expected on honey bees under field conditions, as recommended by the European regulation. Accordingly, effects on honeybee survival, honeybee larvae, honeybee behaviour, and colony survival and development are monitored. The protocols used in the tunnel studies with spinosad allow for a more realistic appreciation of the level of acute effects that can be expected. The selection of cropped plants being attractive to honey bees and sprayed at flowering maximizes interactions of bees with the treated plants and any avoidance behavior can be observed and recorded. The inclusion of a water control and reference items allow to check that bees behaved as expected during the course of the experiment, and that effects of the product of well known toxicity are appropriately displayed during the course of the study. These tests

may or may not reproduce a direct contact of bees with spray droplets, depending on whether the spray is performed during or out of bee activity. However in any case, they reproduce the conditions of exposure of bee colonies that could be placed close to crops treated during flowering and can be considered as worse case with bees being forced to collect their food from the treated flowers.

With regards to the acute effects of fresh and dried residues of spinosad products on honeybees, Itox values indicated a dose – effect relationship when direct contact to spray droplets was allowed. In this analysis a threshold application rate for lethal effects of around 96 g a.s./ha was observed. When allowed to dry, residues of spinosad on flowers do not induce significant levels of honey bee mortality up to and including the highest application rate of 540 g a.s./ha. Assessments performed up to 7 days after treatment made during bee activity confirm the absence of mortality to foragers visiting treated flowers at 96 g a.s./ha. Therefore, this exposure rate can be considered as a threshold for immediate acute toxicity, but at which no long lasting acute toxicity is expected at this application rate or higher.

Such differences of honeybee responses in acute laboratory tests compared to tunnel test can be common and can be explained by the reduced availability of product residues on natural surfaces compared to direct contact in laboratory tests and due to the highly conservative nature of the tier I risk assessment. This phenomenon is well addressed for other non target arthropods and has lead to the development of laboratory tests exposing insects through natural substrates such as leaf discs, seedlings or soil samples as an intermediate experimental step between laboratory tests and semi-field/field tests.[14] For the two standard species used in the risk assessment procedure for non target arthropods, the parasitic wasp *Aphidius rhopalosiphi* and the predatory mite *Typhlodromus pyri*, moving from an inert surface to a natural surface reduces the residual toxicity by a factor of 9.8. Similarly, moving from inert surface exposure conditions to semi-field testing results in 45.1-fold lower residual toxicity (both factors assessed as the geometrical mean ratio of LR_{50} measured in laboratory testing on either inert or natural support and semi-field tests).[15] Spinosad is not systemic and its rapid dissipation from plant surfaces and exposure on natural surfaces (leaves and flowers) may explain the reduced residual toxicity observed.

Effects of a spinosad spray on the parameters representing sublethal effects on honey bees *i.e.* foraging behavior, brood and colony strength were also very limited in tunnel studies at the two application rates tested in detail (76 and 96 g a.s./ha). These observations are consistent with the mode of action of the substance. Spinosad acts by causing excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and finally paralysis. In addition, dissipation data indicate no persistence in environmental media and thus limited exposure duration. This is confirmed through the limited duration of residual toxicity in honey bees and other non target arthropods. Finally, the dissipation of spinosad does not lead to the formation of any active degradation products, which also limits the residual toxicity potency of the product.

The conclusions of the risk assessment appear to be confirmed by the very limited number of published data describing the potential effects of spinosad in pollinating species exposed in field conditions. With only three articles recorded in the last ten years. This could reflect the limited level of concern raised by the frequency of incidents and by the profile of the substance itself, for which the likeliness of long term or delayed effects that could have been missed in the studies having been performed in the context of the regulatory assessments is very limited.

5. Conclusions

The data presented address the potential effects of spinosad, applied as a spray to control various pests, on pollinators and more particularly to the honey bee. The analysis of higher tier studies on attractive crops having been performed for regulatory risk assessment purposes confirm the absence of significant impacts on honey bees even for treatments during flowering, provided that a delay allowing for droplets to dry is respected. Spinosad has been used all over the world for more than ten years in a wide range of crops without a recorded incident to pollinators. The feedback from field surveys is of particular importance as it reflects the potential impact of products on pollinators in realistic exposure conditions, which include interactions with other factors. Therefore the

confirmation of risk assessment outcome through survey data appears as a strong evidence of the actual limited impact of the product and its suitability for sustainable crop protection.

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Effectiveness of method improvements to reduce variability of brood termination rate in honey bee brood studies under semi-field conditions

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Abstract

Quantitative assessments of adverse effects of plant protection products on honey bee brood (*Apis mellifera* L.) may be carried out according to the methods given by the OECD Guidance Document No. 75 (2007). In recent years a number of studies displayed a strong variability in brood termination rates, a key endpoint. Due to these variances no definite conclusions regarding potential brood effects were possible, and the studies needed to be repeated. Due to this, attempts to improve the methodology were initiated by the Working Group 'Honey bee brood' of the German AG Bienenschutz. In 2011, honey bee brood studies adapted to these identified possible improvements resulted in better results compared to historical data. Based on the analysed results, the working group recommends to improve the method by using bigger colonies with more brood, using 4 instead of 3 replicates for better interpretation of data, starting the study early in the season, avoiding major modifications of the colonies shortly before application and using larger tunnels with effective crop areas preferably > 80 m^2. To carry out quicker brood cell assessments to reduce stress for the colonies, it is recommended to use digital brood assessment, which allows marking a higher number of cells (e.g. 200 to 400 cells).

Introduction

One recently used methodology to investigate the honey bee brood development under realistic exposure conditions are semi-field studies according to Schur *et al.* (2003) superseded by the OECD Guidance Document No. 75. In the course of the last few years it became obvious that the brood termination rate (= mortality of bee brood in selected cells on combs) was subject to a certain degree of variation, e.g. resulting in replicates with increased rates up to 100% in the control and reduced rates in the reference item group down to 21%. Additionally, a high variation between replicates within a respective treatment group occurred sometimes. The variability which was distinctly more present under semi-field conditions compared to a field method (Oomen *et al*. 1992) complicates the interpretation of results regarding potential brood effects of the test items with the outcome that some studies were regarded as invalid. The time between BFD 0 (Brood area Fixing Day) and BFD 5 turned out to be the most critical for such variations. To improve the current methodology, the Working Group 'Honey bee brood' of the AG Bienenschutz discussed some aspects of the method, e.g. timing of the experiment, crop area, size and composition of bee colonies, digital comb vs. acetate sheet assessment of brood cells in spring 2011 (Pistorius unpubl.; Becker & Lückmann 2011). The effectiveness of some of these factors were investigated in the subsequent season 2011. First improvements in the experimental procedure were identified which are presented in this paper and which may result in a proposal for an addendum to the existing OECD Guidance Document.

Material and methods

At the meeting in spring the 2011 the following measures for improvement were proposed, summarized in the table below(Table 1).

Tab. 1 Summary of measures to be improved in 2011 and given by the OECD GD 75

Parameter	According to OECD GD 75	Proposed improvement
Colony size	Small test colony (e.g. Mini Plus, nuclei), ~ 3,000 brood cells (≙ 750 cm^2) with brood in all stages, 1 food comb with honey and pollen, ~ 800 g (= ~ 6,000) worker bees	Colonies (nuclei) with 10 frames, 3-5 brood combs, high proportion of capped cells
Crop area	≥ 40 m^2 per tunnel	≥ 80 m^2 per tunnel
Reference item	Insegar, application rate ≥ 600 g/ha ≙ 150 g fenoxycarb/ha (single rate)	single or double rate
Timing	not specified	early start in the season
Irrigation	not specified	if the field is dry
Brood assessment	acetate sheet method; ≥ 100 cells/colony	digital photo method; ≥ 200 cells/colony

For the analysis of potential factors influencing the variability of bee brood studies the following data sets were used:

- period 2002 and 2010: 21 studies with 63 replicates in the control and 54 replicates in the reference item. The data analysis was presented by Becker & Lückmann (2011) and are called 'historical data' in the following.
- 2011: 13 studies (total of 50 replicates) for the control data and 12 studies (total of 43 replicates) for the reference item fenoxycarb (1 study was carried out with dimethoate and was therefore not considered); since in some studies the number of replicates in the toxic reference was lower than in the control, the total number of replicates was different in both groups.

The following endpoints were analysed for its relevance on brood termination rates (BTR) in the control:

- time of the year, expressed as 'day of the year'
- effective crop area
- colony strength

For the reference item the following endpoints were analysed for its influence on the brood termination:

- larval/pupal mortality

Only studies carried out on *Phacelia tanacetifolia* were considered.

Results

a) Influence of study initiation on brood termination rate in the control

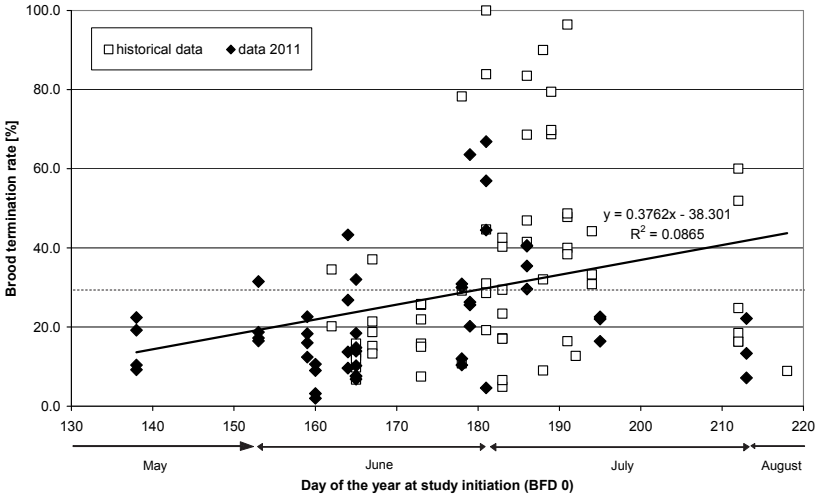

Fig. 1 Influence of study initiation (BFD0) on brood termination rate in the control

The analysis indicate that studies which were initiated before end of June (~ day 181) displayed an increased probability to achieve BTRs ≤ 30% in the control.

b) Influence of effective crop area on brood termination rate in the control

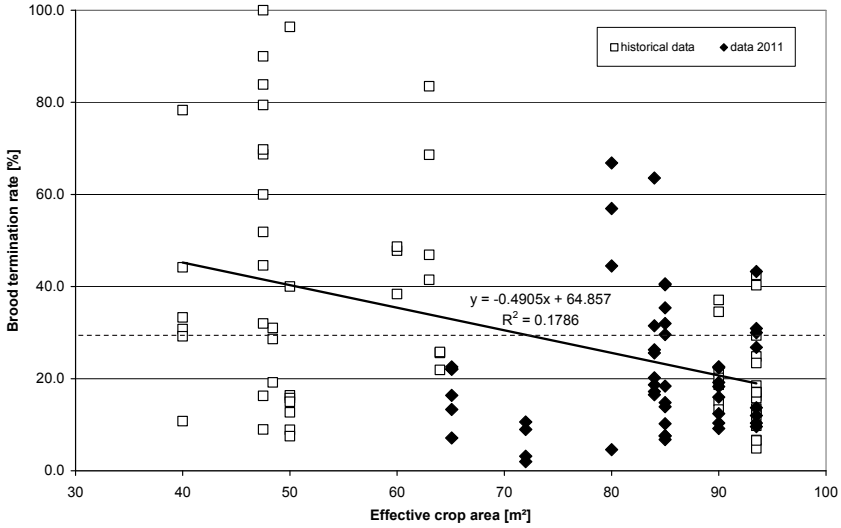

Fig. 2 Influence of effective crop area on brood termination rate in the control

The results show that studies with increased crop areas resulted in higher probabilities to obtain BTRs ≤ 30% in the control.

c) Influence of a combination of study initiation and effective crop area on brood termination rate in the control

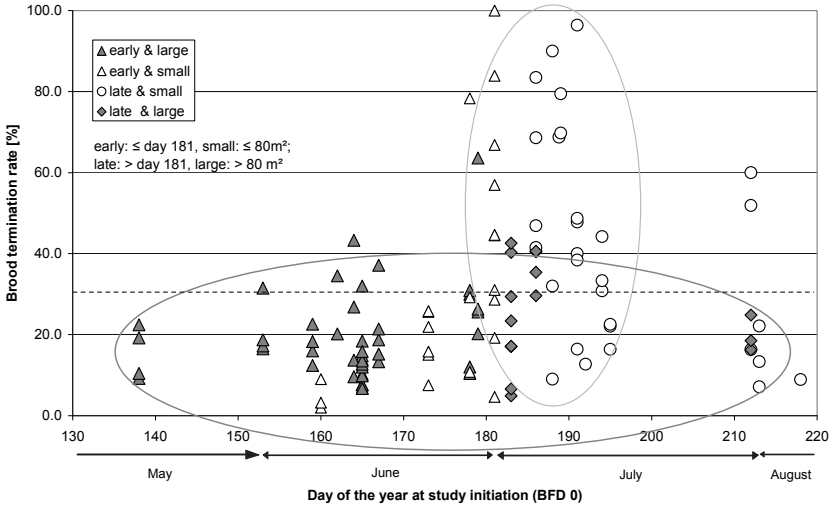

Fig. 3 Influence of study initiation and effective crop area on brood termination rate in the control

A combined analysis of the influence of study initiation and crop area shows that studies which were performed until end of June and/or were performed in tunnels with effective crop areas > 80 m² display higher probabilities to obtain BTRs ≤30%. In contrast, studies which were performed in tunnels with effective crop areas ≤80 m² and carried out after end of June display higher probabilities to result in BTRs > 30%.

d) Influence of colony strength on brood termination rate in the control

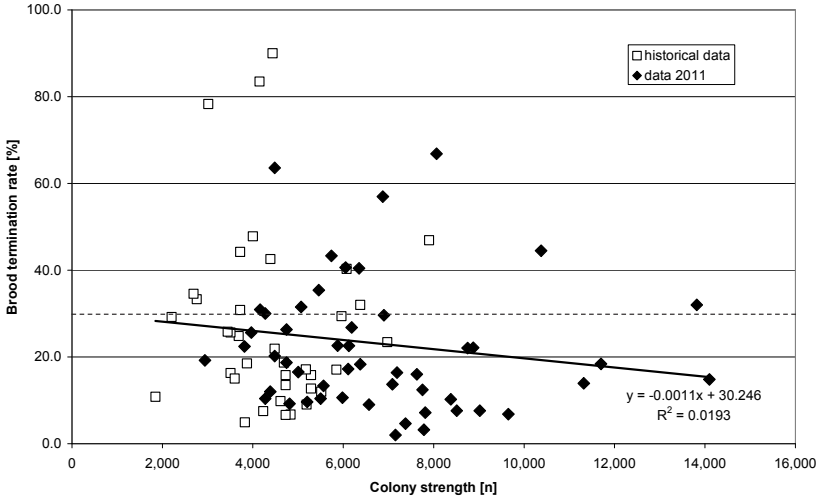

Fig. 4 Influence of colony strength on brood termination rate in the control

The analysis shows that studies which were performed with colony strengths higher than approximately 7,000 bees display higher probabilities to achieve BTRs ≤30%.

e) Influence of decreased brood termination rate on pupal mortality in the toxic reference

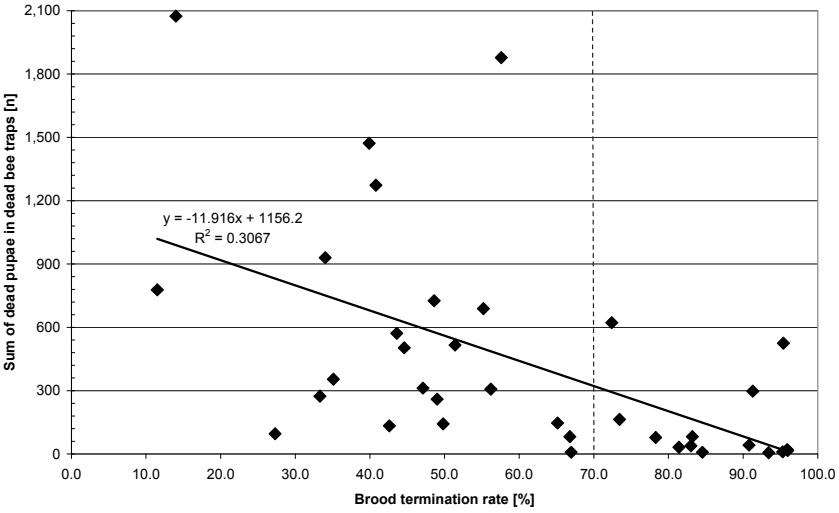

Fig. 5 Influence of decreased brood termination rate on pupal mortality in the toxic reference

The analysis shows that replicates with low BTRs, e.g. < 70% often display an increased pupal mortality, indicating that a sufficient exposure of the honey bees had took place and thus the suitability of the test system to detect potential effects on the bee brood. Only one replicate out of 22 (95.5%) replicates with BTR ≤70% displayed no increased pupal mortality.

Discussion and conclusion

The results show that the suggested improvements led to a reduction of BTRs and variability in the control group of honey bee brood studies in 2011, when compared to the historical data (see Table 2). Nevertheless the proposed measures cannot be a 100% guarantee to obtain always studies with BTRs ≤30%. Even using the proposed improvements (study initiated before end of June and/or use of large effective crop areas) studies in 2011 demonstrated that BTRs might be distinctly higher than 30% due to unknown reasons. For this reason it is important to analyse the importance of further factors in the future.

Tab. 2 Summary and comparison of descriptive statistics of historical and current bee brood studies

	Brood termination rates [%]			
	Historical data		**Data 2011**	
	Control (n=63)	**Toxic reference (n=54)**	**Control (n=50)**	**Toxic reference (n=43)**
Mean	34.7	76.8	21.7	63.7
SD	24.8	24.2	14.8	21.1
Median	25.9	83.4	18.4	65.1
Minimum	4.9	20.9	2.0	11.5
Maximum	100	100	66.8	100
Proportion of replicates ≤30% in the control and > 70% in the toxic reference	55.6	70.4	78.0	41.4*

*95.5% of these replicates display a pupal total mortality > 80 pupae during the entire study period

Based on the experiences and results obtained by the improved honey bee brood studies in 2011, the Working Group 'Honey bee brood' of the AG Bienenschutz recommends:

- to use bigger colonies with 3 to 4 brood combs, containing a high number of capped cells,
- to avoid major modifications of the colonies shortly before application,
- to use 4 instead of 3 replicates for better interpretation of data,
- to start the study early in the season, if possible,
- to use large tunnels, which provide effective crop area > 60 m^2, preferably > 80 m^2,
- to water the crop if dry conditions reduce nectar flow,
- to evaluated termination rate and pupal mortality in the toxic reference item.

Although the digital brood cell assessment has several advantages (e.g. quicker assessments, reduced stress for colonies) there was no correlation between BTR and the use of acetate sheets vs. digital brood cell assessment (Jeker et al. 2011 and 2012, Wang & Classen 2011) and the observation of higher numbers of marked cells. Nevertheless, the Working Group recommends:

- to use digital brood cell assessment,
- to observe 200 to 400 cells.

To verify the improvements and to identify possible additional ones, the work will be continued in 2012. At the end, the authors hope to give recommendations for an improvement of the OECD GD 75 intended to be developed until end of 2012.

Whereas the OECD GD 75 is used as a guidance for honey bee brood studies in the EU the recommendations are based on data from studies carried out in Central Europe, i.e. Germany and Switzerland. Therefore it will be necessary to include experience and recommendations also from others parts of Europe, e.g. Southern Europe.

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Statistical evaluation of regulatory honeybee trials - a pragmatic approach

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Abstract

The sequential risk assessment scheme for honeybees incorporates different levels of testing based on relevant guidelines and guidance documents (OECD 213, 214, EPPO 170 and OECD GD 75/Oomen & de Ruijter test). Within the regulatory process statistical analysis of data derived from these honeybee trials is required. As a consequence, the 'AG Bienenschutz' (a German national expert group on honeybee and plant protection) developed a recommendation for statistical analysis of data from regulatory honeybee trials.

Statistical parameters are well established in standard laboratory trials (OECD 213/214). Depending on the data base, dose response relations (LDx) can be calculated via *e.g.* probit analysis > moving averages > binomial distribution. A NOED (No Observed Effect Dose) can be determined by *e.g.* Fisher Exact Test (corrected according to Holm`s/Bonferroni)

During higher tier bee testing comprehensive data on mortality, foraging activity or generic brood assessment can be evaluated at several levels.

Pre-treatment level: the parameters of the different treatment groups (colonies) before the treatment are determined in order to ensure an equal distribution among the groups (*e.g.* by analysis of variance, Tukey test or multiple t-test, two-sided).

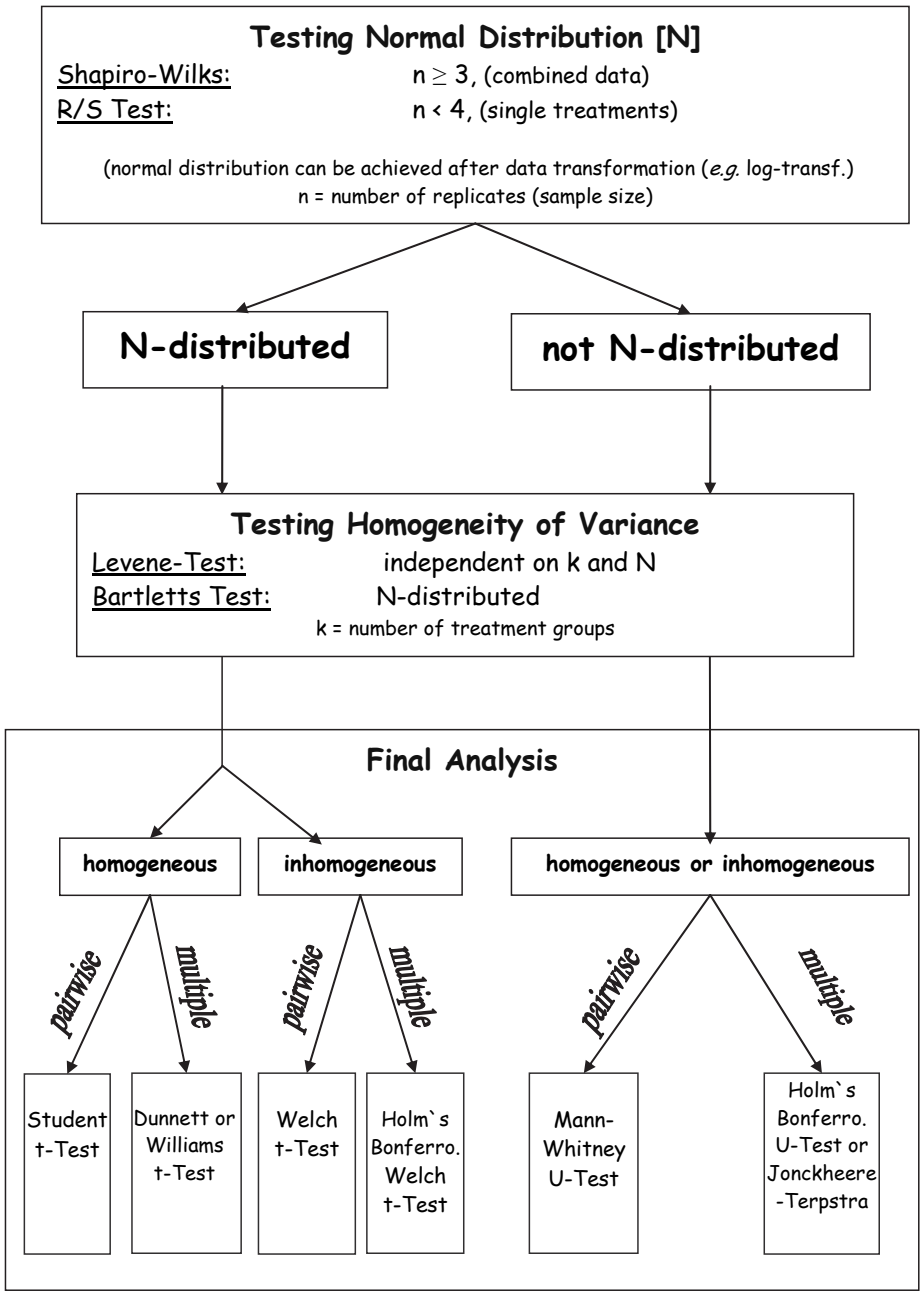
Post-treatment level: effects of the treatment can be analysed *via* comparison to the control group or to the situation before the treatment

A day-wise comparison of the treatment group(s) against the control as well as a comparison over distinct post-treatment intervals can be conducted (pairwise/multiple comparison, one-sided). The following flow chart gives guidance on these analyses of data derived from semi-field and field studies.

It should be generally acknowledged that statistical evaluation of semi-field and field trials is more challenging and sometimes limited. In view of these limits, the interpretation should primarily consider biological relevance of honeybee data. Therefore, a combination of statistical analyses and expert judgement is required.

In future, the current status will be complemented by statistically analysing brood parameters (*e.g.* brood termination rate) or combined data of field trials. Also, new approaches like evaluating the time dependency of data or the use of conceptual models (*e.g.* models excluding irrelevant variance components) will be considered.

Sequential Scheme for Statistical Analysis



IV. Honey bee poisoning incidents and monitoring systems

Monitoring effects of pesticides on pollinators – a review of methods and outcomes

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Abstract

Monitoring studies, in the context of the environmental assessment of Plant Protection Products (PPP) or pesticides, aim at getting feedback regarding the fate and/or effects of active substances and/or their relevant degradation products in/on the environment, when PPP are used under realistic conditions for crop protection. These studies complement the risk assessment performed in application of Regulation 1107/2009/EC, which aims at identifying the conditions of exposure of organisms in the environment, the conditions of occurrence of risks if necessary and propose appropriate risk mitigation measures.

In this context, monitoring studies may be implemented for different reasons. Firstly, they may complement the risk assessment in addressing possible uncertainties that may not have been fully addressed through field studies for time/space scale reasons. Secondly, they may address issues that are out of the scope of the pesticide regulation, as they can explore possible effects in the real life where organisms are subject to other stressors in addition to the product of concern. Thirdly, monitoring studies are also a way to validate or adjust the risk mitigation measures that may have been recommended as a condition of approval of the product. Monitoring studies may also be a source of data that could feed into risk assessment tools and calibrate ecological models. In the frame of Directive 2009/128/EC monitoring data might be used in connection with relevant risk indicators.

To date, there exists no harmonized guidance on monitoring methodology as monitoring has been implemented for the purpose of addressing the questions raised by regulatory authorities. There is no guidance either to define and implement monitoring studies that could be undertaken in a post-registration context as for those recommended in Directive 2010/21/EC. Work is currently being undertaken to address this issue in a dedicated working group of ICPBR for honey bees as well as in a SETAC Advisory Group on Monitoring Environmental Effects of Pesticides (http://www.setac.org/node/483) for terrestrial invertebrates.

This presentation will give an overview of the existing approaches for monitoring, ranging from (i) large scale incident reporting systems implemented at the national level, (ii) pilot apiary focused monitoring aimed at conducting more detailed investigations designed to adjust conditions of use of a product and (3) risk mitigation measures. A proposal on their analysis and respective input into risk assessment procedures and risk management planning is discussed.

Bee poisoning incidents in the Pomurje region of Eastern Slovenia in 2011

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Abstract

In spring 2011 a high number of bee poisoning incidents was recorded during the sowing of maize in the Pomurje region of Slovenia. The sowing of maize in Pomurje started two weeks earlier than normal following an extremely dry period. In contrast to other parts of Slovenia, maize sowing in Pomurje coincidenced with flowering of oilseed rape on adjacent fields. More than 2500 colonies were affected representing nearly 10% of bee keepers in the region. Samples were taken from dead bees, pollen, nectar, honey combs, flowering oilseed rape and maize seeds collected in the field and subsequently analysed for pesticide residues. The active substance clothianidin was most frequently found and was detected in 24 out of 51 samples (47%) of which 12 dead bee samples (86%). Another neonicotoid, thiamethoxam, was found in 4 samples (8%) of which 2 dead bee samples (14%).

The presence of clothianidin in dead bees and pollen in April 2011 is attributed to the sowing of maize treated with the insecticide Poncho Pro. The quality of seed coating for maize seeds treated with the insecticides Poncho or Cruiser collected at different suppliers was tested in a German laboratory. The results showed that abrasion of dust was below the maximum acceptable level of 2 g per 100 kg seeds for 18 out of 19 samples with one sample only slightly exceeding this level. The seed fulfilled the prescribed national quality standards for dust abrasion that were introduced following bee poisoning incidents in 2008. From 29 April 2011 onwards the use of maize seeds and oilseed rape seeds treated with Poncho Pro containing the active substance clothianidin and Cruiser containing the active substance thiamethoxam was prohibited. Further records of bee poisoning in May and subsequent findings of clothianidin and thiamethoxam in dead bees suggest that not all incidents can be attributed to the sowing of maize as route of exposure.

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Quantitation of neonicotinoid insecticide residues in experimentally poisoned honey bees

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Abstract

Background: In cases of poisoning incidents, the standard procedures consider the sampling of dead honeybees to define the active ingredients (a.i.) involved and to determine if the quantity of the residue present can be considered responsible of the death. However one should verify the loss of residues in the period from the discovery of the dead honeybees to their analysis. Since honey bee mortalities due to neonicotinoid insecticides have been recently reported, thiametoxam, clothianidin, imidacloprid residues were determined in experimentally poisoned honey bees.

Results: Oral and indirect contact trials were carried out for each pesticide, using commercial formulations. Honey bees that died during the trials were stored at -18 °C and analyzed through a LC-MS/MS analytical procedure adapted from AOAC methods. The quantity of insecticide residues that were detected resulted much lower than the administered residues. Honey bees that did not die within six hours from the trial start were also analyzed and the quantities of insecticide residues resulted much lower than those found in the dead honey bees.

Conclusion: On the basis of these results, the determination of the Subsequent Residue Level or of a similar index should be required during the normal procedures of authorization for the use of pesticides.

Keywords: poisoning incidents, clothianidin, imidacloprid, thiametoxam

Introduction

The threat posed to honey bees (*Apis mellifera* L.) and other pollinators by most insecticides and some fungicides and herbicides are generally recognized;[1,2] therefore risk assessment schemes had been developed and are currently implemented so as to reduce honey bee losses and inform correctly the users on possible side effects of pesticides.[3,4] Nevertheless unexpected contamination routes, like the harvesting of microencapsulated insecticides by pollen foragers[5,6] or, more recently, the release of dusts from insecticide coated seed when drilled with inadequate machinery,[7-12] may arise; moreover, the incorrect or unauthorised use of pesticides may also result in severe honey bee losses.

In the last decades, different approaches for detecting and monitoring such incidents were devised.[13-16] In any case, the sampling of dead honeybees to single out the active ingredients (a.i.) involved is a crucial point of any procedure, especially when the amount of residue detected is taken into account to determine if the a.i. can be considered responsible for honey bee death. However one should also consider the loss of residues in the period from the discovery of the dead honeybees and their analysis. In former times the determination of the Subsequent Residue Level (SRL) was proposed to quantify to a certain extent these losses,[17] but a somehow more sensitive method could be advisable.

In the recent years, both pollinator decline and colony collapse disorder (CCD) have become serious concerns that could ultimately impair the production of many crops in Europe and in the United States.[18-22] Pesticide (neonicotinoids in particular) use has been identified as a potential contributing factor to CCD and may be one of the environmental stressors contributing to pollinator declines, along with other factors such as new and re-emerging pathogens, habitat loss, pests, and nutritional stress. Most neonicotinoids show a very strong toxicity to pollinating insects and in particular to the honey bee, causing also other effects which are seldom easily identifiable, such as behavioural disturbances, orientation difficulties and impairment of social activities.[2,22-28] Although potential problems could be reduced by appropriate mitigation practices,[1,29] alarming bee mortalities, clearly

due to the use of neonicotinoids either for seed dressing or crop spraying, were recorded in many countries during the past few years, and various limitations in their use were enforced.[9,22]

Therefore, it seemed appropriate to determine clothianidin, imidacloprid and thiametoxam residues in experimentally poisoned honey bees in order to have an estimate of a.i. losses from treatment to sampling and so to gain a better understanding of the role played by these neonicotinoids in poisoning incidents.

Experimental methods

The research was carried out in the laboratory using methods developed at the Di.Va.P.R.A. to test acute oral toxicity (AOT) and indirect contact toxicity (ICT) on honey bees.[30,31] Commercial formulations available in Italy were used: Dantop 50 WG (Isagro Italia s.r.l., Milano, Italy): 50.0% pure clothianidin, hydro dispersible granules; Confidor 200 SL (Bayer S.p.A., Milano, Italy): 17.8% pure imidacloprid, concentrated liquid soluble in water; Actara 25 WG (Syngenta Crop Protection S.p.A., Milano, Italy): 25.0% thiametoxam, hydro dispersible granules. Tests were performed in a dark room at 28-30 °C and 70% relative humidity. Compounds were tested at the highest concentration recommended on the label for crop treatment (field concentration) and they were gradually diluted down to the concentration that caused a mortality not significantly different from that of the untreated controls. Significance was verified with the chi-square test. For each test 40 workers from a single colony were used and the tests were replicated with honey bees from different colonies. Altogether six *A. m. ligustica* colonies from Piedmont (Italy), two *A. m. carnica* colonies from Croatia, and one *A. m. mellifera* colony from France were used. Tested a.i. concentrations and the relative replication number are reported in table 1 for AOT and in table 2 for ICT.

Tab. 1 Amounts of neonicotinoids recovered from dead honey bees after ingestion of decreasing a.i. doses.

			Mortality		Detected amount (DA)	DA/ID
Concentration (mg L $^{-1}$)	Ingested dose (ID) (ng honey bee $^{-1}$)	Replications (n)	24 h mean ± st.dev. (%)	48 h mean ± st.dev. (%)	mean ± st.dev. (ng honey bee $^{-1}$)	mean ± st.dev. (%)
clothianidin						
75	2625	1	100.0	100.0	26.60	1.01
7.5	262.5	1	96.7	100.0	5.40	2.06
1.5	52.5	4	98.7 ± 3.0	99.3 ± 1.5	2.52 ± 1.36	4.82 ± 2.60
0.75	26.25	5	94.0 ± 13.4	94.7 ± 11.9	2.36 ± 1.20	9.00 ± 4.58
0.375	13.125	7	91.5 ± 3.8	92.2 ± 3.0	0.80 ± 0.76	6.10 ± 5.79
0.15	5.25	5	70.8 ± 19.2	71.2 ± 18.7	0.94 ± 0.57	17.31 ± 11.07
0.075	2.625	5	53.4 ± 28.7	56.6 ± 26.8	0.45 ± 0.29	16.11 ± 9.26
0.0375	1.3125	3	20.0 ± 13.7	21.7 ± 13.7	0.30 ± 0.01	22.86 ± 0.76
imidacloprid						
150	5250	3	100.0 ± 0.0	100.0 ± 0.0	159.00 ± 1.00	3.03 ± 0.02
3	105	4	45.8 ± 11.1	82.5 ± 13.2	7.73 ± 2.35	7.36 ± 2.24
1.5	52.5	4	43.8 ± 4.5	61.3 ± 20.7	1.97 ± 1.38	3.75 ± 2.63
0.75	26.25	4	42.3 ± 30.6	51.3 ± 30.1	0.20 ± 0.31	0.78 ± 1.18
0.3	10.5	3	26.7 ± 9.5	26.7 ± 9.5	<0.005	<0.047
thiametoxam						
100	3500	2	100.0 ± 0.0	100.0 ± 0.0	11.61 ± 10.46	0.33 ± 0.30
20	700	1	100.0	100.0	3.24	0.46
10	350	2	100.0 ± 0.0	100.0 ± 0.0	3.56 ± 3.73	1.02 ± 1.07
5	175	1	100.0	100.0	2.30	1.31
2	70	3	100.0 ± 0.0	100.0 ± 0.0	0.71 ± 0.59	1.02 ± 0.85
1	35	4	99.5 ± 1.1	99.5 ± 1.1	0.83 ± 0.36	2.37 ± 1.02
0.5	17.5	8	97.2 ± 5.2	97.4 ± 5.3	0.38 ± 0.23	2.19 ± 1.32
0.2	7	5	67.8 ± 8.0	78.1 ± 13.4	0.01 ± 0.01	0.13 ± 0.13
0.1	3.5	7	41.5 ± 37.6	43.9 ± 37.1	0.005 ± 0.002	0.13 ± 0.05
0.05	1.75	4	7.4 ± 8.7	8.5 ± 9.8	<0.005	<0.286

Tab. 2 Amounts of neonicotinoids recovered from dead honey bees as a consequence of indirect contact tests.

		Mortality		Detected amount
		24 h	48 h	
Concentration (mg L $^{-1}$)	Replications (n)	mean ± st.dev. (%)	mean ± st.dev. (%)	mean ± st.dev. (ng honey bee $^{-1}$)
clothianidin				
75	1	100.0	100.0	59.00
37.5	1	100.0	100.0	28.00
15	2	98.3 ± 2.4	100.0 ± 0.0	5.97 ± 0.24
7.5	3	67.3 ± 0.9	76.7 ± 9.4	1.65 ± 0.66
3.75	2	38.8 ± 10.1	51.3 ± 7.5	0.67 ± 0.52
imidacloprid				
150	1	27.5	60.0	21.00
75	1	7.5	32.5	15.60
30	1	20.0	40.0	10.56
15	1	4.3	22.9	0.91
thiametoxam				
100	1	100.0	100.0	27.00
20	2	98.3 ± 2.4	100.0 ± 0.0	2.02 ± 1.15
10	4	91.0 ± 13.0	98.5 ± 1.4	3.36 ± 1.92
5	4	48.6 ± 15.3	64.9 ± 22.8	1.46 ± 0.52
2	2	3.4 ± 3.4	6.7 ± 6.0	0.27 ± 0.01

The ingested dose was calculated from the tested a.i. concentration taking into account that in AOT tests each honey bee ingests 35 µl of sucrose syrup during the allowed one hour feeding period;[30] on the contrary a.i. amounts that penetrate into the honey bees during the ICT tests could not be evaluated.

Tests started at 12.00 h; mortality was checked at 13.00 h (AOT only), 15.00 h, and 18.00 h on the first day of the trial and at 9.00 h, 12.00 h, 15.00 h, and 18.00 h during the following days. The trials lasted three days and mortality percentages at 24 and 48 h were calculated after correction for mortality of the untreated controls.[32]

At every check, dead honey bees were removed from the cages, stored at -18 °C, and shipped frozen to the analytical laboratory for residue determination through a LC-MS/MS analytical procedure adapted from AOAC methods 2007.01 2007;[33] a LC-MSMS apparatus API 3200, AB SCIEX, 110 Marsh Drive, Foster City, California 94404-1121, USA was used. LOD and LOQ were 0.01 µg kg $^{-1}$ and 0.05 µg kg^{-1} respectively. Linear regression of a.i. detected amounts on tested concentrations was calculated with the statistical package PAST;[34] the Ordinary Least Squares algorithm was used and the regression line was forced through zero.

In order to verify if a.i. residues in honey bees that did not die were different from those present in dead ones, special AOT tests were performed. They were started like standard tests, but dead honey bees were removed from the cages six hours since trial start and all surviving honey bees were captured. Dead and alive honey bees were separately frozen at -18 °C and submitted to chemical analysis.

Results

During the trials, the honey bees showed obvious symptoms of poisoning, such as shaking and tremors, uncoordinated and uncontrolled movements, inability to take up a correct position of the body, and prolonged frenetic movements of the legs and rotation when being in the supine position. Direct observation of the behaviour of the honey bees in cages proved that it was transient at a lower concentration. Moreover, the highest concentrations caused extensive vomiting by honey bees, especially in AOT tests.

The amounts of neonicotinoids recovered from honey bees that died in AOT tests are reported in table 1. Replication number is greater for doses causing mortalities between 100 and 50 % because

the resulting variability tends to be higher at these doses. The detected amount/ingested dose ratio is generally rather low and shows great variations between the three a.i. tested and also between doses of the same a.i.; nevertheless regression lines show fair correlations between tested concentrations - and thus ingested doses - and a.i. amounts detected in dead honey bees, while the probability that the two variables were not correlated is extremely low (figure 1).

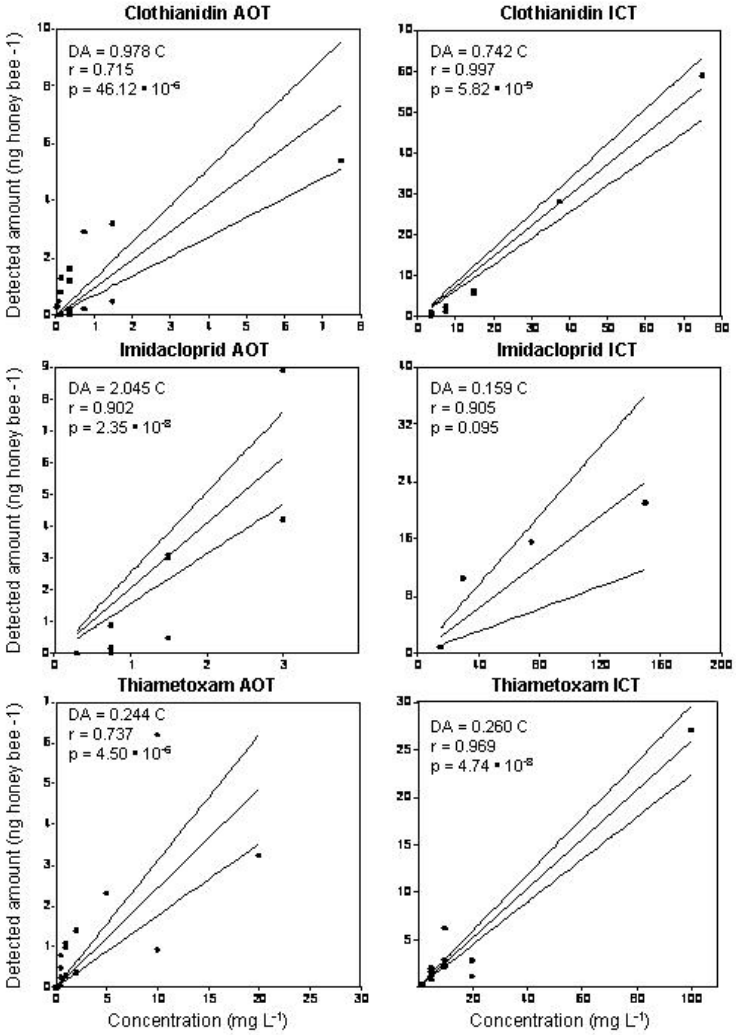

Fig. 1 Linear regression of neonicotinoid amounts recovered from dead honey bees on tested concentrations; 95 % "Working-Hotelling" confidence bands for the fitted lines, Pearson's *r* correlations, and the probability (p) that concentrations (C) and detected amounts (DA) are not correlated are given.

Tests on honey bees taken from colonies belonging to different *A. mellifera* subspecies, although not carried out systematically for every a.i. and every dose/concentration, yielded similar results. The most complete series of data, pertaining to thiametoxam AOT tests on an *A.m.carnica* strain compared with one *A.m.ligustica* colony is shown in figure 2. A Wilcoxon exact test was performed on these data with the statistical package PAST (Hammer, 2001): the probability that the medians of these data are the same is $p = 0.152$.

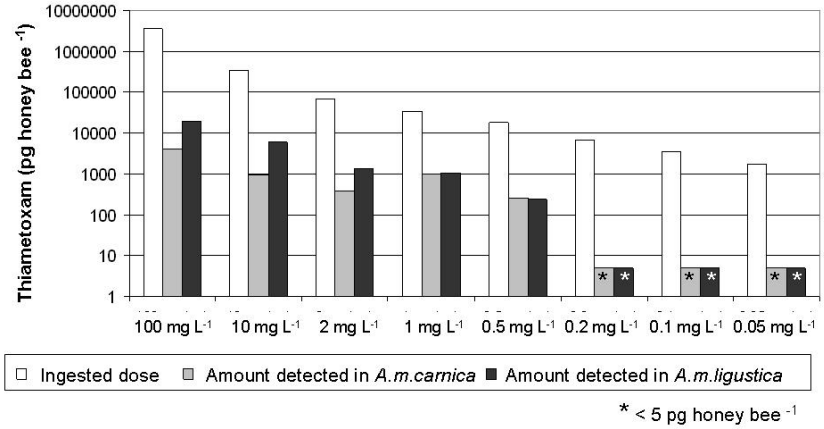

Fig. 2 Amounts of thiametoxam recovered after ingestion of decreasing a.i. doses from dead honey bees that were taken from one *A.m.carnica* and one *A.m.ligustica* hive.

Neonicotinoid amounts recovered from dead honey bees were significantly higher than those recovered from surviving honey bees in each AOT test (figure 3). A Wilcoxon exact test was performed on these data with the statistical package PAST (Hammer, 2001): the probability that the medians are the same is $p = 0.046$. Moreover, half of the alive honey bee samples yielded residue amounts below LOQ.

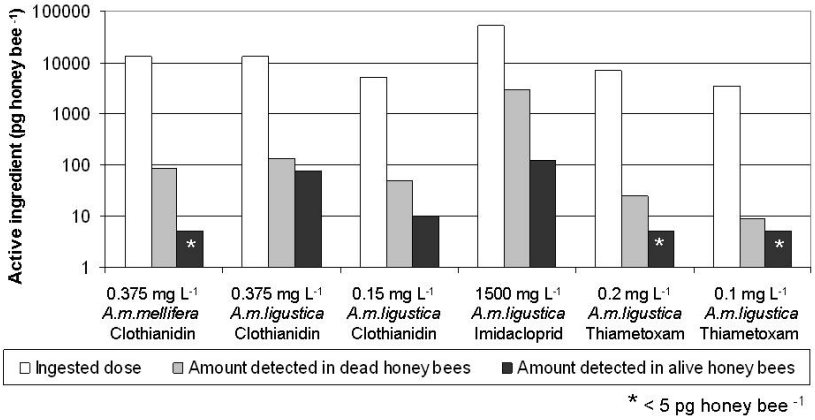

Fig. 3 Amounts of neonicotinoids recovered from dead and living honey bees after ingestion of identical a.i. doses.

The amounts of neonicotinoids recovered from honey bees that died in ICT tests are reported in table 2. Replication number is lower than in AOT tests, but also for ICT most replications were carried out at concentrations that caused intermediate mortalities. The correlation between tested concentrations and a.i. amounts detected in dead honey bees for clothianidin and imidacloprid is better than in AOT tests and the probability that the two variables were not correlated is even lower, while imidacloprid yielded poorer results (figure 1).

Discussion

The poisoning symptoms which were observed during the trials may have reduced a.i. intake by the honey bees mainly in AOT tests and at higher concentrations and may be considered responsible for the somehow irregular results observed. Conceivably that was due to the observed vomiting phenomena and therefore a.i. amounts recovered from dead honey bees which were treated with

field doses were not considered for the calculation of AOT regression lines. Since in ICT honey bees had no opportunity of getting rid of the insecticide through vomiting, results showed a greater regularity and all tested concentrations were used in computations. On the contrary, the ratios between a.i. amounts detected in dead honey bees (DA) and ingested doses (IG) can be calculated only in AOT tests.

The graphs that show regression lines of neonicotinoid amounts recovered from dead honey bees on tested concentrations and the relative 95 % "Working-Hotelling" confidence bands (figure 1) could be used to assess the actual exposure – or a 95 % confidence exposure interval – for honey bees that died on the occasion of a poisoning incident, provided that these honey bees were correctly sampled, stored, handled and analyzed. Such an approach is somehow different from the SRL calculation, which consists in determining the residues present in honey bees after dosing them with one LD_{50} of the insecticide under investigation,[17] and could be readily implemented if dead honey bees were collected and analyzed during the routine determination of AOT LD_{50} of new molecules.

When comparing results of toxicity tests on honey bees performed by different laboratories, substantial differences often emerge and a different genetic response to toxicity tests is sometime advocated to explain such uneven results.[35,36] In the present test, the relevant variation between tested colonies, which belongs to different subspecies and strains, do not appear, but more thorough investigations on response variability between and within subspecies would be welcome.

The rather low DA/IG ratios obtained in the tests could be due to the low stability of the molecules and/or to metabolite formation; both phenomena are well documented[36-38] and probably occurred during the trials. Nevertheless the sharp difference in a.i. amounts recovered from dead and alive honey bees in the same test, even giving allowance for a somehow uneven a.i. intake by different honey bees at the very low concentrations tested, suggests that surviving honey bees were able to better metabolize the insecticide. To better understand the fate of tested neonicotinoids, it would be advisable to determine also the relative metabolites in the dead honey bees, but, to do so, a substantial increase in sample size should be required.

Conclusions

In cases of poisoning incidents, the mere quantitation in dead honey bees of the a.i. involved do not allow to define the real amount of a.i. honey bees were exposed to; therefore it is often impossible to prove that the a.i. is responsible for the observed losses. SRL determination or the implementation of some more refined indexes should be advisable during the normal procedures of authorization for the use of pesticides, so that field survey results could be interpreted correctly.

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Project 'APENET: monitoring and research in apiculture'

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Abstract

APENET: monitoring and research in apiculture', is a two year research project funded in 2009 by the Italian Ministry of Agriculture and completed in September 2011. The main objectives of APENET were:

to give explanation about the colony losses and high bee mortalities reported in the recent years in many countries;

to evaluate the efficacy of the recently introduced law, regarding the suspension of seed dressing, for mitigation of colony losses.

The project was structured in 7 research topics:

1: Monitoring honeybee state of health in Italy;

2: Improving the reduction of the dust dispersion during maize sowing;

3: Honeybees and agrochemicals;

4: The honeybee colony as a biosystem and its health: evaluation of the effects of the micro- and macro- environmental factors and their influence on pathogen development;

5: Evaluation of the synergic effect of different factors on honeybee health;

6: Honeybee pathologies;

7: Studies on the immune response of *Apis mellifera* and its modulation by means of biotic and abiotic stress.

Six universities and several other research institutions from all over Italy were involved. Collaboration of beekeeper organisations was also very important. The present contribution is aimed to expose the main results obtained during the APENET project.

Dust drift during sowing of maize - effects on honey bees

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Abstract

Background: Bee poisoning incidents in southern Germany in 2008 revealed drift of insecticidal dusts of treated maize seed during sowing on adjacent areas with flowering bee forage plants as a considerable route of exposure. Consequently, improvements have been proposed for seed dressing quality regarding dust abrasion taking into account residue content of dust and for the sowing techniques as possible risk mitigation measures. To assess potential effects on honey bee colonies following insecticidal dust drift on adjacent non-target areas, in 2010 and 2011 two large-scale drift experiments were carried out during maize sowing using seed batches from two different years (2010: seed batch from 2008; 2011: seed batch from 2011).

Results: Despite improvements of seed dressing quality regarding dust abrasion comparing the two seed batches (Heubach value 0.86 in 2010 and 0.45 in 2011) and the use of a precision air seeder with drift reducing deflector, in both experiments bee mortality was clearly increased, especially in semi-field conditions.

Conclusions: Drift of insecticidal dusts during sowing of maize may result in a risk for honey bees in field conditions. To exclude adverse effects on bees, especially during sowing of maize further improvements of seed treatment quality and machinery is needed.

Keywords: maize, seed treatment, clothianidin, dust drift, honey bee poisoning

Introduction

Systemic insecticides like neonicotinoids are commonly used as seed treatments in important crops like maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.) and winter oil seed rape (= WOSR; *Brassica napus* L.)[6]. Neonicotinoids are used in maize for control of wireworms (*Agriotes* spp.), cutworms (*Agrotis* spp.), Western Corn Rootworm (*Diabrotica virgifera* Le Conte) and of aphids and leafhoppers[1].

In 2008, in parts of southern Germany treatment of maize seeds with a high application rate of clothianidin (125 g a.s./ha[7]) was used to control the larval stages of the emerging quarantine pest Western Corn Rootworm. During sowing of maize, more than 11.500 honey bee colonies from about 700 beekeepers in the Upper Rhine valley, Baden-Wuerttemberg and approximately 460 colonies from 36 beekeepers near Passau, Bavaria showed symptoms of insecticide poisoning[7].

The temporal and spatial link between affected bees and sowing of maize was soon confirmed by residue analyses of samples of dead bees and of flowering bee forage plants from adjacent areas[7].

Poor seed treatment quality and the use of precision air seeders led to drift of dust containing insecticides which contaminated flowering winter oil seed rape, fruit trees and weeds (e.g. *Taraxacum* sp.). Contaminated flowers are considered to be the most important route of exposure of bees to toxicants[2].

Since 2010, two large-scale drift experiments during sowing of clothianidin-treated maize were carried out to investigate the link between abrasion potential of treated maize seed (determined by Heubach-values[4]), dust drift and resulting residues in adjacent flowering crops as well as the effects on honey bees exposed to these contaminated flowering crops.

Experimental methods

Both drift experiments, 2010 and 2011, were performed on wind-exposed areas near Braunschweig (Wendhausen and Lucklum). Due to the strong dependence on local wind conditions and a limited availability of test fields, two different field set-ups were used (Fig. 1).

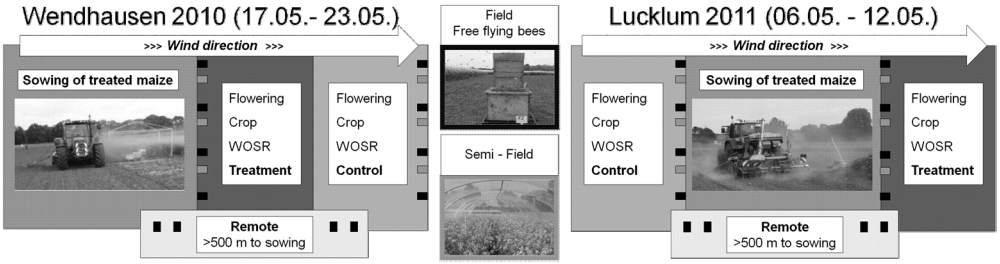

Fig. 1 Field set-up in drift experiments during sowing of maize in 2010 and 2011

In the preceding autumn 2009 and 2010 at both plots, winter oilseed rape (*Brassica napus* L.) was sown for the drift trials. In spring, this WOSR served as a flowering bee attractive crop in the drift experiments during sowing of maize. The test site Wendhausen in 2010 was located in an area without notable alternative bee attractive crops, while the test site nearby Lucklum in 2011 was in a region with a high portion of flowering WOSR.

In both drift experiments a total of 18 colonies were used (Hohenheimer Einfachbeute, Zander, 10 frames). Each contained at least six combs with brood in all stages (eggs, larvae and sealed brood) including sufficient honey and pollen storage (Tab. 1).

Tab. 1 Average numbers of bees in colonies in the two drift experiments before the start

Variants	Wendhausen 2010	Lucklum 2011
Field / Remote (n = 4)	~17.000	~8.500
Field / Control (n = 4)	~21.500	~11.500
Field / Treatment (n = 4)	~16.000	~11.000
Semi-Field / Control (n = 3)	~10.00	~7.500
Semi-Field / Treatment (n = 3)	~11.500	~9.500

Two days before set-up of hives at the trial site (five days before start of the experiments), an assessment of the colony and brood nest size was carried out using the Liebefelder evaluation method[5] to determine the development status of the experimental colonies. Further assessments of colony and brood development were made between the 14 -17th day and 27-30th day after the initial evaluation.

Since highest risk for foragers is expected in adjacent flowering crops next to the sown acreage, in both 'worst case' scenarios bee colonies were placed in semi-field and field and set-up along the edges of the sown area.

In the field four colonies each were placed at distances of 0 m (='Treatment'), 50 m (= 'Control') and more than 500 m (='Remote') to the windward directed border of the WOSR field (Fig. 1) In the semi-field approach in both variants 'Treatment' and 'Control' three gauze-covered tunnels (16 x 6 m) were set up at the field edge on the flowering WOSR crop and each provided with a bee colony (Fig. 1, 'Semi-Field'). Due to the limited food supply in the tents, smaller bee colonies were used in semi-field, while in field larger colonies were used (Tab. 1).

In order to assess bee mortality, each hive was fitted with a modified dead bee trap[3] (Type 'Gary', W x L x H: 435 x 400 x 300 mm; mesh size: 8 x 8 mm; Fig. 1, 'Field').

Before bee flight activity, the hive entrances of semi-field colonies were closed and the gauze removed from the tents. After sowing, the tents were covered again and the hives reopened. The hive entrances of the bee hives in the field experiment were not closed during sowing. In both experiments treated maize of different quality was sown using a precision air seeder with drift reducing deflector (Tab. 2).

Tab. 2 Seed treatment of maize, abraded dust and sowing technology

Experiment	Wendhausen 2010	Lucklum 2011
Arable crop	Maize (*Zea mays* L.)	Maize (*Zea mays* L.)
Seed treatment with application rate	Poncho Pro (clothianidin: 125 g/ha)	Poncho 600 FS (clothianidin: 50 g/ha)
Content of active substance clothianidin in Heubach filter dust [%]	10.6	19.1
Average Heubach-Value [g/100 000 kernel]	0.86	0.45
Sowing technology	pneumatic precision air seeder, type Kverneland Accord Optima NT e-drive, converted and drift reduced	

As maize sowing was conducted during bee flight activity, in the field experiments foragers leaving and entering the hives were continuously exposed to contaminated dust, both directly to dust in the air and indirectly through contaminated pollen and nectar of flowers of adjacent downwind WOSR as well as to uncontaminated flowers in the upwind area. As the colonies in the semi-field experiments were not actively foraging during sowing, the foragers were only exposed to contaminated nectar and pollen and dusts deposited on flowers after reopening the entrances of the hives but not directly exposed to the dust drift.

In both drift experiments sampling and assessment of dead bees (workers) from bee traps (Type Gary) was conducted once a day starting the day before sowing (= DBS) until the 6th - 7th day after sowing 5 times a day on the day of sowing date and 3 times the first day after sowing. Samples of dead bees were separately packed and stored at -20 °C until transport to the analytical laboratory of the JKI in Berlin-Dahlem.

Results and discussion

Semi-field trials

The semi-field variant is a 'worst-case' scenario, which allows bees to forage only on limited crop inside the tent.

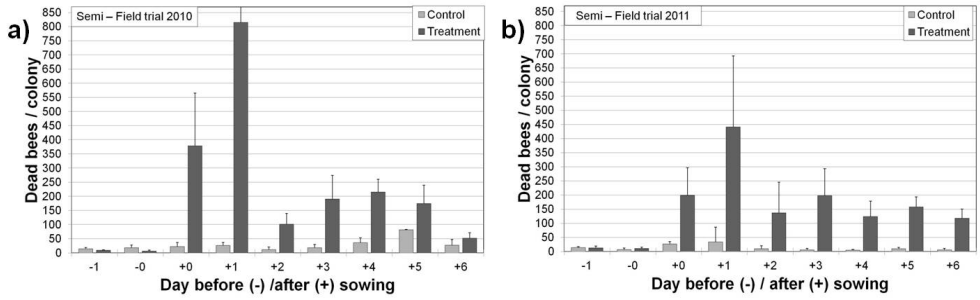

Fig. 2 Mortality in semi-field colonies (n = 4) during the drift trial a) using maize seed from 2008 (high Heubach-value, Wendhausen 2010) and b) from 2011 (with low Heubach-value, Lucklum 2011)

The semi-field variants 'Treatment' of both experiments showed similar trends of mortality within the experimental period compared to the field approaches, but at a much higher level (Fig. 2 and Fig. 3). After a peak in mortality was detected in the dead bee traps at the assessments in the morning of the first day after sowing (2010: 815 (Fig. 2a); 2011: 440; (Fig. 2b)), the mortality in 'Treatment' colonies was lower in the following days but still showed a treatment related, clearly increased mortality until the end of the experiment.

The mortality in the 'Control' variant was on a low level throughout the study. In 2010, a slightly increased mortality on DAA+5 was likely caused by climatic conditions. In 2011 (Fig. 3b.) , on the day of sowing and on the first day after sowing the mortality was slightly increased; this can probably be attributed to slightly increased stress for colonies due to enclosure until sowing process was finished in the late forenoon. Due to the availability of uncontaminated forage in control and worst case exposure to only contaminated forage in the semi-field experiment, the differences in mortality between control and treatment are considerably pronounced. A higher increase of mortality was observed in 2010 compared to 2011.

But also the semi-field study does not allow any differentiation in the proportion of bees poisoned by contact toxicity due to gathering of contaminated pollen and bees poisoned by the consumption of contaminated nectar and pollen. However, it may be concluded that the majority of dead bees in the hives and dead bee traps have been not caused by direct exposure to contaminated dust during forage flights, but occurred after foraging of nectar and pollen on the treated crop and after consumption of contaminated food by hive bees

Field trials

The mortality of the colonies before sowing was on a comparable level for all colonies in 2010. In 2011, on the day of sowing before start of sowing a comparably high mortality was observed with a mean of 125.5 bees/colony, caused by a highly increased mortality of single colony. Although a multi-residue analysis was conducted with the sample, it was not possible to conclude on the reason for the increased mortality. After sowing, the mortality of this colony returned to a mortality level comparable to other treatment colonies. Especially during the first 24 hours after sowing, the 'Treatment' of both experiments revealed considerably higher mortality compared to the control. Thereafter, the mortality was lower but still showed a treatment related increase for several days after sowing (Fig. 3b).

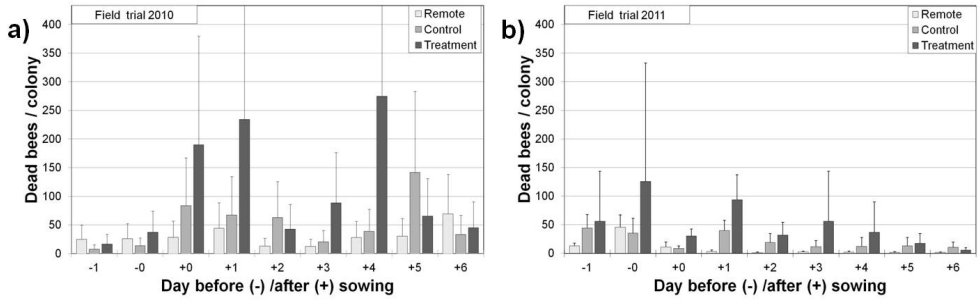

Fig. 3 Mortality in field colonies (3 variants: Treatment directly bordering to exposed flowering WOSR, Control in 50 m distance, Remote < 500 m distance; each n = 4) during the drift trial a) using maize seeds from 2008 (high Heubach-value, Wendhausen 2010) and b) from 2011 (low Heubach-value, Lucklum 2011)

Due to the short distance of the 'Control' (ca 50 m) to the contaminated WOSR area, also a peak in mortality was observed after 24 hours, but mortality was lower than in the 'Treatment'. As the free

flying colonies of control and treatment could forage both on the contaminated but also on the uncontaminated side of the field, it is not possible to conclude if the higher mortality was caused due to exposure of bees to toxic amounts taken up during flying or by a increased portion of bees foraging on the contaminated side of the field. Only the mortality of the 'Remote' colonies with little chance to forage on contaminated WOSR always remained at a low level which was considered as the range of natural mortality.

In general, across all field variants in 2010 (Fig. 3a) much higher increase of mortality was observed compared to the experiment in 2011 (Fig. 3b), in line with the results from the semi-field studies Fig. 2a and 2b). To some extent, this may be attributed to the improvement of seed quality of maize but may also to be due to slightly different sizes of hives used in the experiments (in average: 2010 > 2011; Tab. 1). However, the differences in the field experiments are also caused by differences in the vegetation surrounding the trial sites and the available alternative forage.

While the drift experiment 2010 at Wendhausen was carried out in an area with low alternative nectar availability, the experimental site of the drift experiment 2011 at Lucklum was located in an agricultural region with a high portion of WOSR and other alternative forage (e.g. other WOSR crops, maple trees and dandelion). Thus, in 2011 the foragers of the field colonies may have been partially distracted from the contaminated WOSR crop, which is in line with local observations of lower flying activity in the WOSR crop during the test period. Furthermore, it was confirmed by analyses of randomly selected pollen loads from samples of both experiments that bees have also been collecting pollen e.g. from maple trees.

In field trials, a differentiation of the proportion of bees which died from direct or indirect contact exposure during flying, collecting toxic dust particles and contaminated pollen or the consumption of contaminated nectar and pollen was not possible.

Conclusion

The results of both experiments showed a clear treatment related increase of bee mortality, especially in the worst-case semi-field trials, but also in the field trials. The bee mortality of both variants in 2011 was slightly lower than in 2010, but still on a high level. Presumably the exposure of bees to residues on the crop may have been on a similar level, taking into account the Heubach-values and the residues in the dusts. The experiment 2010 was located in a region with few alternative bee attractive crops, whereas the experiment 2011 was in an area with a wide range of flowering. Thus, in the field experiments, one reason for smaller effects observed at the bee colonies in 2011 was sufficient alternative forage in the surrounding of the fields, distracting foragers from the contaminated field site. In both experiments there was a clear impact of abraded dust from clothianidin-treated maize seeds on the mortality of bees. This indicates a necessity for further improvements of the seed treatment quality of maize and/or of the sowing technique to exclude adverse effects on bees.

Acknowledgement

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Dust drift during sowing of winter oil seed rape - effects on honey bees

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Abstract

In 2008 a large-scale honey bee poisoning in parts of southern Germany occurred during sowing of maize. This incident was caused by contamination of flowering bee forage plants with drift of dust from the insecticidal seed dressing containing the active substance clothianidin. This ingredient is also used in the seed dressing of other crops such as oil seed rape, sugar beet and various grains (wheat, barley, rye).

Two large-scale drift experiments were conducted in 2009 (Wendhausen) and 2011 (Lucklum). In both drift experiments winter oil seed rape seeds treated with clothianidin was sown; the drill area was surrounded by two experimental areas with flowering mustard. The oil seed rape was sown by a pneumatic sowing machine with compressed air installations with at least 90 % drift reduction due to a deflector.

In 2009, on both sides directly along the edge of the mustard (distances to the contaminated mustard area: 0 and 50 m), 4 hives for the field exposure as well as 4 gauze-covered tents (4 x 4 m) with bee hives for the semi-field experiment were exposed, with the side exposed opposite to the wind direction used as control. Before sowing, the bee hives in the tents were closed and the gauze from the tents at the distance of 0 m to the drilling area was removed. Immediately after sowing, which took about 1 hour, the tents were covered again and the hives reopened. The hive bees in the field trial were free flying and not enclosed during the drilling process, so that they were continuously exposed to the contaminated dust. Besides the free flying hive bees directly bordering the exposed area, other hives were exposed in about 50 and 800 m distance from the exposed mustard.

In 2011 a similar approach was used with 2 x 3 tunnels (6 x 16 m) located in mustard in wind direction and opposite to this and outdoor bee hives in distances of 0, 50 and 500 m to the exposed mustard. Here before sowing the gauze from all tunnels was removed.

The contamination of adjacent flowering mustard and the impact of dust drift on bee colonies in semi-field and field trials were examined by assessing flight activity and mortality in Gary-bee traps as well as population development. Dead bees were documented, collected, frozen and analyzed for residues.

Both experiments show that even in worst case scenarios, sowing of winter oilseed rape with the modified seed technology had no adverse effects on bee colonies.

Keywords: honeybee, *Apis mellifera,* bee poisoning, clothianidin, dust abrasion, dust drift

Acknowledgement

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Do residues of imidacloprid in surface water cause honeybee colony losses?

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Abstract

Honeybee colony mortality is a problem at a world wide scale. Insecticides in agriculture and horticulture might contribute, especially the neonicotinoid imidacloprid, which is a commonly used systemic insecticide that might induce several effects at sublethal concentrations. Furthermore it is very persistent in soil and water. At several locations in the Netherlands imidacloprid was found in the surface water in relatively high concentrations. The exceeding of the acceptable levels was suggested to be causally related to honeybee colony losses, and several groups in society are concerned.

The aim of this study is to determine whether concentrations of imidacloprid in surface water influence honeybee mortality in the Netherlands. Therefore monitoring data of honeybee mortality from 2005, 2006, 2007 and 2009 and several covariates are used. Data of honeybee mortality are linked to maximum values of imidacloprid concentrations in surface water. For a realistic risk valuation, three foraging distances are used, i.e. 1000 meters, 3000 meters and 7500 meters.

Peak concentrations of imidacloprid within a radius of 7500 meters around the colonies appeared negatively correlated with honeybee mortality. However, imidacloprid was aliased with the factor beekeeper, so the earlier shown correlation is possibly a disguised beekeepers effect. A negative effect of imidacloprid in surface water on honeybee colony survival in the Netherlands is therefore not shown in this study.

Although it can not be proven that imidacloprid has no influence on honeybee mortality, it seems unlikely that imidacloprid in surface water as a single factor is relevant to the current problem. Possible interactions between imidacloprid and factors that undermine the vitality of colonies are not taken into account in this study.

Reference

The full report of this study is available (in Dutch):
http://documents.plant.wur.nl/pri/bijen/imidaclopridresiduen.pdf.

V. Bumblebees and other pollinators

Aspects determining the risk of pesticides to wild bees: risk profiles for focal crops on three continents

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Abstract

In order to conduct a proper risk assessment of pesticides to bees, information is needed in three areas: (i) the toxicity of the pesticide; (ii) the probability of bee exposure to that pesticide; and (iii) the population dynamics of the bee species in question.

Information was collected on such factors affecting pesticide risk to (primarily wild) bees in several crops in Brazil, Kenya and The Netherlands. These data were used to construct 'risk profiles' of pesticide use for bees in the studied cropping systems. Data gaps were identified and potential risks of pesticides to bees were compared between the crops.

Initially, risk profiling aims to better identify gaps in our present knowledge. In the longer term, the established risk profiles may provide structured inputs into risk assessment models for wild and managed bees, and lead to recommendations for specific risk mitigation measures.

Keywords: pesticide, exposure, risk, wild bees, risk profile

1. Introduction

1.1 Importance of pollination

Pollinators contribute greatly to food security. Effective pollination results in increased crop production, better commodity quality and greater seed production. In particular, many fruits, vegetables, edible oil crops, stimulant crops and nuts are highly dependent on animal pollination.

In the three countries included in this study, Brazil, Kenya and The Netherlands, the economic value of pollination services is undeniably important. The value of Brazilian export of eight important agricultural commodities dependent on pollinators is estimated at € 7 billion annually.[1] The annual economic value of insect pollination in East Africa has been estimated at € 900 million.[2] In the Kenyan district of Kakamega alone, 40% of crop production (€ 2.4 million) could be attributed to bee pollination.[3] The value of animal pollination for Dutch agriculture is estimated at € 1 billion annually.[4]

1.2 Role of wild pollinators

Honey bees and bumblebees, often managed, are among the most important pollinators of crops in both temperate and tropical areas.[5] However, wild bees, both social and solitary species, are also essential for pollination of many crops, especially in the tropics and in cropping systems which include a high diversity of crops within the same area. In some cases, wild bees complement pollination done by honey bees, but for many tropical crops wild bees are the principal or only pollinator.[6,7,8,9]

For example, in the Kenyan district of Kakamega, 99% of the crop production value attributable to pollination was provided by wild bees.[3] The main effective pollinators of passion fruit (*Passiflora edulis* Sims) in Brazil are carpenter bees of the genus *Xylocopa* Latreille.[10] The importance of wild pollinators was recently also underlined in oilseed rape and other crops in Europe[11,12] and New Zealand.[13]

1.3 Threats to pollinators

There is increasing evidence that insect pollinators, both wild and managed, are in decline in many regions of the globe, with the clearest cases documented in Europe and North America.[14] Various causes for this decline have been identified, including loss, fragmentation and degradation of habitats, reduction in resource diversity, pests and pathogens of pollinators, competition by introduced pollinators, climate change, reduced genetic diversity, and pesticide use – all potentially causing direct and indirect adverse effects on pollinator populations. There appears to be agreement that not one of these pressures is primarily responsible for the observed pollinator decline, but that interactions among multiple factors are likely in effect.[14,15,16,17] Both managed and wild pollinators face many common threats, and both are subject to significant declines.[5]

Losses in wild bee diversity and numbers are particularly strong under intensive agricultural management.[18] A recent large study in winter cereals showed that insecticide use had a significant negative effect on bee species richness and abundance.[19] So far, no large honey bee losses have been reported from Africa, Australia or South America[20,21], but increasing agricultural expansion and intensification pose a significant risk to both managed and wild pollinators on these continents.[21,22,23] This is illustrated by the fact that pesticide imports have increased by 38% in Kenya between 2003 and 2008 [24], and pesticide sales in Brazil have tripled between 2000 and 2010.[22]

1.4 Pesticide risk assessment

To address the impact that pesticides may have on pollinators several tools have been developed. These tools vary from relatively simple hazard assessments (evaluating only pesticide toxicity) to more sophisticated risk assessments (where a combination of pesticide toxicity and potential exposure to the pesticide is assessed). Since risk assessment integrates pesticide toxicity and bee exposure, it is generally considered to be more relevant for the estimation of potential impact than a hazard assessment. However, not in all cases will appropriate estimates of exposure be available, and a hazard assessment will then provide an initial indication of the likelihood of adverse effects of the pesticide to bees.

Pesticide hazard and risk assessment for bees in the EU, USA or Australia have so far focused on managed western honey bees *(Apis mellifera* L.*)* alone.[25,26,27] However, honey bees may have different intrinsic susceptibility to pesticides than other bees. They may also be exposed in a different manner due to variations in behaviour and life history, and bee populations may respond in varied ways to pesticides because of differing population dynamics. Consequently, the pesticide risk assessment procedures currently applied for managed honey bees are not necessarily directly applicable to other bees. Only recently have pesticide risk assessment methods for bees other than honey bees received more attention[28], but no clear consensus on risk assessment procedures has yet been established.

1.5 Purpose of the study – pesticide risk profiling

In order to conduct a proper risk assessment of pesticides to bees, information is needed in three areas: (i) the toxicity of the pesticide; (ii) the probability of bee exposure to that pesticide; and (iii) the population dynamics of the bee species in question.

Pesticide toxicity data have mainly been generated for the western honey bee, but much less so for other *Apis* species or non-*Apis* bees (either native or managed). Increasingly, however, toxicity tests are being done with bees other than *A. mellifera*, although not all of these have found their way to the international published literature.

The probability and degree of exposure to pesticides depend on cropping and pesticide application practices, pesticide properties, attractiveness of the crop to bees, and certain aspects of bee biology (in particular phenology and behaviour). Data on these aspects of exposure, for a given crop in a given country or region, may be available from agricultural extension services, pesticide registration authorities, bee experts, agronomists and environmental scientists.

Finally, the population dynamics of the bee species will determine how an observed effect of the pesticide (either lethal or sublethal) will affect long-term survival of the population. This includes such factors as the population size of the bee at the time it is exposed to the pesticide, its population growth rate, and the migration capacity of the bee, among others.

In this assessment, we have attempted to collect information relevant to pesticide risk for (primarily wild) bees that are important on a limited number of focal crops. Because this is not a conventional risk assessment, we use the term 'risk profile'. Initially, risk profiling aims to better identify gaps in our present knowledge. In the longer term, the established risk profiles may provide inputs for risk assessment models that consider wild and non-*Apis* managed bees, which may lead to recommendations for specific risk mitigation measures.

2. Methods

2.1 Focal crops

A limited number of economically important focal crops were chosen for developing a risk profile (Table 1). Focal crops were selected because of their dependence on pollination by wild and/or managed bees, and/or because wild bees were known to be active in these crops.

Tab. 1 Focal crops for which pesticide risk factors were assessed.

Country	Brazil	Kenya	Netherlands
Focal crops	Melon Tomato	Coffee Cucurbits (watermelon & squash) French beans Tomato	Apple Tomato (greenhouse)

Cucurbits, such as melon *(Cucumis melo* L.*)*, watermelon *(Citrillus lanatus (Thunb.))* and squash *(Cucurbita moscata (*Duchesne ex. Lam.*))* are highly dependent on bee pollination and reduced production by more than 90% can be expected when lacking animal pollination.[6] Both honey bees and other bees are important pollinators.

Highland coffee *(Coffea arabica* L.) is self-pollinating, but both honey bees and other bees have been shown to increase yields by over 50%.[6,9,29] Lowland coffee *(Coffea canephora* L.*)* is self-incompatible, and animal pollination is of great importance for berry production.[6,30]

Tomato *(Solanum lycopersicum* L.*)* is self-compatible, but requires wind- or insect-mediated vibration of the flower anthers for pollination (e.g. by buzz pollination).[6] Bumblebees, some stingless bees and some solitary bees are good buzz pollinators.

French beans *(Phaseolus vulgaris* L.*)* are self-compatible, but increases of up to 10% in yield may be possible with optimal pollination. Furthermore, pollination of French beans may improve the quality and uniformity of seed set.[31]

The production of apple *(Malus domestica* Borkh.*)* greatly depends on insect pollination, and honey bees, bumblebees and solitary bees all have been found to increase fruit yields.[6]

2.1 Risk factors

A preliminary list was established of the main factors considered to potentially influence pesticide risk to bees (Table 2). Factors may have different possible effects on pesticide risk to bees. In some cases, a clear correlation between a given factor and an increase or reduction of risk can be assumed. In other cases this relationship is less clear and requires more detailed information on bee biology or the cropping situation. On the basis of this list, a simple questionnaire was designed to collect information on risk factors for focal crops in the three participating countries.

Tab. 2 Pesticide risk factors and their possible effects on bees.

Risk factor	Possible effect on the risks of the pesticide to bees
Exposure – crop factors	
Surface area under crop:	
- overall size	Larger surface area under the specific crop ➔ higher exposure risk
- patchiness	lower fraction of the crop in the overall area ➔ lower exposure risk
Period(s) in the growing season when pesticides are applied to the crop	*Determinant for factors below*
Period(s) in the year when the crop flowers	If overlap between flowering of crop and pesticide applications ➔ higher exposure risk
Period(s) in the year when bees are foraging or collecting nesting materials	If overlap between bee activity in crop and pesticide applications ➔ higher exposure risk
Period(s) when weeds are flowering in the crop which may be attractive to wild bees	If overlap between flowering of weeds and pesticide applications ➔ higher exposure risk
Crop has extrafloral nectaries	If extrafloral nectaries present in crop ➔ higher exposure risk
Crop is regularly infested with honeydew producing insects	If honeydew producing insects present in crop ➔ higher exposure risk
Drinking water is available in the crop	If drinking water in the crop ➔ higher exposure risk
Exposure – bee biology factors	
Location of nest in relation to crop field	In-field and field-border nests ➔ higher exposure risk Off-field nests ➔ lower exposure risk (depending on distance)
Bee foraging range	If in-field and field border nests: shorter foraging range ➔ higher exposure risk If off-field nests ➔ risk depends on distance between nest and sprayed field
Time spent foraging, or collecting nesting materials, per day ('time-out-of-nest/hive')	More hours out-of-nest/hive ➔ higher exposure risk
Period of the day when foraging or collecting nesting materials.	Early/middle in the day ➔ possibly lower exposure risk (if pesticide is applied afterwards and has very low persistence) All-day/late in the day ➔ higher exposure risk
Number of days spent foraging on the crop (for an individual bee)	More days spent foraging ➔ higher exposure risk

Risk factor	Possible effect on the risks of the pesticide to bees
Number of days spent foraging on the crop (for the colony)	More days spent foraging ➔ higher exposure risk
Number of different nectar and pollen plant species used during crop flowering	Fewer species ➔ higher exposure risk
Quantity of pollen collected per day	Higher quantity ➔ higher exposure risk
Quantity of nectar collected per day	Higher quantity ➔ higher exposure risk
Quantity of nectar consumed per day	Higher quantity ➔ higher exposure risk
Body weight	Higher body weight ➔ possibly lower exposure or impact risk *Determinant for other factors*
% of pollen self-consumed	More self-consumed ➔ higher exposure risk to adult
% of pollen fed to brood	More fed to brood ➔ higher exposure risk to brood
% of nectar self-consumed	More self-consumed ➔ higher exposure risk to adult
% of nectar fed to brood	More fed to brood ➔ higher exposure risk to brood
Collective pollen and/or honey storage in the nest (social bees)	If collective pollen and honey storage ➔ lower exposure risk due to mixing, maturation and microbial action; ➔ possibly higher exposure risk if pesticides are concentrated in honey
Exposure & impact – pesticide use/application practices	
Formulation type	Some formulations types (e.g. micro-encapsulation, sugary baits, DP, WP) ➔ higher exposure risk
Pesticide is systemic	Specific exposure/impact assessment
Pesticide is an insect growth regulator (IGR)	If IGR ➔ specific impact on brood
Mode of application	Some modes of application (e.g. dusting, aerial application) ➔ higher exposure risk Some modes of application (e.g. seed/soil treatment with non-systemic pesticide; brushing) ➔ lower exposure risk
Application rate	For the same pesticide product: higher application rate ➔ higher exposure/impact risk
Application frequency	Higher application frequency ➔ higher exposure risk
Systemic pesticides are applied as soil treatment or seed treatment to a previous rotational crop	If systemic pesticides applied to a previous rotational crop ➔ possibly higher exposure risk
Impact & recovery – pesticide properties	
Contact LD_{50} (adult)	Lower LD_{50} ➔ higher impact (for similar exposure levels)
Oral LD_{50} (adult)	Lower LD_{50} ➔ higher impact (for similar exposure levels)
Oral LD_{50} (brood)	Lower LD_{50} ➔ higher impact (for similar exposure levels)
Foliar residual toxicity	Higher residual toxicity➔ higher impact (for similar exposure levels) & ➔lower likelihood of recovery after pesticide impact
Impact & recovery – life history and population dynamics factors[1]	
(Worker) metabolic rate	Higher metabolic rate ➔ lower impact (increased detoxification)
Degree of sociality	High degree of sociality with one or more reproductive queens and separate foragers ➔ lower risk of impact to the population/colony because pesticide effects primarily on foragers (except for IGRs)
Fraction of population/colony active out of the nest/hive (social bees)	Higher fraction of population of colony active out of the nest/hive ➔ higher risk of impact for the whole population/ colonyr5

Risk factor	Possible effect on the risks of the pesticide to bees
Time to reproductive age of queen/reproductive female (egg-adult)	Shorter development time ➔ lower exposure risk (if development partly overlaps with flowering)
Number of offspring per queen/reproductive female	Greater number of offspring ➔ greater likelihood of population recovery after pesticide impact
Number of generations per year	Greater number of generations per year ➔ greater likelihood of population recovery after pesticide impact
Population growth rate [note: is product of previous 3 factors]	Higher population growth rate ➔ greater likelihood of population recovery after pesticide impact
Number of swarms per colony per year	More swarms per year ➔ greater likelihood of population maintenance, if swarming occurs before pesticide impact & ➔ greater likelihood of population recovery after pesticide impact
Migration distance of swarms	Greater swarm migration distance ➔ greater likelihood of population recovery after pesticide impact (if cropping is patchy)

2.3 Data collection

In Brazil, cropping and bee data were collected through discussions with crop and pollination experts and by consulting published and unpublished literature. Pesticide use information was obtained from crop experts and the pesticide registration authority (Ministério da Agricultura, Coordenação-Geral de Agrotóxicos e Afins) through the Sistema de Agrotóxicos Fitossanitários – Agrofit.

In Kenya, cropping and bee data were collected through discussions with crop and pollination experts and by consulting published and unpublished literature. Pesticide use information was obtained from crop experts and the Kenya Pest Control Products Board (PCPB). In addition, an extensive survey was carried out on pollinator knowledge and crop protection practices covering approximately 150 farmers in Machakos, Kirinyaga and Kiambu counties.

In the Netherlands, cropping and bee data were collected through discussions with crop and pollination experts and by consulting published and unpublished literature. Pesticide use information was obtained from Statistics Netherlands (CBS).

Acute LD_{50} values for the western honey bee (*A. mellifera*) were obtained from a recently developed database, compiled from multiple regulatory and non-regulatory data sources.[32] The lowest (generally 48h) LD_{50} value of both oral ingestion and contact tests, as calculated using the rules defined for the database, was used in this report. When LD_{50} values were not available in the database, the Footprint Pesticide Property Database[33] and the Footprint Biopesticides Database[34] were consulted. Results from brood tests, or sublethal toxicity tests, have not been taken into account. Acute LD_{50} values for bumblebees were taken from a recent review.[35] Pesticide toxicity data for bees other than *A. mellifera* and *Bombus* (Latreille) are still limited. No public database appears to exist for such bees and toxicity data for other bees were therefore not included in this assessment.

3. Results

Detailed results of the study are provided elsewhere.[36]

3.1 Presence of bees

The main groups of bees visiting the focal crops in the three countries are listed in Table 3. In all focal crops, except melon in Brazil and tomatoes in the Netherlands, wild bees may contribute significantly to pollination. This is in addition to, or instead of, the honey bee. Furthermore, in all focal crops, the groups and/or species of bees that are regular visitors appear to be relatively well known. In many cases, important pollinators have been identified, although for some crops the role of wild bees as pollinators requires more study.

Tab. 3 Main groups of bees visiting the focal crops, and their role as pollinator of those crops

		Bee group/species visiting the crop	
Country	**Crop**	**Important pollinator**	**Visitor; not an important pollinator**
Brazil	Melon	*Apis mellifera* L. (honey bee)	*Xylocopa* Latreille (carpenter bees) *Frieseomelitta doederleini* (Friese) (stingless bee)
	Tomato	*Bombus transversalis* (Olivier) (bumblebee) *Bombus atratus* Franklin (bumblebee) *Bombus morio* (Swederus) (bumblebee) *Xylocopa grisescens* Lepeletier (carpenter bee) *Augochlora* Smith (sweat bees) *Exomalopsis auropilosa* Spinola (long-horned bee) *Melipona* Illiger (large stingless bees)	*Apis mellifera* L. (honey bee)
Kenya	Cucurbits	*Apis mellifera* L. (honey bee) Halictidae (sweat bees) (e.g. *Lasioglossum* Curtis)	*Xylocopa* Latreille (carpenter bees)
	Coffee	*Apis mellifera* L. (honey bee) *Patellapis* (Friese) (sweat bees) *Xylocopa* Latreille (carpenter bees) *Megachile* Latreille (leafcutter bees)	
	French beans	*Xylocopa* Latreille (carpenter bees) *Megachile* Latreille (leafcutter bees)	*Apis mellifera* L. (honey bee)
	Tomato	*Xylocopa* Latreille (carpenter bee) *Lipotriches* Gerstaecker (sweat bees)	*Apis mellifera* L. (honey bee)
Netherlands	Apple	*Apis mellifera* L. (honey bee) *Osmia rufa* L. *(=O. bicornis)* (red mason bee) *Bombus* Latreille (bumblebees) (mainly *B. terrestris/lucorum* L.*; B. pascuorum* Scopoli*; B. lapidarius* L.*)* *Andrena* Fabricius (sand bees)	
	Tomato	*Bombus terrestris* L. (bumblebee)	

3.2 Risk factors

3.2.1 Exposure – crop factors

Various crop-related factors may increase bee exposure to pesticides, such as overlap between the presence of bees in the crop area and flowering of the crop or weeds, overlap between bee activity on the flowering crop and pesticide application, or the presence of extrafloral nectaries, insects producing honeydew, or drinking water in the crop area. These factors are summarized for the focal crops in Table 4.

The main factors influencing risk are probably the overlap of pesticide applications with crop flowering or with bee activity in the crop area. In all but one crop, pesticides are applied during flowering and bee activity. Only in coffee production in Kenya, pesticide applications during flowering are explicitly being avoided. In most crops, weeds are being mulched or otherwise controlled, and only in apple in the Netherlands there is risk of exposure of bees foraging on Dandelion flowers just before the apple flowering period.

Of the focal crops, only French beans have extrafloral nectaries. Some cucurbits also have them, but the relevant cucurbit crops in Kenya do not. Most crops are regularly infested by honeydew producing insects such as aphids, whiteflies and scale insects. In all three countries these pests are

controlled with insecticides, and to what extent bees will be attracted to such pests to forage honeydew requires further study. In general, bees will use nectar as the main drinking water source. However, in the Netherlands, bumblebees may drink (potentially contaminated) condensed water from the greenhouse walls after the sugar water provided in the colony boxes is depleted.

Tab. 4 Factors related to cropping practices that may influence the risk of bee exposure to pesticides.

	Brazil		**Kenya**				**Netherlands**	
Exposure – crop factors	**Melon**	**Tomato**	**Cucurbits**	**Coffee**	**French beans**	**Tomato**	**Apple**	**Tomato**
Pesticide application overlaps with the flowering period of the crop	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Pesticide application overlaps with the flowering period of weeds in the crop	No	No?	No	No	No	No	Yes	No
Pesticide application in the crop overlaps with the period when bees are actively foraging or collecting nesting materials in the crop	Yes	Yes	Yes	No?	Yes	Yes	Yes	Yes
Crop has extrafloral nectaries	No	No	No	No	Yes	No	No	No
Crop is regularly infested with honeydew producing insects	No?	Yes?	Yes	Yes	Yes	Yes	Yes	Yes
Crop may be visited by bees for collection of water	Yes	Yes	-	-	Yes	Yes	Yes	Yes
Overall likelihood of exposure	**high**	**high**	**high**	**low**	**high**	**high**	**high**	**high**

- = data not available; ? = possibly

Overall, the likelihood of bee exposure to pesticides used in the focus crops, based on crop-related aspects, can be considered high. The only exception is coffee in Kenya, where pesticides tend not to be applied in the period when bees are foraging.

3.2.2 Exposure – bee biology factors

Bee biology, such as period, duration and range of foraging, nest location, and nectar and pollen consumption, may affect the risk of bee exposure to a pesticide (Table 2). Many of the listed factors are highly variable for individual species, and even more so among groups of bees. For instance, foraging ranges will depend on the availability of suitable flowering plants, but are also determined by bee size. The timing of foraging may be greatly influenced by weather conditions. The quantity of pollen and nectar collected depends on the size of the colony, the size of the bees, and also on the sugar content of the nectar.

Detailed results for all countries and focal crops are available elsewhere. [36] Here, an example of the results for the focal crops in Kenya is provided (Table 5). Information on the African honey bee was available, but it was more limited for *Xylocopa* (carpenter bee) and sweat bees (Halictidae). No information on relevant bee biology factors could be obtained for local leafcutter bees (*Megachile* Latreille) and the sweat bee *Patellapis* (Friese). Based on the limited bee biology data available, there is no reason to expect higher pesticide exposure for *Xylocopa* than for European honey bee in Kenya, but some key factors could not be quantified. The likelihood of exposure to pesticides of African honey bees is probably similar to European honey bees.

Tab. 5 Factors related to bee biology that may influence the risk of bee exposure to pesticides in the focal crops in Kenya.

Exposure – bee biology factors	**Coffee Cucurbits French beans Tomato**	**Coffee Cucurbits French beans Tomato**	**Coffee**	**French beans Coffee**	**Tomato Cucurbits**
	Apis mellifera scutellata	***Xylocopa***	***Patellapis***	***Megachile***	**Halictidae**
Location of nest in relation to crop field (approximate distance from crop field)	Inside and in field borders (50–100 m)	Outside and in field borders; fringes of woodlands	-	-	Outside and in field borders; fringes of woodlands
Average bee foraging range (maximum distance from nest)	~1500 m (10 km)	700–1000 m (6 km)	-	-	50–100 m
Time spent foraging or collecting nesting materials	~10–15 trips/d; 4–11 hrs d^{-1} (individual nectar forager); ~1.5 hrs d^{-1} (individual pollen forager)	1–2 hrs d^{-1} (individual bee); Median flight duration 30 min	-	-	4–10 hrs d^{-1}? (individual bee)
Period of the day when foraging or collecting nesting materials	(Early) morning/all day (on cool days)	Early and late in day	-	Mid-day	Entire day
Time spent foraging on the crop (for an individual bee)	5–15 d	Coffee: 30 d French beans: 100 d Tomato: 90 d	-	-	60 d
Time spent foraging on the crop (for the colony)	Coffee: 30 d Cucurbits: - French beans: 100 d Tomato: 90 d	n.a.	n.a.	n.a.	n.a.
Quantity of pollen collected	200–300 mg d^{-1}	-	-	-	<30 mg d^{-1}
Quantity of nectar collected	250 µL d^{-1}	-	-	-	-
Quantity of pollen consumed	~6.5 mg d^{-1} (nurse bee)	-	-	-	-
Quantity of nectar consumed	80–320 mg d^{-1} (forager)	-	-	-	-
Body weight	60–120 mg (worker)	> honey bee	-	-	3–95 mg
% pollen self-consumed by adult	Limited (early adult stage)	-	-	-	-
% pollen fed to brood	Most; stored and transformed	Up to 100%	-	-	Up to 100%
% nectar self-consumed by adult	Some; most stored and transformed, and consumed as honey	-	-	-	-
% nectar fed to brood	Most; stored and transformed and consumed as honey	-	-	-	-
Collective pollen and/or honey storage in the nest	Yes	Limited?	-	No	Limited?
Overall likelihood of exposure compared to the European honey bee	**Similar**	**Similar?**	**Unclear**	**Unclear**	**Greater?**

- = data not available; ? = possibly; n.a. = not applicable; d = day; hr = hour; min = minute; mg = milligram; mL = millilitre; µL = microliter; Sources of the data in this table are provided elsewhere.[36]

Based on bee biology factors, it can be inferred that sweat bees (Halictidae) on tomato in Kenya may be more exposed to pesticides than the honey bees on the same crop. This is because the nests of sweat bees are located close to the field which, in combination with the more limited foraging range, is likely to increase exposure risk. Furthermore, sweat bees are generally smaller than honey bees and individual foraging time appears longer. Finally, almost 100% of collected pollen is fed directly from the field to the brood, which may lead to higher pesticide exposure of offspring than is the case in honey bee or other pollen-storing bees, like stingless bees (Meliponini). When in storage, microorganisms and added nectar in pollen may accelerate breakdown of pesticides.

Overall, the study shows that there are still major data gaps regarding elements of bee biology that influence exposure risk of bees to pesticides in all three countries. For most bee groups, information was available on daily and seasonal flight activity and on foraging patterns. On the other hand, information was lacking on foraging duration, quantities of pollen/nectar collected and amounts consumed by the foraging adults.

3.2.3 Exposure – pesticide use and application practices

The numbers of pesticide products and active ingredients (a.i.'s) registered and/or used on the focal crops in the three countries are summarized in Table 6.

Tab. 6 Number of pesticides registered and/or used in the focal crops.

	Brazil		**Kenya**				**Netherlands**	
	Melon	**Tomato**	**Cucurbits**	**Coffee**	**French beans**	**Tomato**	**Apple**	**Tomato**
Number of active ingredients registered for use on the crop	64	130	11	9	17	23	72	61
Number of active ingredients used per crop	--	--	29	12	20	29	57	66
Number of active ingredients used in period when bees are active in the crop	--	--	25	0?	20	22	54	60
Number of insecticide/acaricide active ingredients used in period when bees are active in the crop	--	--	13	0?	11	15	13	21
Systemic pesticides are applied as soil or seed treatment to a *previous* rotational crop	yes	yes	?	n.a.	?	?	n.a.	n.a.
Number of systemic pesticides used or registered per crop	35	49	14	5	10	12	28	24
Number of insect growth regulators used or registered per crop	4	15	0	0	0	0	3	6

? = data not available; n.a. = not applicable

A large number of pesticides were registered on tomato in Brazil, but it could not be ascertained which were used in periods when bees were active on the crop. Systemic pesticides were confirmed to be applied by soil or seed treatments to previous crops, which might pose a risk for exposure of bees to contaminated pollen or nectar in the subsequent melon or tomato crops.

In Kenya, a considerable number of pesticides were used on cucurbits, French beans and tomato, and a large fraction of these were used throughout the crop cycle, so potentially exposing bees. In coffee, however, most pesticides were used only after flowering, i.e. when bees were either not or less active in the coffee crop.

In the Netherlands, a large number of pesticides is used while bees are active in both apple orchards and on tomato. Greenhouse tomato production always starts with fresh substrate, and previous crops are therefore not relevant. The use of systemic pesticides in previous rotational crops is not relevant in perennial crops such as apple.

3.2.4 Impact and recovery – pesticide properties

Acute toxicity data for *A. mellifera* are reported for most pesticides, as these tend to be required for pesticide registration. However, in many cases, only acute contact and oral test results obtained on adult worker bees are available.

On average, acute LD_{50} values for honey bees were available for 94% of the a.i.'s used in the various focal crops (Table 7). For only 70% of a.i.'s used on tomato in the Netherlands an acute LD_{50} could be found. This was partly due to the relatively large number of bio-pesticides and general disinfectants being used in that crop. Only few acute LD_{50} values for bumblebees were available.

Tab. 7 Number of acute LD_{50} values available for honey bee and bumblebee in the focal crops, and their associated hazard.

Country Crop	Number of pesticides registered or used	Number of pesticides with an acute LD_{50} for honey bee	Number of pesticides with an acute LD_{50} for bumblebee	% pesticides (no.) which are		
				Highly toxic[1] (LD_{50} < 2 µg bee^{-1})	Moderately toxic (2 ≤ LD_{50} ≤ 11 µg bee^{-1})	Practically non-toxic (LD_{50} > 11 µg bee^{-1})
Brazil						
Melon	64	61	4	28% (17)	13% (8)	59% (36)
Tomato	130	119	13	36% (43)	5% (6)	59% (70)
Kenya						
Coffee	12	12	2	42% (5)	8% (1)	50% (6)
Cucurbits	29	29	9	52% (15)	7% (2)	41% (12)
French beans	20	20	5	40% (8)	5% (1)	55% (11)
Tomato	29	28	7	50% (14)	7% (2)	43% (12)
Netherlands						
Apple	57	52	5	10% (5)	11% (6)	79% (41)
Tomato	66	52	5	21% (11)	8% (4)	71% (37)

[1] Based on the hazard classification for honey bees according to the US-EPA.[27]

Since application rates were not available for all crops, only a comparison of hazards was made of the pesticides used in the different focal crops. The LD_{50} values (the lowest of the oral or contact LD_{50} was used) were classified according to the US-EPA hazard ranking for honey bees[27] (Table 7). The hazard classification for honey bee was then applied as a surrogate for all bees in this study.

The majority of pesticides used in both focal crops in the Netherlands were classified as practically non-toxic to bees. In Kenya the largest fraction of pesticides used was classified as highly toxic to bees, and this concerned all four crops. Both Brazilian crops were intermediate as to the hazard of the pesticides being used. Of the crops assessed in this study, the highest pesticide hazard to bees was found to be in cucurbits and tomatoes in Kenya; the lowest hazard in apple in the Netherlands.

The US-EPA toxicity classification primarily addresses the hazard of pesticides applied as a spray. Systemic pesticides applied as seed or soil treatment are not explicitly covered. However, a relatively large number of systemic pesticides are also being used on the focal crops (Table 6). The worst case toxicity–exposure ratio (TER), as defined by EPPO for pesticides with systemic action, was also calculated.[25] It was found that whenever this systemic TER resulted in a high risk classification, the pesticide had already been categorized as highly toxic by the EPA oral/contact toxicity classification. One can therefore conclude that the EPA hazard classification is also 'protective' for bees when systemic pesticides are concerned, at least for the compounds evaluated in this study.

Tab. 8 Factors related to the bee's life-history and population dynamics which may influence the impact of a pesticide to bees in the focal crops in Brazil.

Impact – bee life history and population dynamics factor	**Brazil**					
	Melon Tomato	**Tomato**				
	Apis mellifera (Africanized)	*Bombus*	*Xylocopa grisescens*	*Augochlora*	*Exomalopsis auropilosa*	*Melipona*
(Worker) metabolic rate	Hybrids < non-hybrid African or European subspecies	-	-	-	-	-
Degree of sociality	Eusocial	Primitively eusocial	Parasocial	Solitary	Parasocial	Eusocial
Fraction of adult population/colony active out of the nest/hive (social bees)	~35%	< 100%	Up to 100%	100%	100%	< 100%
Time to reproductive age of queen/reproductive female (egg-adult)	~33 d	-	35 – 69 d	-	-	-
Number of offspring per queen/reproductive female	8 – 12 offspring colonies/ parental colony yr^{-1}	-	5 – 8 yr^{-1}	-	-	-
Number of generations per year	3–4	-	1 – 4	-	-	-
Population growth rate [note: is product of previous 3 factors]	16-fold colony increase yr^{-1}	< honey bee	< honey bee	< honey bee	< honey bee	-
Number of swarms per colony per year	Up to 60	n.a.	n.a.	n.a.	n.a.	-
Migration distance of swarms	> European subspecies (=500–600 m; max. 1600 m)	n.a.	n.a.	n.a.	n.a.	-
Overall likelihood of pesticide impact compared to the European honey bee	**Lesser**	**Greater**	**Greater**	**Greater**	**Greater**	**Unclear**

? = data not available; n.a. = not applicable; d = day; m = metre; yr = year; Sources of the data in this table are provided elsewhere.[36]

Insect growth regulators (IGRs) tend to have a relatively low toxicity to adult bees, but may be very toxic to the larvae. A hazard classification based on acute LD_{50} obtained from adult bees is then not appropriate and toxicity data on bee brood are required.[25] Relatively few IGRs are being used on the focal crops (Table 6), and therefore no specific assessment of their risk was conducted.

3.2.5 Impact and recovery – life history and population dynamics

The life-history and population dynamics of the bee species will determine to a large extent how its populations will resist to or recover from such pesticide impact (Table 2).

As an example, information compiled on factors related to life history and population dynamics of the bee groups present on the focal crops in Brazil is shown in Table 8. Information for the other study countries is provided elsewhere.[36]

Limited specific information was available for Africanized honey bee and the carpenter bee *Xylocopa grisescens* Lepeletier, in Brazil. The Africanized honey bee has a considerably higher population growth rate and swarming rate than the European subspecies. As a result, it can be expected that the Africanized honey bee can recover quicker from pesticide-induced adverse effects on the population than the European honey bee.

It can be assumed that population growth rates of all the listed solitary and parasocial bees, will be lower than that of the honey bee. Also, the fraction of the total population which will be out of the nest foraging or collecting nesting materials will be greater for the solitary, parasocial and primitively eusocial bees, than for honey bees and stingless bees. As a result, it is likely that pesticide impact on individual bees will affect more of the populations of the carpenter bees, the solitary sweat bees, the long-horned bees and to a lesser extent the bumblebees, than of the more social bees. In addition, the lower population growth rates would result in less rapid population recovery of these groups.

4. Discussion and conclusions

4.1 Data availability

With respect to the presence of bees in the focal crops, generally it was known which groups of bees were active on the crop, although in a number of cases identification was only known along fairly broad taxonomic groups. The role of the wild bees as pollinators was relatively well known for melon in Brazil, coffee and French beans in Kenya, and tomato in the Netherlands. The lack of data for the other crops underlines the importance to obtain better insights on the exact role of wild bees as pollinators.

With respect to exposure, data were generally available for crop factors and for pesticide use and application factors, although in many cases these data were not complete. Data were limited or lacking especially for factors related to bee biology. As a consequence, it is generally possible to infer the overall likelihood of exposure of wild bees in the focal crops. However, it is often not possible to further qualify or quantify the degree of exposure of individual bee taxa.

With respect to impact and recovery, toxicity data were available for most pesticides used in the focal crops. However, these were mainly limited to acute toxicity to honey bees. Few toxicity studies have been published for bumblebees, and even less so for other bee species. Availability of data on life history characteristics and population dynamics of, in particular, wild bees was poor or completely absent. Much of the research needed on pollination biology would also be of high value to pesticide risk profiling and assessment. Given the limited resources available for such research, it seems important that pesticide ecotoxicologists and pollination biologists seek active collaboration to optimize and mutually complement on-going and planned research efforts.

4.2 Risk profiles

The risk profiling approach used in this study was developed because a comprehensive risk assessment method for wild bees, or even for honey bees in non-temperate cropping systems, is not yet available. The results of this study indicate that important data gaps still exist with respect to, in particular, bee biology and quantification of exposure that may preclude the establishment of a

proper risk assessment procedure for wild bees in the near future. However, the elaboration of a risk profile, as outlined in this study, may provide a preliminary qualification of the risks of pesticide use to (wild) bees in specific crops.

There are important differences between a risk assessment and a risk profile. A risk assessment for bees, conducted for the registration of a pesticide, tends to focus on a specific pesticide product, includes a quantitative estimate of exposure and of effect, and refers to explicit acceptability criteria (e.g. the hazard quotient or toxicity-exposure ratio, in the EU/EPPO approach).

A risk profile, on the other hand, focuses on the cropping system. It includes (where possible) a quantitative measure of effects, but generally comprises only a qualitative (or semi-quantitative) estimate of exposure, and can therefore not quantify risks. As a result, explicit acceptability criteria are not used.

We consider risk profiling a particularly useful approach to:

- conduct a qualitative evaluation of pesticide risks to bees in specific cropping systems;
- compare potential risks of pesticide use to bees among cropping systems;
- facilitate discussion among researchers, regulators, farmers and beekeepers on pesticide risks to (wild) bees;
- identify data/information gaps;
- set priorities for further research (e.g. with respect to crops, bee groups, types of pesticides); and
- set priorities for risk mitigation.

In the absence of agreed quantitative risk assessment procedures for wild bees, or honey bees in (sub-) tropical cropping systems, establishing a risk profile provides a structured assessment of potential risks of pesticides to bees in a given crop situation while making explicit any knowledge gaps. This forms a good basis for discussion among researchers, regulators, farmers and beekeepers on how to value potential pesticide risks to bees and pollination in specific cropping systems.

The establishment of a risk profile further helps to set priorities for research, by identifying crops, species or groups of bees, or types of pesticides that merit additional study. For instance, additional research efforts would clearly be justified for pollinator-dependent cropping systems, where there is a great likelihood of exposure of bees to pesticides, and a large fraction of moderately toxic pesticides is being used, i.e. for which the resulting impact on bees may not be clear. Another priority example for research would be a pollinator-dependent crop, in which many highly toxic pesticides are being used, but where the likelihood and extent of exposure of bees is not clear. The focus of research would be different according to the uncertainties that need to be clarified for the cropping system in question.

Even though risk profiling will often lead to less concrete conclusions about risk than formal risk assessment, the establishment of a risk profile could also lead to risk mitigation. In a number of cases, the outcome of a risk profile will be clear enough to warrant risk mitigation measures to be developed and/or to be taken. This would, for instance, be the case if there is a great likelihood of exposure of bees to various highly toxic pesticides in a highly pollinator-dependent crop. The risk of adversely affecting pollinators and crop production in such cases is so great that immediate implementation of risk mitigation measures is justified. The requirement for risk mitigation should, in such high risk cases, not be made conditional to the generation of further data.

Table 9 provides suggestions for priority setting for research and for developing (additional) risk mitigation on the basis of the outcome of a risk profiling exercise. Priorities are mainly based on the likelihood of exposure of bees on the one hand and the toxicity of the pesticides used in the crop on the other. Priorities are also based on the pollination dependency of the crop and the population dynamics of the bee.

Tab. 9 Priority setting for research or for (additional) risk mitigation, based on the outcome of a risk profile for a given cropping system.

Priority for research 'R'**, or for (additional) risk mitigation** 'M' (if in brackets [ ], the priority is secondary to the main priority)		**Crop dependence on pollination**						
		High			**Limited**			**No**
		Likelihood of exposure of bees to pesticides			**Likelihood of exposure of bees to pesticides**			
		High	**Low**	**Unclear**	**High**	**Low**	**Unclear**	
Severity of impact / Large fraction of the pesticides used in the crop are:	Highly toxic	M [R]		R [M] §	M §		R §	
	Moderately toxic	R [M] §		R §				
	Practically non- toxic	R §						

§ In particular if bee population dynamics or life history are likely to increase the severity of pesticide impact or reduce the speed of recovery

It is important to realize that this type of priority setting is relevant to risks of pesticides to bees in crops, in particular those that are to some extent dependent on pollination. It does not guide research or risk mitigation priorities unrelated to crop pollination, e.g. which focus on biodiversity protection. Other criteria are important for such aspects of bee conservation.

This structured profiling exercise of pesticide risks to (wild) bees in different cropping systems on different continents has, according to current knowledge, not been carried out previously. The list of risk factors (Table 2) used in the assessment is definitely not exhaustive, and the possible effects these factors may have on pesticide risks to bees will clearly need further research. It is hoped that this present work can be used as a basis for conducting similar studies elsewhere. Over time, this should result in a more precise set of risk factors, and progressively generate a more comprehensive database of risk profiles for different cropping systems and situations. In the long term, risk profiling is expected to contribute to the development of formal risk assessment procedures for wild bees and for honey bees in non-temperate ecosystems.

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Losses of Brazilian bees: an overview of factors that may affect these pollinators

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Abstract

The Neotropical region to which Brazil belongs, has a great and rich diversity of natives bees, up to a total of 3.000 species including the allochtone genus *Apis* that by natural crossing among European and African races produced a hybrid called Africanized honeybee. In this way, beekeeping enjoys a spectacular moment with good production mainly of honey and propolis from *Apis mellifera*, causing Brazil to be recognized around the world as a country with great potential. Brazilian bee losses nevertheless remain a question, compared with countries of the northern hemisphere where several reports show that the vanishing of honeybees is associated with diseases caused by *Varroa*, *Nosema*, virus or pesticides. We can suggest different issues in the Brazilian situation that are directly influencing the honeybee population. Given the extension of the territory and rich flora, all possible food resources and nest sites for the good development of bees may be considered present.

However, we verified that annual bee losses in the Southeast can reach 20-30%, mainly due the genetic mechanisms of swarming (nest abandon). Many times the major factor leading to nest abandon is lack of food, often taken mistakenly by the untrained beekeeper as death of the hive caused by diseases or pesticides. Although in Brazil diseases do not represent an important problem for Africanized honeybees, some cases of presence of *Nosema ceranae* and *Varroa destructor* led the specialists to precaution and monitoring the colonies. In spite of this, the Brazilian beekeeping is managed without use of any acaricide or antibiotic, producing contaminant-free products.

As to pesticides, Brazil has a particular climatic and soil condition that might differently affect the risk of exposure of bees to xenobiotics. For example, comparing the dynamics of carbamate pesticides in soil between Brazil and Europe, it was found that in our condition ten-fold more time is needed to obtain the metabolites sulfone and sulfoxide, both more soluble and toxic than it precursor. Comparing the pesticides consumption, currently Brazil has become the world leader followed by USA, with a total spending of 44.9% herbicide, 28.5% insecticide and 22.1% fungicide. Even with this consumption Brazil still belongs to the group that uses a relatively small amount of active ingredient per hectare, less than Japan and France. However cultures like tomato, potato, citrus, cotton and coffee that are often visited by bees during bloom, are also those where the use of pesticides is needed for the pest control. Thus, little is known yet about pesticide losses of the Brazilian bees! What are real effects of pesticides, toxic plants, diseases, genetic improvement, beekeeping management, starvation or interactions among these? Therefore, our local group on ecotoxicological assessment is trying to increase the knowledge on the pesticides hazard to bees (*Apis* and non-*Apis*) in order to protect these.

Keywords: Brazilian bees, overview, pesticide, diseases, environment, protection.

1. Introduction

The commercial beekeeping for obtaining products such as honey and wax is one of the most ancient activities developed by man, as reported since the beginning of the pre-history till the contemporaneous time[1]. When it comes to the origin of bees and particularly about *Apis mellifera* L.,

1758 (Hymenoptera: Apidae), several studies suggest the African continent as the center of origin, with at least two great natural dispersions by migration (from Europe and Asia) and other artificial introductions provided by man during the process of colonization of the new world[2].

In Brazil, the first reported introduction of the subspecies *A. mellifera mellifera* was in Rio de Janeiro city in 1839. Between 1870 and 1900, the Italian bee *A. mellifera ligustica* Spinola, 1806 was introduced in the Southern region of the country[1]. Little more than 100 years after the introduction of these subspecies indigenous to Europe, non-adapted to the Neotropical region, some queens of the African subspecies *Apis mellifera scutellata* Lepeletier 1836 were imported through a genetic improvement program established in 1956, and introduced in colonies in the town of Rio Claro, São Paulo. In 1957, some colonies of this African subspecies swarmed and crossed under natural conditions with the resident European subspecies, giving rise to a fertile intraspecific hybrid called Africanized honeybee (AHB) in which the behavioral characteristics of the African bees and not of European bees[3] predominate.

In addition to the bees of the genus *Apis*, there is in Brazil a great and rich range of native bees, either eusocial or solitary, responsible for a great part of pollination and reproduction of a number of plant species. Through the surveys already accomplished, estimates lead to believe that in Brazil over 3000 species of native bees exist, that is, up to now over 1500 of them have described[4,5]. Within this group, it are the bees belonging to the tribe Meliponini and the genera *Melipona* Illiger 1806, *Tetragonisca* Moure 1946, *Scaptotrigona* Moure 1942, *Nannotrigona* Cockerell 1922, characterized by the absence of a sting and that can be handled in a rational way for honey production and pollination, mainly of greenhouse crops (protected environments)[6]. Owing to the importance and preservation of the native bees, a federal law was created and approved which has as a basic principle the protection of the keeping of these pollinators[7].

Thanks to the large terrestrial biomass formed by the Amazonia, Cerrado (tropical savanna), Atlantic Forest (Mata Atlântica), Araucaria moist Forests (Mata de Araucárias), Caatinga (xeric shrubland), Pampas (lowlands) and Pantanal (tropical wetland), the favorable climatic conditions (annual average of 20-25°C), the learning of management of the AHB, the investment in research etcetera, Brazil has stood out and obtained recognition of the international market for its products, mainly honey and propolis. In the latest three years Brazil has exported on average over 21,000 tons of honey from a total amount of product estimated as more than 50,000 ton/year. Of the propolis marketed in Japan 90% is of Brazilian origin:

(ABEMEL, 2012 (http://abemel.com.br/portal/) and

(SEBRAE, 2012 (http://www.sebrae.com.br/setor/apicultura).

In comparison with countries in the Northern hemisphere where losses can reach up to 30 to 50% of the colonies of *A. mellifera*[8] (colony collapse disorder and other phenomena), there is little information in Brazil about hive decline and similar phenomena and on the possible consequences of bee losses. Different from North America and Europe where beekeeping is intensely managed, it is given high government investments, among other for genetic improvement. In Brazil, the beekeeping of *A. mellifera* is done mostly by small farmers with little technical and scientific knowledge, making the exploitation of the potential of the AHB and the existing vegetation unviable.

Therefore, the particular conditions for the Brazilian bee such as plants of bee importance, genetics, behavior, diseases, pesticides and management should be studied thoroughly in order to obtain reliable information about the AHB and to exploit the full potential of these bees in Brazil.

2. Genetics/behavior

Differently from the European honey bee subspecies (EHB) of *A. mellifera*, AHB possesses particular characteristics resulting from the hybridization process as well as from the environment (Ruttner, 1988). The adaptation strategies of the AHBs as compared with the EHBs can be summarized in three regards: (i) climate, (ii) defense of predators and (iii) interaction between available resources and hive behavior[9]. Without doubt, the factor climate is one of the most important to be evaluated, affecting directly the hive's development by the availability of resources in the environment and its direct

reflections on production. A comparative survey between EHB and AHB found that the subspecies originating from temperate regions (Northern hemisphere) co-evolved by surviving and adapting themselves to the conditions of cold. The subspecies from the African continent have evolved by creating mechanisms necessary for colony defense against predators and parasites, which accounts for the success of the colonization by the AHB in the tropical region of the Americas and the non-adaptation to environments of temperate climate such as, for example, central regions of the Argentina and USA[2, 3,10].

In the tropical region of the Americas, the AHB has presented in general two dispersal mechanisms, one reproductive and another of abandon. In the former case, it is found that in times of food abundance, the colonies show a fast population growth and production of several reproductive swarms in a short time period. In the other case, the absence of suitable food resources causes the hive to start swarming[11]. In this way many cases of hive losses by either inadequate management or food absence are confounded as losses caused by diseases or pesticides. For instance, during winter in the Southeast region (e.g. South of Minas Gerais), colony losses can reach 20-30%, mainly due the incorrect management of hives (starvation) (Carvalho SM, 2011, pers. comm.). Similar examples are also found in other regions in Brazil. In the state of Rio Grande do Sul, the loss of 40% of the colonies between the winter and spring in 2006 was ascribed to extreme climate factors such as low temperatures and excessive rain[12].

3. Diseases, parasites and predators

Similar to the other countries where *A. mellifera* is present, it is possible to find several bee diseases in Brazil, except for example the American foulbrood (AFB) caused by *Bacillus larvae* ssp. *larvae*. Up to the present, only two cases of AFB spores were reported in Brazil, that is, one in 2001 in Rio Grande do Sul state and other in 2006 in Paraná state. In both cases, the AFB spores were found in bee products (honey/pollen) from illegal imports. Afterwards, all measures for the control and monitoring of the diseases were taken, in addition to notifying the World Organization for Animal Health/OIE[12]. In this manner, Brazil remains one of the few areas in the world free of AFB, even bordering to countries such as Argentina and Uruguay, where this disease is present since 1989[13]. One important fact is that the use of chemicals (e.g. acaricide, antibiotic, etc) is prohibited by the Brazilian law, producing contaminant-free products and so achieving world recognition.

On the other hand and not causing great problems, it is possible to find in several parts in the country (spring/summer) characteristic symptoms of European foulbrood (EFB), which is caused by a complex of disease agents (*Melissococus pluton, Bacillus pluton, Bacillus orpheus, Bacillus eurydice, Bacillus laterosporus* and *Streptococus apis*)[14]. Also in states of the South/Southeast the occurrence of chalkbrood disease was reported, which was introduced a little more than 10 years ago through the import of pollen contaminated with spores of a fungus (*Ascosphaera apis*)[13]. In most of the cases reported, this disease does not present a severe risk as has been found in countries of Europe and the USA, likely due to the warm climate and more intense hygienic behavior of the AHB.

Out of the several bee viruses, only few are reported in Brazil. Likely the low incidence is not linked to the absence of the virus, but rather to the difficulty of diagnosis from the part of the beekeepers. Following a metagenomic analysis in bee samples of the Southeast region[15], four viruses were found in Brazilian bees, namely *Acute Bee Paralysis Virus*, *Black Queen Cell Virus*, *Deformed Wing Virus* and *Israeli Acute Paralysis Virus*. Also the microsporidians *Nosema apis* and *Nosema ceranae* were still found, which are believed to be closely related to the CCD in the USA. In the particular case of *N. apis*, recent monitoring lead to believe that its presence is linked to colder regions of Brazil, as it was found only in samples obtained in the states of Paraná and Santa Catarina (Teixeira EW, unpublished).

For a long time, it was believed that the larval death of *A. mellifera* occurring in the Cerrado region during late winter and early spring was caused by *Sac Brood Virus* (SBV). However, studies found that the symptom was not a disease but rather the toxic effect of the pollen of *Stryphnodendron polyphyllum* (Fabaceae, Mimosoideae)[16] (Figure 1), named *Brazilian Sacbrood-like Disease* (BSBD). It is important to remember that the pollen of S. *polyphyllum* is not only toxic to the AHB, but also to the

native bees *Scaptotrigona depilis* (Moure 1942) and *Tetragonisca angustula* (Latreille 1811)[17] and also that the pollen and nectar of several other species are regarded as toxic to all bees in Brazil[6,18,19].

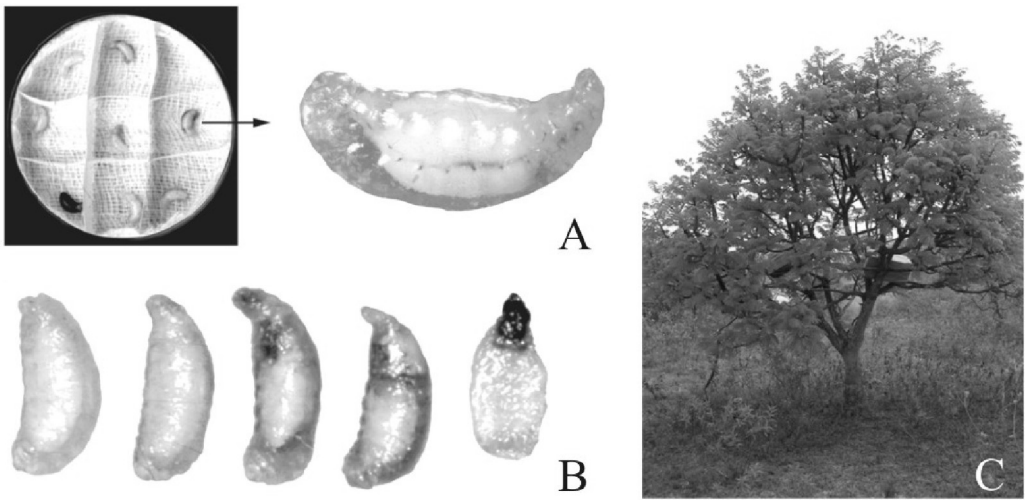

Fig. 1 Symptoms of Brazilian Sacbrood-like Disease in *A. mellifera* larvae fed *in vitro* (A) with pollen of *Stryphnodendron polyphyllum* and samples collected in hives during bloom (B) (from Carvalho et al., 2004); (C) a tree of *Stryphnodendron polyphyllum* on the Cerrado near Lavras, Minas Gerais, Brazil (Photo: Stephan Carvalho).

Among several parasites of bees, we found the mites *Acarapis woodi* (Rennie 1921) and *Varroa destructor* Anderson & Trueman 2000. The first case of occurrence of *A. woodi* in Brazil took place in the state of Rio Grande do Sul in 1970, likely from material coming from Uruguay where this mite had already been reported since 1953[20]. Nowadays, little is spoken about the presence and damages caused by this mite, and it is deemed an unimportant parasite of AHB under Brazilian conditions. Confirming this hypothesis, studies found that an important mechanism of resistance of bees against *A. woodi* is autogrooming, for which the AHB is more resistant than the EHB as it breaks the migratory cycle but not the reproductive stage of the parasite [21,22].

In relation to the ectoparasite *Varroa destructor*, the early reports in Brazil date back to 1978[1], while at present found in every region. An important characteristic of the AHB against this parasite compared with other subspecies of *A. mellifera*, is the more intense hygienic behavior, conferring increased tolerance[23]. In addition, factors as high tropical temperatures affect directly the reproductive rate of this mite, besides a little rigorous and relatively short winter[24]. Also the short development time of AHB (egg-larva-pupae-adult) can affect the life cycle of *V. destructor*[25]. In this sense, pioneering works showed that even with an average infestation of 5.0% in AHB hives in Brazil, the mite *V. destructor* does not cause significant damages to bees[26]. However, after the occurrence of CCD in the USA where it is believed that one of the main factors of the hive loss is the presence of *V. destructor*[27], the question about the real situation of the mite in Brazil was again risen. Recently, a study showed that even with low population levels of *V. destructor* on AHB, rates up to 23.0% of mortality of larvae and pupas are found[28].

Not less important than the questions cited above, for Brazil in the Neotropical region with a rich diversity of insects it is found that lots of these insects are important predators which may become enemies of bees. Indisputably, the ants (Hymenoptera: Formicidae) are the main causes of damages both to *A. mellifera* and the native bees, with emphasis on species of the genus *Camponotus* Mayr, 1861 [14]. There are also reports about attacks by ants of the genus *Solenopsis* Westwood, 1840[6]. In addition, other animal also can attack the hives such as wasps, armadillos and birds. Fortunately, in

Brazil there are no reports about the occurrence of new pests and predators as *Vespa velutina* Lepeletier 1836 (Hymenoptera: Vespidae) in France[29] and *Aethina tumida* Murray 1867 (Coleoptera: Nitidulidae) in the USA and Europe[30].

4. Pesticides

Brazil possesses a great and competitive farming sector, which for its climatic and soil characteristics is considered a food barn to the world. In that context, we can mention crops of soybean, sugar cane, fruits, vegetables etc. Factors contributing to the Brazilian agricultural success are the increase of yield per area and the increase of the planted area, brought about mainly by investments in research and technology, in particular the genetic improvement and control of pests, diseases and weeds[31]. Official data show that in the agricultural year 2010/11 a total area of 61.89 million of hectares was grown, while only grains (rice, bean, corn, soybean and wheat) occupied 46.27 million of hectares, with a yield of 142.9 million of tons. Estimates for the agricultural year 2020/21 point to a growth of 9.5% in the planted area (reaching 50.66 million de hectares) with an increase of 23.0% of the total product, reaching 175.7 million of tons[32,33].

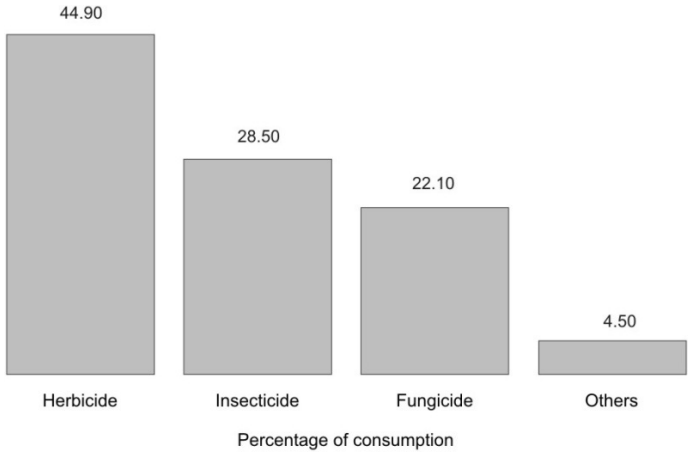

Fig. 2. Pesticide consumption in Brazilian crops[32].

Because of the expanding agribusiness, Brazil has become the greatest world market of pesticides, passing other countries as for example, the USA. In the year of 2007, 673.9 thousand tons of formulated products were consumed, with a total billing of US$7,125 billion against 646.0 thousand tons of products and US$ 6.000 billion in the USA. Out of the total consumed in Brazilian market, 44.9% were herbicides, 28.5% insecticides, 22.1% fungicides and 4.5% others (Figure 2)[34]. Considering that Brazil lies into a tropical region and has natural resources available across the year as adequate sunshine, water and temperature, it is possible to have two crops per year, causing Brazil to be the largest market in total sales and not in consumption of pesticide per area[33]. Another important characteristic is the amount of food produced on the basis of the investment in pesticides. In the ranking, Brazil takes the sixth place with expenditure of 7.39 US$ per ton of food produced, preceded by the USA < Argentine < EU (without France) < France < Japan (Figure 3) (Sindag, 2011*).

* Data from FAOSTAT and AMIS Global, available on the Sindicato Nacional da Indústria de Produtos para Defesa Agrícola/SINDAG at http://www.sindag.com.br/index.php - access in october/2011.

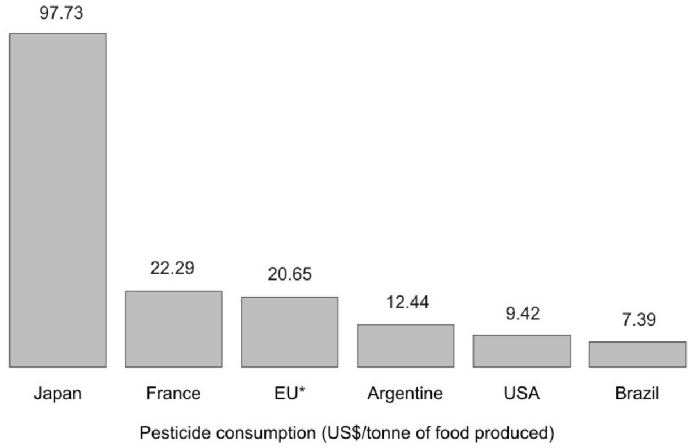

Fig. 3 Pesticide spent (in US dollar) per ton of food produced in different countries. *European Union without France (SINDAG, 2011[*]).

Several characteristics of the Brazilian agriculture are important and needed discussion, mainly climate and soil. For example the amount of organic matter and soil microbiology, are of extreme importance to the mobility, stability and metabolism of pesticides. In comparison with countries of non-tropical climate (e.g. Europe and North America), the organic matter content in Brazilian soils is inferior, which increases the mobility of molecules with low octanol-water partition coefficients[35]. So not only the mobility but also insecticide metabolism (e.g. carbamates) in Brazilian soils is different. Studies show that in Brazil both oxidation and production of toxic metabolites (e.g. sulfone and sulfoxide) need ten-fold more time than in soils of Europe and the USA, suggesting that such characteristics are directly linked to the low organic matter content of the Brazilian soils, consequently a short microbiological activity[36,3738].

Considering the accidents with bees brought about by the application of pesticides, little is known about the real Brazilian situation, for which it is difficult to predict whether bees are safe or not in relation to the effect of pesticides. Several reports about the possible contamination or death of bees by pesticides are mentioned in the states of Minas Gerais, Piauí and Rio Grande do Sul[39]. One particular accident occurred in the state of São Paulo as a result of application of thiamethoxam over a citrus crop. After aerial application, one hundred hives near to the treated area were found dead. Samples of bees collected *in loco* had residues of this neonicotinoid in the order of 0.04 mg/kg[40]. Early results suggest that the AHB can be more tolerant to some organophosphate and pyrethroid insecticides than the European subspecies[41].

Relative to the risks of exposure of bees to pesticides, factors like type of formulation (powder, liquid, fumigation etc), mixtures of compounds (insecticide + fungicide), environmental and application systems cannot be neglected. Differently from what occurs in Europe and the USA, where there is a periodical inspection of equipment for pesticide application, there is no such regulation in Brazil and no law or educational campaign aiming at the responsible use of machines and equipment. In a country with such a great farming area, increased consumption of pesticides and use of new technologies of application (e.g. aerial spraying) studies on the deleterious effects on bees are necessary[42].

5. Concluding remarks and future works

Like any other country with a booming agriculture, the particular characteristics in Brazil can affect native and exotic bee populations under natural conditions. Reports about decline of bee populations are notified in several parts of the country. There are however no technical and scientific confirmations and only a very small percentage of these cases is elucidated. The actual monitoring of

diseases in colonies of *A. mellifera* show that there is not any predominant disease which may account for a systematic bee decline[43]. One can further question the effect of nectar or pollen from plants which are admittedly toxic to bees, as is the case with *S. polyphyllum*, *Spatodea campanulata* (Bignoniaceae) etcetera. In relation to pesticides, the increase of consumption of these compounds in Brazil begins to generate discussions of the risks on the populations of beneficial insects and pollinators.

In conclusion, the situation of the bee populations in Brazil remains unknown, as it is not possible to state with certainty whether these are at risk or not. Therefore research for genetic improvement, improvement of beekeeping practices, food supplements, synergism among pesticides, disease monitoring, influence of the surrounding area (e.g. toxic plants) etcetera, need to be developed to contribute to the preservation of this important insect group.

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Bees require protection for sustainable horticultural crops production in Kenya

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Abstract

Background: Horticulture is the fastest growing agriculture subsector in Kenya, with the majority of production consumed locally and only less than 10% is exported. Intensive farming is currently practiced. Majority of Kenyan farmers have small land size, less than 1 ha where they grow not only horticultural crops but other food crops and livestock keeping. This paper highlights aspects of bees that relate to horticultural production and suggests reasons why bee protection is essential in Kenya.

Results: Kenya is rich in diversity of horticultural crops. Only few of these are traded in domestic and export market. The rest are consumed locally and have not been promoted for commercial gains. Wide range of fruits and vegetables require xenogamous pollination to enhance yields. Farmers have reported reduced honey bee visits on their crops though they are less aware of pollination needs of their crops. Also, pollination management is not included in their crop management practices.

Conclusion: Bees are an essential component of horticultural production but their survival has been threatened by farm practices in Kenya amongst other factors. Policy support is suggested to minimize the bee decline trend and to increase their use for pollination provision.

Keywords: farm practices, honey bees, non-Apis bees, pollination, stingless bees

1. Introduction

1.1 Role of horticulture in Kenyan economy

Horticulture is the fastest growing agricultural subsector in Kenya with recorded tremendous expansion of both domestic and export markets[1]. In 2010, it was second after tea in terms of foreign earning. The production is by small scale farmers and large multinational companies with more than 60% of the growers being smallholders[2], who usually have less than 0.5 ha of land allocated for horticulture production.

It is now appreciated that horticulture plays a major role in supporting Kenyan livelihood[3]. The subsector directly and indirectly employs over six million Kenyans[2]. In appreciation of the role of horticulture, funding to manage horticultural challenges in Kenya has been on increase to aid the realization of the economic potential of this subsector[4].

In Kenya, horticultural crops consist of vegetables, fruits, medicinal and aromatic plants as well as ornamental flowers (floriculture). These large categories have a wide range of crops that have different production requirements. The climatic conditions in the country are varied, allowing production of these crops. Floriculture is mainly by commercial large scale companies with a very small proportion coming from small-scale farmers. Vegetables are mainly grown outdoors by small-scale farmers.

1.2. Key constraints to effective horticultural production in Kenya

Horticulture in Kenya is faced by many challenges especially in smallholder farmers. These are exacerbated by the system of production, which is largely small-scale in operation, implying that the investment to production is minimal. Pests are the main constraint, responsible for a large proportion of production costs. Main pests are exotic and most of these are of quarantine importance to the importing countries from Kenya. Farmers reportedly apply pesticides more than usual in controlling the pests while others use concentrations higher than those recommended[5,6,7]. Lately, the export segment has been faced with stiff regulations that require monitored pesticide use, including utilization of only specific pesticide molecules[8]. The effect of these regulations has increased

awareness by farmers on the negative effects posed by pesticides not only to the health of the consumers but also to themselves. Farmers are increasingly adopting Integrated Pest Management (IPM) strategies to manage pests.

Unreliable rains and harsh weather conditions also pose a major threat to the horticulture sector development in Kenya. The country has experienced extreme weather patterns, away from the usual rainfall patterns. There is less rain in a season and frequent floods. Water harvesting is not a common practice by small scale farmers though farmers are slowly adopting water conservation measures to mitigate the unreliable rain patterns.

Depleting soil fertility is a major challenge in horticultural farms. Use of inorganic fertilizers many times is advocated but these are expensive and unaffordable to many smallholder farmers. Animal manure is usually used to supplement the fertility provision though not always.

Capital and funds for managing production costs is usually a major challenge as it affects the choice of inputs farmers use to mitigate the production problems. Kenya currently boosts of high numbers of micro finance enterprises and commercial banks that exist and provide farmers with loans, which are repayable on negotiated terms. The Kenyan government has played a key role in ensuring a favorable environment to support micro financiers as well as to protect borrowers.

2. Results: role of bees

2.1 Horticultural crops pollinated by bees in Kenya

Amongst the exported fresh produce, ornamental flowers take the largest percentage by volume and value. For example, in 2005, they accounted for 45% by volume and 57% by value of all exported fresh produce[1]. Vegetables are the second most important fresh produce, which was about 35% by volume in 2005 with the rest covered by fruits. For market purposes, the ornamental flower plants do not require pollinators since flowers are the target market commodities. However, bees and other flower visiting insects can access nectar and pollen from such plants, hence awareness of visiting insects is important for protection of bees. The advantage of the cut flower production system is that most of them are grown in-doors and are thus rarely accessible by the bees.

The green beans (French beans or Snap beans) are the dominant vegetables for fresh export market. Other major ones include sugar snaps, snow peas and runner beans. Common Asian vegetables include okra, karela, dudhi, chilli and aubergine. However, for domestic market, there is wide range of important vegetables e.g. tomatoes and wide range of cucurbits. More than 90% of vegetable production is for domestic market and almost all are grown outdoor.

In Kenya, few fruits are exported compared with those that appear in the domestic market. Exported fruits include, avocadoes, mangoes, pineapples, passion fruits, bananas, and strawberries[1]. The list is not static and has been steadily growing, as well as the amount exported due to new market access.

Demand for herbs and spices has been on the increase both in the domestic and export markets. Currently Kenya exports lemon grass, basil, dill, sweet Marjaram, oregano, parsley, rosemary, thyme, sage, chamomile and tarragon, (herbs). In addition, it exports spices that include garlic, ginger, coriander, chillies, paprika, turmeric and cumin. Some of these crops require pollination to optimize their yields.

There exists in Kenya a wide range of indigenous fruits and vegetables many of which are not traded[9,10,11]. However, their use by local inhabitants is critical as they form part of household food source.

A new greenhouse farming technology for smallholder farmers introduced in early 2000s and currently in high demand by farmers has been compounded by lack of pollinators. Different non-Apis bees are being targeted to provide pollination services since honey bees are less efficient for those crops grown. Currently, only stingless bees (e.g. *Meliponula* spp, *Hypotrigona* spp, *Pleibena* spp) are promising. A main limiting factor in using honey bees is the small size of the greenhouses, which can be overcome by use of the stingless bees.

2.2 Pollination requirements

Studies in Kenya show that horticultural crops that are traded and consumed for their fruits and seeds require pollination to enhance yields[12,13]. The studies have shown evidence that there is increased fruit and seed yield when bees are provided, for those crops dependent on bee pollination. Such evidence is similar to scientific reports in other parts of the world[14,15,16, 17].

In terms of income resulting from utilization of bees, Kasina et al.[12] showed that there is high gain when bees are provided to pollinate crops in Kenya. Such gains would reportedly be lost if bees do not pollinate the crops. The evidence in other parts of the world is similar[18,19,20]. It is now common knowledge that honey bees are not the major bee pollinators in a wide range of crops [21,22]. In contrast, an array of native bees provides more pollination service to crops compared with honey bees[12,22]. However the ability to domesticate, and the sheer number of honey bee workers provide a reliable pool of pollinators to satisfy given a flower patch, thus satisfying farmer interests and intent of pollination. Apart from honey bees in Kenya (and other tropical countries), stingless bees are the only unmanaged pollinators that can provide large number of bees for pollination when required. The domestication of the other bees is a challenge and their presence is mainly dependent on habitat management.

2.3 Crop pollination management

Currently in Kenya there are no pre-planned strategies for managing pollination service in small-scale farmers, who form the bulk of horticulture producers. However, commercial growers are known to keep honey bees for pollination of their vegetable and fruit crops. Experience by the author and interaction with these growers over time has shown that the growers have no idea of who the best pollinator of their crop is, and they do assume that honey bees will do the pollination work. Honey bee pollination business has existed for some time in Kenya, where growers rent honey bee colonies to pollinate their crops. Unlike in USA and probably other developed countries, Kenyan pollination rentals have loose contractual agreements. There is no policy support for pollination rentals. In addition, there is minimal colony movement since there are no migratory beekeepers.

Awareness about pollination management is increasingly becoming important in Kenya. In the past 10 years, scientists have highlighted the importance of pollinators in agriculture. The recently concluded project supported financially by Germany (2001-2010), BIOTA East Africa, showed the effects of land fragmentation not only to native and wild bee species but also of bee pollination on crop productivity[13,23,24, 25,26]. The project was the first major initiative to highlight the plight of pollinators in the country. Contributing to awareness of pollination, the Uvima project (2008-2011) developed fact sheets and keys for 21 bee genera in East Africa that describe, in simple terms, the different bees, crops they pollinate and how to protect them[27,28]. In continuation of the achievements by these projects, the Global Pollination Project (2009-2013) is undertaking studies to enhance utilization of pollinators in agriculture[29]. In addition, KARI has been training farmers, extension service providers and other stakeholders on aspects of pollination and pollinator management. Recently concluded stakeholder analyses have reported pollination as a major challenge in horticultural production in Kenya[9,10,11].

The challenges faced in Kenya that are preventing full utilization of bees in horticultural production include the farming systems. Most of the producers are small scale in operation, with farm sizes of less than 1 ha where they grow a variety of crops. For example, one of the main fresh produce exporters and processors currently runs contract farming where each contact farmer must have a minimum area of 19 x 19 m (described as single unit). Farmers can choose to increase the units depending on their farm size and capital base. The unit size is a reflection of the status of the land size situation in Kenyan small-scale horticultural farms. With such farms, managing pollination is quite difficult, considering that farmers with similar crop interest may not occupy areas in same vicinity.

2.4 Threats to bees

Several factors come into play when considering current threats to bees in Kenya. Firstly, while bees are important providers of pollination to agriculture, they are perceived only as source of honey. That implies only honey bees are recognized for such role since domestication of stingless bees, which also produce honey, has not fully operationalized. As the importance of pollination becomes clear, the role of bees is expanding and farmers are likely to take steps to manage pollination. Another aspect is that Kenyan honey bees are aggressive, and they sting at slight disturbance. This has made many Kenyans to develop a negative opinion on utilization of bees. Beekeepers are few due to this perception of honey bees and many farmers are hesitant of using honey bees for crop production. However, it is lack of awareness on honey bee behavior and its handling that prevent farmers from utilizing the full potential of honey bees. Thus overcoming these fears could drastically contribute to protection of bees. Pollination in Kenya has largely been feral, bees coming from nearby habitats within the agriculture landscape. However, farmers have continued to open up land to cultivation with no effort in maintaining undisturbed areas. This has over time denied bees places to nest and forage, contributing to their decline in the farmlands. The Government of Kenya has developed policies to prevent further habitat loss through creation of the environment management authority[30] (EMCA, 1999) and protection of wildlife[31,32]. It has also encouraged private conservation of wildlife. Recently the government developed a policy to encourage all farmers to have at least 10% of their farm size left aside for trees and other life forms. Many farmers are considering this and are developing structures to implement the policy. The main threat to bee existence in Kenya are currently farm practices that are not favorable to bees. While habitat loss is a key threat, bees are able to survive well by utilizing farm pockets suitable for nest establishment, and presence of food resources all year round. However, current practices do not support such safety pockets. High use of a wide range of synthetic broad spectrum pesticides is common[33] which contributes to decimation of bees and or their foraging and nesting sites. Main tillage practice used by farmers is soil pulverization, which has effect on the soil nesting bees. Flooding in horticulture is common irrigation practice to supplement rains and this has also effect on soil nesters.

3. Discussion

There is clear and documented benefit of crop pollination by bees in Kenya[12,34,35]. However, bees are under-utilized for pollination purpose mainly due to lack of awareness by farmers and extension service providers on these benefits, and lack of available options to manage the bees. In horticulture, pollination use is critical to ensure the quantity and quality of the produce.

Threats that exist to bees in Kenya can be minimized through establishment and promotion of government policies, stakeholder participation and farmer implementation of changed farm practice behavior. The Global GAP implementation in the country has enabled farmers to adopt practices that are friendly to bees such as Integrated Pest Management (IPM). This has worked in farms that are growing crops purposely for export market. For the domestic market, which is the main outlet for more than 90% of horticulture produce in Kenya, there are no standards yet for produce. However, attempts have been made to develop Kenya GAP, modeled towards the Global GAP perspectives. However, this standard only becomes effective to farmers targeting small market segment in the country (mainly supermarkets and commercial grocers). Awareness creation will provide extra avenues to support the need for farmers to manage pollinators.

Worldwide, scientists, regulators and industry are considering additional perspectives in protecting pollination[36]. For example, the protection goal for honey bees has focused mainly on colony survival for production of honey and other hive products but now an additional protection goal on pollination has been considered. Also, only honey bees have been used to represent other bees in pesticide evaluation studies but addition of other non-*Apis* bees is being considered[36].

The participation in global bee meetings is essential for Kenya to grasp the current trends in bee benefits and protection. Different stakeholders in Kenya continue to participate in international meetings representing scientists, beekeepers and their associations and regulators. The pesticide industry in Kenya is mainly an extension of its parent company located in Europe, USA or China and

India among others. Thus participation of this Kenyan segment of stakeholders in international meetings has not been forthcoming, probably because their partners do participate. This may not be a key threat if their parent companies are able to cascade bee aspects. Locally, the industry participates well in meetings that discuss bee aspects.

4. Conclusion

Kenya is home to a wide range of horticultural crops, both exotic and native, which benefit immensely from pollination by bees. Most farmers grow horticultural crops in an area of less than 1 ha and do not practice pollination management. In contrast, commercial farmers keep or rent honey bees for pollination, but do not consider whether it is the most efficient pollinator for their crops. The majority of smallholder farmers is not aware of the role of bees in crop production, which may be one of the reasons why they do not manage pollination. The main threats to bee protection are the current farm practices particularly those related to use of synthetic pesticides, tillage and agronomic practices since these affect bees, their nesting and forage resources. Considering that farmers do gain from protecting bees, it is suggested that policies that support bee protection at farm level should be promoted. In addition, pollination rentals need policy consideration to ensure beekeepers are motivated to provide colonies for pollination while at the same time the growers are obliged to use practices that protect bees at times of flowering periods. Aspects of 'in situ' pollination rentals need to be considered to minimize bee movements within the country.

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Side-effect of acetamiprid in adult Africanized honeybee

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Abstract

The insecticide acetamiprid is widely used in many crops in Brazil like cotton, beans, citrus and rice to control many kind of pests and ensure high yields. Honeybees (*Apis mellifera*) may frequently become exposed to such chemicals as a consequence of their foraging activities (collecting water, natural resins, pollen and nectar) or even by spray drift, since in some of these cultures this neoniconinoid is applied by aircraft. Intoxication resulting from exposure to xenobiotics products can be lethal, which is easily identifiable, or cause effects on the physiology and insect behavior. These effects, caused by sublethal doses are difficult to measure (such as paralysis, disorientation or behavioral changes), and can compromise the entire social structure of the colony.

To improve the knowledge about the action of low doses of insecticides in honey bees, the effects of acetamiprid was studied in adult Africanized honey bees. To achieve this goal was used two behavioral protocols: proboscis extension reflex (PER) and locomotor activity. Bees were obtained from adequately fed, healthy and queen-right colonies. Adult worker bees were collected from frames without brood. After insecticide application, the bees were kept in plastic containers (250 mL) and held in a incubator at a temperature of 32°C and relative humidity around 70% and were fed with cândi solution. Initially, LD_{50} and LT_{50} were estimated. The PER and locomotor activity were analyzed 1, 4 and 24 hours after topical application of 1µl in doses corresponding to LD_{50}, $LD_{50}/10$ and $LD_{50}/100$.

The PER was impaired 1 and 4 hours after application of LD_{50} and $LD_{50}/10$. The locomotor activity behaviour was impaired 1 hour after application of LD_{50} and 4 hours after application of LD_{50} and $LD_{50}/10$. It was not observed impairment 24 hours after topical application. The greater impairment was found when the behavioral analyses were made close to the period determined by LT_{50}. These results can be justified, probably, due to the presence of a detoxification mechanism. Some studies show that metabolites of acetamiprid were not toxic to honeybee which is compatible with the time of action of the insecticide observed in this study.

Determination of fipronil LD_{50} for the brazilian bee *Melipona scutellaris*

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Abstract

To better understand the sensitivity of the models represented by *Apis mellifera* L., 1758 in toxicology studies of insecticides to bees, the aim of this study was to determine the LD_{50} of fipronil by topical application on the stingless bees *Melipona scutellaris* Latreille, 1811. Foraging bees were collected at the nest entrance and in laboratory anesthetized with CO_2 for applying 1.0 µL of fipronil solution on the pronotum. Each group of treatments was made with thirteen bees divided in three cages, while in the control treatments the bees received only acetone. During the assay, the behavior and the number of dead bees were registered. The results showed that the insecticide fipronil applied topically was harmful to *M. scutellaris* and for *A. mellifera*, where the LD_{50} for 48 hours was 0.41 ng a.i./bee or 4.1 ng a.i./ g of bee. Comparing the LD_{50} values here obtained with the stingless bee *M. scutellaris* and those of *A. mellifera* in literature, we can conclude that the native bees are more sensitive to fipronil than the allochtonous bee, suggesting that further studies should be accomplished to determine the real hazard of pesticides to natives bees.

Keywords: stingless bees, pesticides, phenylpyrazole, toxicity, LD_{50}

1. Introduction

The insecticide fipronil (phenylpyrazole - $C_{12}H_4Cl_2F_6N_4OS$) acts on the nervous system of insects by blocking chloride channels through the receptor of gamma-aminobutyric acid and glutamate. Due to its mode of action it is considered a new generation insecticide, being a broad-spectrum systemic insecticide and effective at low application rates. Widely used in Brazil and more than 70 countries it is considered highly toxic to bees, which is why its use is banned in France since 2004[1-10].

Toxicological studies with bees use mostly the model species *Apis mellifera* L., 1758 (Hymenoptera: Apidae) and it has been observed that sublethal doses of fipronil can cause behavioral changes, to these bees, related mainly with tasks such as feeding and foraging, fundamental for the survival of colonies[11-18]. However, native bees exposed to pesticides may be at a different risk, because differences in tolerance among species of bees have been observed by several authors[19-26] and most show that wild bees are more sensitive to the insecticides than the honeybee *A. mellifera*[27,28].

Among the stingless bees within the tribe Meliponini, *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae) is popularly known. It is endemic in northeastern Brazil and distinguished by its ease of domestication and management, honey production and significant potential for replication on a large scale for pollination in greenhouses and open field, beyond its ecological importance as pollinators of native plants in Brazil[29-34].

Seeking to diversify the species of bees to better understand and compare the sensitivity of the models represented by honeybee *A. mellifera*[27], the objective of this study was to determine the topical LD_{50} of the insecticide fipronil for the stingless bees *M. scutellaris*.

2. Material and Methods

Three colonies of *M. scutellaris* from the Universidade Estadual Paulista (UNESP) *campus* Rio Claro, were used in the experiment. The hives were kept in protected room where the bees had free access to the external environment through a plastic tube that connected the nest entrance and the outside. All time, the colonies were surveyed to assess the health, queen laying capacity, foraging activity and

food availability. To promote the survival of colonies during a dry season, 60 % of sucrose solution prepared with lemon juice was provided[35].

2.1 Acute toxicity test (LD_{50})

Assays were carried out at the Center for the Study of Social Insects of UNESP with some modifications on the directives of the Organization for Economic Cooperation and Development[36].

To determine the topical LD_{50} of the insecticide fipronil (95% of purity, Bayer CropScience, Brazil) to foragers of *M. scutellaris*, a stock solution (1000 ng a.i./µL acetone) was prepared and next a range of several concentrations between 0.5 to 5.0 ng of a.i/µL acetone. The control treatment received only acetone, after its low toxicity had been assessed in preliminary assay compared with water.

To facilitate the handling and application, the bees were anesthetized with CO_2 (ten seconds). With a repetitive micropipette the volume of 1.0 µL of solution was applied on the thorax of the bees. To ensure the variability among the colonies and to obtain a realistic and reliable value of LD_{50}, the bees from each repetition were taken directly from a single colony. In each treatment (=concentration) we had three distinct groups of bees which originated from different colonies. Thus, each treatment (group of bees from one concentration) consisted of three replicates with ten bees, in total thirty specimens. During the assay, bees were fed *ad libitum* with sucrose-solution (50%), and cages were kept in climatic room at 29±2ºC, relative humidity of 70±5% and darkness.

2.2 Data collection and analysis

Along 72 hours after the application of fipronil on *M. scutellaris*, assessments were made one, four and every twenty-four hours, with registration of all behavior, as well as the number of dead bees. Statistical analysis to determine the LD_{50} value were performed using a log-logistic model from the package "drc" (Analysis of Dose-Response Curves) [39] compiled by the statistical software R[40].

3. Results and discussion

Bioassays performed in order to compare the toxicity of acetone and water showed that this organic solvent was no toxic to *M. scutellaris* foragers. Already, the insecticide fipronil topically applied was considered highly toxic to *M. scutellaris* foragers, with a LD_{50} for 48 hours of 0.41 ng a.i./bee ($CL_{95\%}$ = 0.23 - 0.58; D.F. = 16 and χ^2 = 9.8238, Figure 1). Comparing this result with the LD_{50} of fipronil established for other species of bees, foragers of *M. scutellaris* were more sensitive to fipronil than *A. mellifera* (1.9- 6 ng a.i./bee), *Megachile rotundata* Fabricius, 1787 (4 ng a.i./bee), *Nomia melanderi* Cockerell, 1906 (113 ng a.i./bee) and *Scaptotrigona postica* Latreille, 1807 (0.54 ng a.i./bee)[13,18,37,38,41, 42]. Likewise, taking into consideration that the workers of *M. scutellaris* have a mean weight of 0.1g, recalculating the LD_{50} we got a LD_{50} of 4.1 ng a.i./g of bee. In this way, *M. scutellaris* remains more susceptible to fipronil than *A. mellifera* (103 ng a.i./g of bee), *M. rotundata* (132 ng a.i./g of bee) and *N. melanderi* (13,190 ng a.i./g of bee)[41].

The doses of fipronil from 1.5 to 5 ng a.i./bee for 48/72 hours and 5 ng a.i./bee for 24 hours, caused 100 % of mortality, respectively (Figure 2) The dose of 1.5 and 1.0 ng / bee also had high rates of mortality after 48 hours of intoxication, with 96% and 85% of dead bees, respectively.

Still, the bees in the group treated with 5.0 ng of fipronil/bee showed signs of intoxication: after 4 hours this group had bees with their wings vibrating. This same behavior was observed in *A. mellifera* treated with 0.1 ng fipronil/bee and after 11 days of exposure dose of 0.01 ng of fipronil/bee[11]. According to these authors the behavior of vibrating wings is accompanied by the emission of alarm pheromone, which causes attacks among individuals, also observed in this study. After 24 hours of contamination, surviving bees in the groups treated with the higher doses of fipronil (2.0, 2.5 and 5.0 ng a.i./bee) had tremors followed by paralysis and death. These same signals were also observed in honeybee *A. mellifera* treated with sucrose solution contaminated with fipronil 2 g/Kg of diet[12].

These results are consistent among the diversity of bees, which differ in their vulnerability to exposure to insecticides[28]. Several studies[19-26] show differences in tolerance and/or sensitivity between species of bees and pesticides, most of these results show that the species honeybee *A.*

mellifera was more resistant compared to species of stingless bees which corroborates the suggestion that wild bees are a pollinating group at particular risk for exposure to pesticides[27].

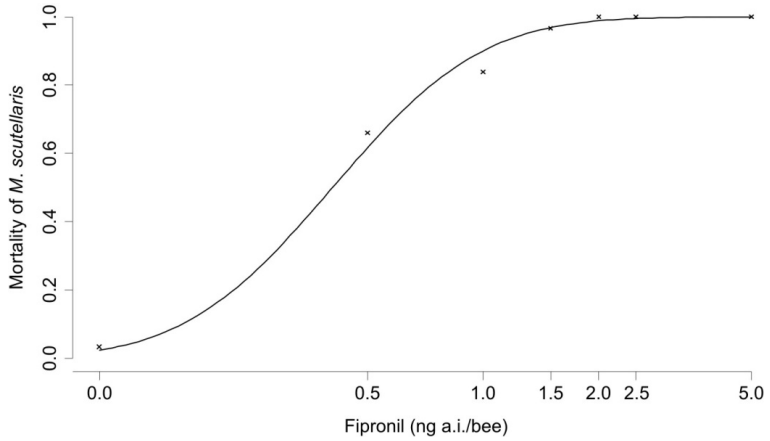

Fig. 1 Acute toxicity (48 hours) by topical application of the insecticide fipronil to foragers of *Melipona scutellaris*.

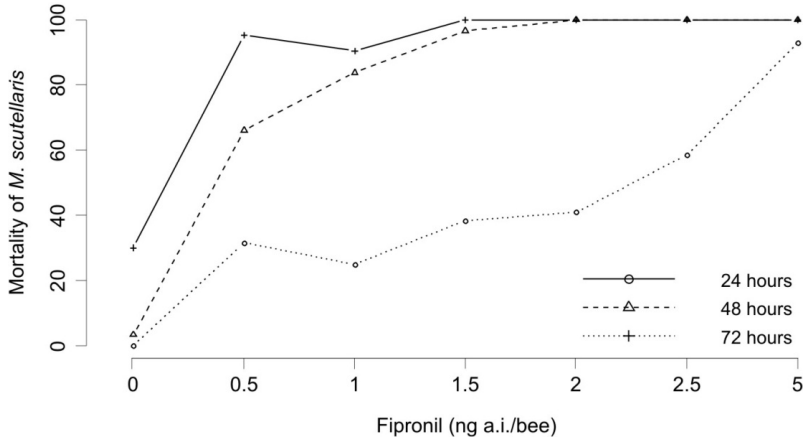

Fig. 2 Mortality evolution of foragers *Melipona scutellaris* when exposed at different doses of fipronil.

The findings also support the idea that ecotoxicological studies on diverse pollinating species can be used to obtain a better understanding of how the sensitivity of a model representative of honeybees (*A. mellifera*) can be compared to other species of bees[43].

The bee species *M. scutellaris* is considered a potential effective pollinator species for production on a large scale as a pollinator in greenhouses and the open field, with ease of maintaining strong hives, which can be easily transported and multiplied[30]. Brazil has a high diversity of bees that interact with numerous plant species[44] and it is believed that 33% of the crops that provide food for the human population depends on pollination by bees[45].

We believed than poisoning by insecticides is one of the causes of high mortalities of bees, especially in areas in southern and southeastern Brazil, where the disappearance of bees caused by insecticides

has become a concern. Between 2008 and 2010, about 5000 bee hives of Africanized *A. mellifera* were lost in the central region of São Paulo. Hives of native bees were not included[46].

Since Brazil has a high diversity of native bees, endemic in the tropics and sensitive to low temperatures, studies on the toxicity of insecticides in Brazil should focus on these species[47].

4. Conclusion

The insecticide fipronil was highly toxic to foraging stingless bee *M. scutellaris* under laboratory conditions, with a topical LD_{50} in 48 hours of 0.41 ng a.i./bee (4.1 ng of fipronil/g bee). It is suggested that bees of *M. scutellaris* are more sensitive to fipronil than *A. mellifera* (Africanized and Italian), *M. rotundata*, *N. melanderi* and *S. postica*. Tremor followed by paralysis were the main signs of intoxication observed in the groups treated topically with the highest dose of fipronil.

The LD_{50} results determined in this work are being used to assess behavioral changes through the Proboscis Extension Reflex (PER) and locomotor activity, in particular which doses of fipronil causing sublethal effects in foragers of *M. scutellaris*.

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Is the European honeybee (*Apis mellifera mellifera*) a good representative for other pollinator species?

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Abstract

Pollinators are important components of biodiversity and provide a key ecosystem service through pollination (Klein et al., 2007). Honeybees, mainly *Apis mellifera*, are the most economically valuable pollinators for crop monocultures worldwide (Wantanabe, 1994), however, for several high-value crops, e.g., coffee, *Apis* pollination is less effective than pollination by local wild pollinator species (Klein et al., 2003).

Worldwide an increase of high-value crop farming and an accompanying increased dependency on pollination services occurs. For instance, data from Brazil indicate that total cropping area has grown with 70% and as a consequence pesticide use increased by 700 %. The current pollinator risk assessment is based on the European honeybee (*Apis mellifera mellifera*) and it is not clear if this is representative for other pollinator species.

In a first attempt to test if *Apis mellifera mellifera* is a good representative for other pollinators a first-tier contact LD50 test using dimethoate and deltamethrin was performed with several pollinator species originating from The Netherlands, Brazil, and Kenya, respectively. Thus acquired LD50 data will be used to construct a Species Sensitivity Distribution curve ranking the different species by their response to direct contact with the toxicant.

Tested species comprised European honey bee, bumble bee, Africanized honeybee, *Scaptotrigona postica* (stingless bee), African honeybee, *Melliponula ferruginea*. The presentation will present SSD curves for both dimethoate and deltamethrin and will discuss the results in the context of pollinator risk assessment.

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Effect of agrochemicals on the pattern of visitation of honey bees (*Apis mellifera)* in melon (*Cucumis melo)* flowers in Brazilian Northeast

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Abstract

Background: Lately pollinators of the main crops have presented a decline with considerable economic impacts. Among the probable causes of this problem is the inadequate use of pesticides, mainly in monocultures. The culture of melon (*Cucumis melo*) is dependent on pollination services by honey bees (*Apis mellifera* L.). However, agrochemicals are not applied with special care to the visitation of bees in cultivated areas at the irrigation pole of Petrolina-Juazeiro, located in Brazilian Northeast. Therefore, the objective of this study was to evaluate the effect of agrochemicals pulverization on the pattern of visitation of honeybees to melon flowers.

Results: In the dry season the honey bees' visits occurred since 6 a.m. in larger numbers than in the wet season. Indeed, in the dry season, there was a negative effect of the application of agrochemicals on the frequency of visitation by *A. mellifera*, after the 1st, 2nd, and 5th day of pulverization. This effect was more intense on the 1st day, when there were registered average values smaller than one visit, independently of the flower type and time of the day. Moreover, the first visits were observed only from 8 a.m. On the 5th day the average numbers of visits were still lower than in the dry season, but higher than on the previous days.

Conclusion: Comparing both seasons, we found that the frequency of visitation observed in wet season was lower than that found in dry season. This indicates that the productivity of the melon in the first period can be reduced by the excessive application of agrochemicals, since there will be a reduction of the pollination services.

Keywords: melon, pollination, agrochemicals, honey bees, *Apis mellifera*

1. Introduction

Pollinators of the main crops apparently are in decline with considerable economic impacts. The inadequate use of pesticides, mainly on the monocultures, is one of the probable causes of this problem. Apart of the lethal effect, easily percepted, the impact of pesticides on pollinators, especially bees, can cause more subtle alterations on their behavior. These changes may culminate with the rupture of the division of labor and the social exclusion of the contaminated bees, and eventually cause serious problems for the colony[1,2].

The role of pollinators as promoters of agricultural production is undeniable. However, the current production systems, inputs and agricultural practices have caused negative effects on these agents, both on their diversity and abundance and on pollination efficiency[1].

The melon (*Cucumis melo* L. – Cucurbitaceae) crop is dependent on pollination services carried out by *Apis mellifera* L. Simultaneously, many pesticides are used to control plagues and diseases manly during the wet season, when pests are more frequent. Nevertheless, the consequences of agrochemicals application on the pattern of visitation of bees, and its relation with the fruit production is unknown. Thus, this study aimed at quantifying and comparing the visitation pattern of *A. mellifera*, in two periods of the year, the dry season without the use of agrochemicals, and the wet season with the application of agrochemicals.

2. Material and methods

The work was performed in a commercial area at the irrigated perimeter of Mandacaru, in Juazeiro (09º24'S, 40º26'W), Bahia, in Brazilian Northeast. For the observations seeds of melon (yellow kind, 10/00) were used. The seeds were cultivated in a conventional system with mulching, drop irrigation, without the introduction of honey bee hives.

In order to register the flower visitors, daily and simultaneous observations were done on the two kinds of flowers (hermaphrodite and male) in two periods of the year. The timetables for the agrochemicals applications were different, depending on the climatic and plant phytosanitary conditions. In the dry season (July 2010) 10 flowers were observed (n= 5 ♂ and n=5 ♂♀), and in the wet season (March 2011), 32 flowers (n= 16 ♂ and n=16 ♂♀). The frequency and behavior of the flower visitors were observed from 5 a.m. to 6 p.m., and the average numbers of visitors were calculated per time and per floral type.

3. Results and discussion

In the dry season it was verified that the visits of *A. mellifera* occurred from 6 a.m. in both kinds of flowers. For hermaphrodite flowers, the average number of visits of *A. mellifera* varied from 1.5 visits (recorded from 5 p.m. and 6 p.m.), to 17.5 visits (observed from 10 a.m. to 11 a.m., and from 1 p.m. to 2 p.m.). For male flowers, this number varied from 1.6 visits, which was recorded early in the morning (from 6 a.m. to 7 a.m.), and in late afternoon (from 5 a.m. to 6 p.m.), to 12.8 visits (from 11 a.m. to 12 a.m., Figure 1). In general, in all intervals, hermaphrodite flowers were more visited than male flowers. These results are in agreement with a work[4] carried out previously with the same melon variety in an experimental area.

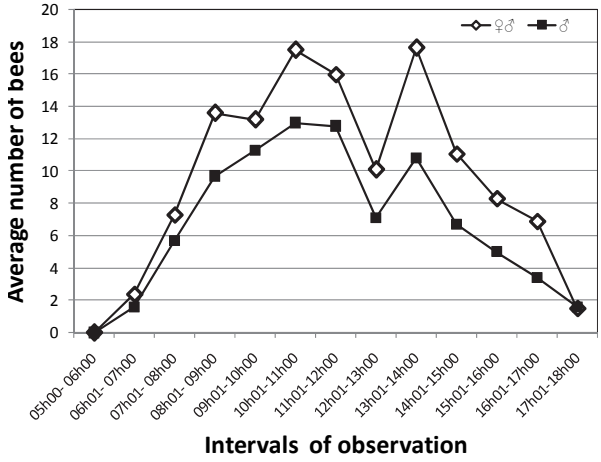

Fig. 1 Average number of honeybees visiting melon flowers (hermaphrodite: ♀♂ and male: ♂) in the dry season, in Juazeiro.

In the wet season (Figure 2), in general, it was verified that the average number of visits of *A. mellifera* for each interval of observation was much smaller than the average observed in the dry season. This result may be related to several factors. One could be the flowering of the surrounding vegetation which would compete for the visitors' attraction but, at least close by, flowers were not observed during the experimental period. The other aspect could be the negative effects of the agrochemicals, since these are used more frequently (daily) and in greater quantities during the wet season, due to a higher incidence of pests and plant diseases. Comparing the frequency of visits at the 1st, 2nd and 5th day, after pulverization, it was observed that the effect of the agrochemicals application was higher at

the first day, when average values below one visit were recorded, regardless the floral type and time. Moreover, the first visits were observed only from 8 a.m. (Figure 2).

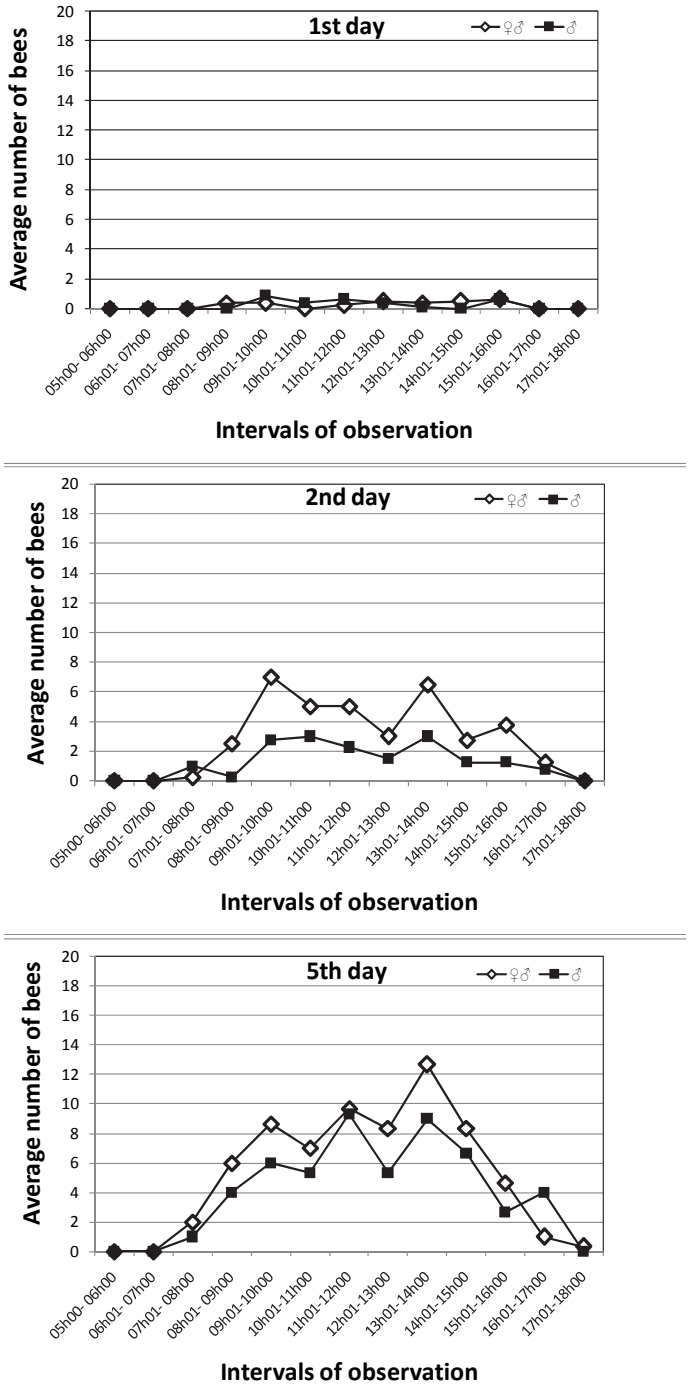

Fig. 2 Average number of honeybees visiting melon flowers (hermaphrodite: ♀♂ and male: ♂) in the wet season, on the 1st, 2nd, and 5th day after pulverization, in Juazeiro.

On the second day, the visits were recorded from 7 a.m. when the frequency of visits varied from 0.25 to 7.0 for hermaphrodite flowers, and from 0.25 to 3.0 for male flowers. On the fifth day there was no difference between the time of the first visits, however the frequencies were higher for both hermaphrodite (1.00 to 12.67) and male (1.00 to 9.33) flowers. According to other studies[3], for the development of fruits with commercial characteristics, 10 to 15 bee visits are necessary. This number of visits was registered only on the fifth day, which indicates that during all this period the fruits that were developing probably did not fulfill the commercial standard parameters. As the producton of hermaphrodite flowers occurs during a short period of the culture cycle[4], the indiscriminate use of agrochemicals in this period may compromise fruits' production.

4. Conclusion

Comparing both periods, it was found that the frequency of visits recorded in wet season was lower than that recorded in dry season. This indicates that the melon productivity during the wet season can be affected by the excessive application of agrochemicals, due to a consequent decrease of the pollination services.

Acknowledgments

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Assessment of lethal and sublethal effects by spinetoram on *Bombus terrestris*

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Abstract

Nowadays the worldwide use of bumblebees as pollinator of several horticultural crops has resulted that they fulfil both an ecological and economical role. Consequently, exposure to pesticides is not unlikely. In general naturalyte insecticides as spinosyns are a major widely applied class because they are more selective than conventional pesticides, however, toxicity of spinosyns A and D (spinosad) has been reported on honeybees and bumblebees. In the field bumblebees can be exposed to pesticides by contact and by the consumption of contaminated food.

In this project we assessed the potential hazards of a novel naturalyte insecticide spinetoram consisting of spinosyn J and L. Three different experiments were conducted in the laboratory wherein workers of the bumblebee *Bombus terrestris* were exposed to different concentrations starting form the maximum field recommended concentration (MFRC) and then different dilutions (1/10-1/10,000). First, via direct contact with wet and dry residues of spinetoram severe worker loss was observed; the respective LC_{50}-72h values were 50 µg/l and 21 µg/l. Typically, intoxicated bees showed symptoms of tremors and paralysis. Second, oral exposure via contaminated sugar water in micro-colonies demonstrated that the MFRC caused 100% worker loss after 4 weeks, whereas this was only 54% with 1/10 of the MFRC after 11 weeks. For worker mortality the calculated acute (72 h) and chronic (11 weeks) LC_{50} values were 21 µg/l and 2.5 µg/l, respectively. At 1/100 of the MFRC no lethal effects were observed. Next to lethal effects, sublethal effects were evaluated. In the nests exposed to the MFRC and to 1/10 of the MFRC the numbers of drones produced were significantly ($P<0.05$) reduced when compared with the control group (57 ± 4 drones). However at lower concentrations starting at 1/100 of the MFRC no sublethal effects were seen on the reproduction. Third, we assessed for potential sublethal effects by spinetoram (1/100-1/10,000 of the MFRC) towards foraging behaviour. Here we used the bioassay as developed to assess foraging effects by neonicotinoids (Mommaerts et al., 2010). Here no change in the behaviour of the workers was seen.

In conclusion, the highest concentrations of spinetoram (MFRC and 1/10 of the MFRC) caused lethal mortality of exposed workers and this resulted in a loss of progeny. But when compared with spinosad, spinetoram is safer. Interestingly, no negative effects towards foraging behaviour were scored in the laboratory foraging bioassay. However, before making final conclusions about the compatibility of this compound with *B. terrestris* side-effects should be evaluated under more realistic field conditions with queen-right colonies.

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Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers

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Abstract

Aims: The aims of this study were to design universal markers for different protozoan parasites of *Bombus* spp. based on the phylogenetic position of two important bumblebee parasites *Crithidia bombi* and *Apicystis bombi*.

Methods and results: Standard PCR and extraction techniques were used to amplify and sequence 18S rDNA. Phylogenetic analysis of the rDNA was performed in order to predict the parasite-range of the primers.

Conclusion: *C. bombi* phylogenetically clusters with the trypanosomatids with slowly-evolving SSU-rRNA sequences (SE), while *A. bombi* is the closest sister group of *Mattesia*. A multiplex was designed containing an internal control and two broad-range primer pairs, detecting *C. bombi* and other SE trypanosomatids and also *A. bombi* and other neogregarines.

Significance and impact of study**:** Sequence data generated will further improve the current systematics of insect trypanosomatids and gregarines which remain troublesome. Broad-range markers for bumblebee parasites are necessary tools enabling the screening of commercially imported colonies and thus controlling their worldwide distribution and to discover related emerging parasites.

Detection of viral replication in bees

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Abstract

Recently foraging bees were discovered with pollen loads infected with honey bee viruses uncorrelated with the infection status of the bees itself. This observation has wide implications on the broadly used PCR viral detection techniques. False positives results could be obtained if viral remnants from infected pollen in the bee gut are detected. Integration of the real time PCR technology could help to eliminate these false positive results, however techniques detecting viral replication ultimately prove presence of active viruses. We demonstrated that current minus strand detection methodology often is not selective enough to differentiate between positive RNA strands (inactive virus) and minus RNA strands (replication virus) and provide possible solutions

VI. Session - Biocontrol methodology using bees

Pesticide sprays compromise pollination and biocontrol services on strawberry

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Abstract

During the past five years we have developed a successful and reliable method of protecting strawberry and raspberry ccultivations from grey mould (*Botrytis cinerea*) attacks, by using honey-bee disseminated biocontrol with the commercial antagonist *Gliocladium catenulatum* (Prestop Mix). This has allowed growers to fully abandon grey mould fungicide sprays and increase their marketable berry yields. Some growers still prefer to use, in addition to the bee-vectored biocontrol, chemical fungicides, and many more are spraying against insect pests at the time of flowering. The pesticide sprays may decrease the efficiency of the pollination and biocontrol services, provided by our 'entomovector technology'.

In our second study year (2007) we assessed in detail the honeybee foraging patterns on strawberry, primarily to determine whether adequate flower visitation was taking place in the target area (strawberry and raspberry cultivations) for effective biocontrol of the grey mould. Flower visits by pollinators - including honeybees from the hives for entomovectoring - were monitored on five farms throughout the flowering season and at different times of the day. Periods of observation lasted in one spot usually 20 or 30 minutes, and at each spot about 10-15 flowers were observed at one time. In total over 11 hours of observations were made, including 445 individual flowers.

At the beginning of flowering, an average of 1.5 honeybee visits per flower in one hour were counted, throughout the day (an average of about 10 honeybee visits/flower/day). This rose to about 3 or 4 visits/flower/hour at the end of the flowering season, despite abundant appearance of competing flowers. However, at the critical times in the middle of flowering honeybee visitation rates on strawberry flowers declined, being lowest at 0.5 visits/flower/hour about two weeks after onset of flowering. This coinceded with the peak spraying of fungicides and insecticides on those farms, which were using both the bee-dsseminated biocontrol and chemical pesticides. On several farms, 2-3 sprays were carried out within 5 days, and we observed clear decline in pollinator visitation after the sprays. After some days, the visitation rates started to increase again. Practically no honeybee visits occurred during the day following insecticide sprays, presumably because of a repellent effect of the insecticide.

We believe that pesticide use on strawberries severely compromises the efficacy of bee-disseminated biocontrol, and the associated pollination services. Although combining the biocontrol and pesticides produces the highest level of grey mould control, the marketable strawberry yields were highest in our study from treatments where only the bee-vectored biocontrol was used, indicating that pesticide sprays were not necessary, and likely were harmful to the efficacy of the bees in providing optimum pollination and biocontrol on the crop.

Laboratory miniature dispenser-bioassay to evaluate the compatibility of powder compounds in the entomovectoring with *Bombus terrestris*

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Abstract

The application of new plant protection strategies such as the entomovector technology (Mommaerts et al., 2011) demands the design of an appropriate bioassay in order to guarantee pollinator safety. Indeed in the context of the entomovector technology vectors such as bumblebees of *Bombus terrestris* are exposed to powder formulations containing the active ingredient which they need to disseminate into the target crop.

In this study we report on the development of a miniature dispenser (MD)-test allowing to evaluate lethal and sublethal side-effects of powder formulations of (bio)pesticides. Five model compounds were used: Prestop-Mix, Signum, kaolin, wheat flour and cellulose. Following a tier approach, the products were first placed in a one-way MD connecting a microcolony and an empty nest box containing the food. So workers walked through the powder product when leaving the nest in search for food and when returning back to their nest. Second, a two-way MD-test was conducted where the setup was similar to the one-way MD-test except that workers returned to their nest with food via another route free of exposure to the powder product. Third, the second setup was extended with a flight cage instead of an empty box. Finally, the flight cage was replaced by a greenhouse compartment to assess the side-effects under more field-related conditions with queen-right hives.

In general, the results demonstrated that the two-way MD-bioassay provides a reliable assessment of hazards of powder formulated products. For example severe toxicity was observed for the carrier kaolin as 89% of the workers were dead after 5 week, whereas for the other 4 products mortality was below the IOBC threshold of mortality (<25%). This toxicity profile was in agreement with the results obtained from the higher tier experiments. Furthermore, data confirmed that cellulose can be used as a negative control, while kaolin as a positive, in future risk assessment experiments.

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VII. Plenary discussion on regulatory issues and ICPBR working groups

Discussing protection goals for bees – an EFSA approach

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Protection goals defined in EU regulation

In Regulation (EC) No. 1107/2009, a high level of protection is required, which is expressed as 'no unacceptable effects on the environment' where it concerns plant protection products and of 'no serious risk to the environment' where it concerns treated seeds. However, often a 'translation' into precise goals to guide the development and application of risk assessment methodology is difficult. In particular, clarifications are needed to define specific protection goals with respect to ecological, temporal and spatial scales: in-crop versus off-crop; multiple stress and uncertainties.

Ecosystem Services

Overview of Ecosystem Services in agricultural landscapes for both in-crop and off-crop situations which are potentially affected by pesticides (EFSA Panel on Plant Protection Products and their Residues (PPR), 2010).

Ecosystem service category	In crop area	Off crop area
Provisioning	Food	Food
	Fibre and fuel	Genetic resources
		Fresh water
Regulating	Pollination	Pollination
	Pest and disease regulation	Pest and disease regulation
		Water regulation
		Erosion regulation
		Water purification
Cultural	Education and inspiration	Education and inspiration
	Recreation and ecotourism	Recreation and ecotourism
	Cultural heritage	Cultural heritage
		Aesthetic value
Supporting	Primary production	Primary production
	Photosynthesis	Photosynthesis
		Habitat provision
		Soil formation and retention
		Nutrient cycling
		Water cycling

Example: Protection goals for birds and mammals

In-field, ecosystem service: education, inspiration, recreation, ecotourism and (food). When we asked the risk managers in Brussels for protection goals, we got the following answer:

- The population should be protected, no visible dead birds and mammals

The birds and mammals working group translated this question in:

- The population should be protected, no dead birds and mammals

What could we do at this meeting?

Define specific protection goals for bees, other non-solitary bees and solitary bees for:

- In-field and off-field situations,
- Different ecosystem services: pollination, food production (honey, wax & propolis) and, biodiversity (genetic resources, education, aesthetic values), education and inspiration.

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ICPBR-Working Group Risks posed by dusts: overview of the area and recommendations

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Abstract

Background: In 2008 the poisoning of about 12000 bee colonies was reported from Germany. These poisonings were caused by the drift of dust particles containing the insecticidal substance clothianidin following the seeding of maize seeds, inadequately treated with the insecticide Poncho Pro.

Results: Investigations were done on the dust load contained in seed packages of different crops, on the experimental abrasion of dust from treated seeds using the Heubach-Dustmeter as well as on the actual dust drift during the sowing operation of treated seeds with different machinery under field conditions. Resistance to abrasion of treated seeds and subsequent dust drift during sowing operations differ significantly between crops, coating recipes and facilities. Furthermore dust drift depends on particle size, sowing technology as well as on environmental conditions (e.g. wind speed, soil humidity).

Conclusions: The drift of dust from treated seeds may pose a risk to honeybees, which needs to be appropriately considered within the authorization process of pesticides. The total quantity of abraded dust as well as the actual emission of dust during the sowing operation can be significantly reduced by technical means (e.g. coating recipe and facility equipment, deflector technology) and by additional mitigation measures (e.g. maximum wind speed).

Keywords; honeybee, poisoning, risk, seed treatment, dust, drift

1. Introduction

In 2008 the poisoning of about 12000 colonies of honeybees was reported by German beekeepers in parts of South-West Germany. According to the findings of investigations immediately initiated, these poisonings were caused by the insecticidal substance clothianidin following the seeding of maize treated with the insecticide Poncho Pro[1,2,3]. Extreme exposure of honeybees to clothianidin was caused by

- bad seed dressing quality (high quantities of dust within seed bags, low resistance of the treated seeds to abrasion, high concentration of active substance within the dust),
- massive emission of dust by seeders (especially vacuum-pneumatic seeders with air outlet at the top/to the side),
- sowing at a time of full flowering of adjacent areas (e.g. oil seed rape, fruit orchards, dandelion; high numbers of contaminated borders on landscape level in the South of Germany),
- strong wind during time of drilling.

The risk assessments and management actions taken subsequently by the German authorities were illustrated by the Federal Office of Consumer Protection and Food Safety (BVL) on the 10th

International Symposium of the ICPBR-Bee Protection Group in Bucharest in 2008. All uses of neonicotinoid pesticides for the treatment of seeds were re-assessed and authorisations of pesticides for maize seeds were immediately suspended in Germany.[4] In Italy and France, scientists deduced from a spatial and temporal correlation between spring mortality of bees and the sowing of maize seed dressed with imidacloprid, thiamethoxam or clothianidin a causal connection between both of these factors.[5,6] Due to the numerous reports of bee poisonings in a number of Member States of the European Union over nearly one decade, the Commission Directive 2010/21/EU was adopted in the year 2010.[7] In this Directive the basic conditions for seed treatments with some neonicotinoids and fipronil concerning seed treatment, seeding technique and risk labeling are stipulated. Based on the analysis of available data (Fent G, 2011, unpublished) it was concluded that during the sowing of pesticide dressed seeds, abraded dust particles are emitted into the environment, including adjacent off-crop areas and effects on non target species, especially honeybees, cannot generally be excluded. In the current paper the data available so far are discussed and recommendations for the authorization of seed dressings are suggested.

2. Results

2.1 Sources of dust

Data on the dust load contained in seed packages of different crops, on the experimental abrasion of dust using the Heubach-Dustmeter [8,9] as well as on the actual dust drift under field conditions have been presented by the Julius Kühn-Institut (JKI) as well as by the plant protection industry, e.g. at the 'European Workshop on Seed Protection', on the 10th and 11th May 2011, in Paris (Heimbach U and Stähler M, 2011, unpublished; Heimbach U et al., 2011a, unpublished, Kubiak R et al., 2011, unpublished) and in the course of a *webinar* on 'Risks for honeybees by using insecticide treated seeds' organized by the US EPA, on 27th July 2011 (Heimbach U et al., 2011b, unpublished Pistorius J et al., 2011b, unpublished).

2.1.1 Free dust from seed bags

The JKI analyzed the amount of free dust from seed bags of several crops (Table 1).

Tab. 1 Amount of free dust from seed bags of several crops (Heimbach U and Stähler M, 2011, unpublished)

CROP/Year of treatment	Target drilling rate of seeds[a] (kg or No. ha^{-1})	Fine-grained dust[b] < 0.5 mm (g ha^{-1})	Coarse-grained dust[b] > 0.5 mm (g ha^{-1})	N
Cereals 2009				
Barley	180	11.3 (31)[a]	46.0 (116)	30
Wheat	250	9.5 (28)	6.7 (19.2)	31
Rye	150	5.1 (24)	6.6 (32.9)	23
Maize	100000			
2008		4.5 (25.6)	6.1 (47.3)	82
2009		1.99 (5.8)	3.5 (12.1)	45
OSR	700000			
2007		0.81 (4.72)	-	22
2008		0.27 (0.88)	-	24
Sugar-beet 2008	100000	0.035 (0.125)	-	22

[a] Cereals given in kg ha^{-1}; [b] Amounts given in mean (max) g ha^{-1} normalized for target drilling rates of 1 ha

The findings, normalized for 1 hectare, indicated that seed bags of different crops contained very different total amounts of dust. Seed bags of cereals contained more dust than maize, oil seed rape

(OSR) or sugar-beet. The maximum mean amount of fine-grained dust of barley seed bags was more than 300 times higher than the amount of fine-grained dust of sugar-beet bags. Additionally the results for maize and OSR show lower amounts of dust in 2009 compared to 2008, indicating first improvements of the seed dressing quality.

Sieving of dust from maize seed bags showed a great variation of particle sizes. These varied over a broad scale, the smallest smaller than 80 microns, the biggest ones over 500 microns.

2.1.2 Resistance of treated seeds to abrasion

Further investigations of the JKI using the Heubach-Dustmeter revealed that the resistance of treated seeds to abrasion can be regarded as a key factor for the amount of dust potentially contained in the seed packages (Table 2). Sugar-beet turned out to show the best resistance to abrasion, followed by OSR, maize and cereals.

Tab. 2 Resistance of treated seeds to abrasion using the Heubach-Dustmeter for several crops (Heimbach U et al., 2011a, unpublished)

CROP/Year of treatment	Target drilling rate of seeds[a] (kg or No. ha^{-1})	Heubach-value[b] (g ha^{-1})	N
Barley 2009-2010	180	2.25	51
Wheat 2009-2010	250	2.84	131
Rye 2009-2010	150	0.86	37
Maize		1.11 (4.15)	53
2008		0.42 (0.91)	81
2009	100000	0.33 (0.66)	43
2010		0.18 (0.4)	34
2011			
OSR 2009-2010	700000	0.08	212
Sugar-beet 2009	100000	0.03	22

[a] Cereals given in kg ha^{-1}; [b] Amounts given in mean (max) g ha^{-1} normalized for target drilling rates of 1 ha

Further to these findings the JKI showed that concentrations of the active substances may vary between treatment facilities, supposedly depending of the individual treatment procedures, recipes (especially additives, stickers) and the implementation of effective dedusting equipment. As part of a quality improvement initiative of the German professional treatment facilities for maize, the resistance of the treated seeds to abrasion was significantly improved, showing mean normalized Heubach-values of 1.11 g ha^{-1} in 2008 compared to 0.18 g ha^{-1} in 2011. This optimization is also reflected in the maximum normalized Heubach-values for maize seeds that were reduced by about 90 % from 4.15 g ha^{-1} in 2008 to 0.4 g ha^{-1} in 2011.

2.2 Drift of dust - Exposure assessment

According to a literature study prepared at the University of Essen (Höke S and Burghardt W, 1997, unpublished) drift of soilborne particles of different nature into adjacent areas increased, if wind speed exceeded 5 m s^{-1}. Furthermore the size and shape of particles affect the potential of drift with respect to distance and duration. While particles of 1000 down to 70 microns creep, jump and roll over short distances of 1 to 1000 meters, particles of less than 70 microns may be subject to suspension and are spread over longer distances. The knowledge about the size and transport dynamics of dust particles from treated seeds is currently insufficient and therefore needs further consideration.

2.2.1 Studies on dust drift from treated seeds and subsequent ground deposition

Experimental data on dust drift have been presented by the Julius Kühn-Institut (JKI) as well as by the plant protection industry e.g. on the European Workshop on Seed Protection, 10th and 11th May 2011, in Paris (Heimbach U and Stähler M, 2011, unpublished; Heimbach U et al., 2011a, unpublished, Kubiak et al., 2011, unpublished).

In the latest comprehensive compilation of dust drift data, the available data for ground deposition following seeding of maize, cereals, OSR and sugar-beet were analysed (Fent G, 2011, unpublished). The analysed data base comprised experimental data from field studies carried out in Germany, Italy and France until 2009. Studies were carried out mainly on behalf of companies of the plant protection industry (i.e. BASF, Bayer CropScience, Syngenta Agro) and the JKI. The data quality requirements applied to the studies available ensured sowing was carried out according to agricultural practice and wind speed was below 5 m sec^{-1}, LOQ and LOD were reported and analytical performance of deposits was state of the art. Applying these criteria, the results of in total 115 field experiments were selected for further scrutiny. Further it was assumed that both dust transport and deposition (dispersal and quantity of active substance retrieved in the collectors) is not product specific and therefore the active substance can be used as a dust drift tracer. However, no analytical data were reported on the concentration of active substances within the dust prior to the drift experiments, e.g. from abrasion tests. A typical study design to investigate the drift of dust particles is given in Figure 1.

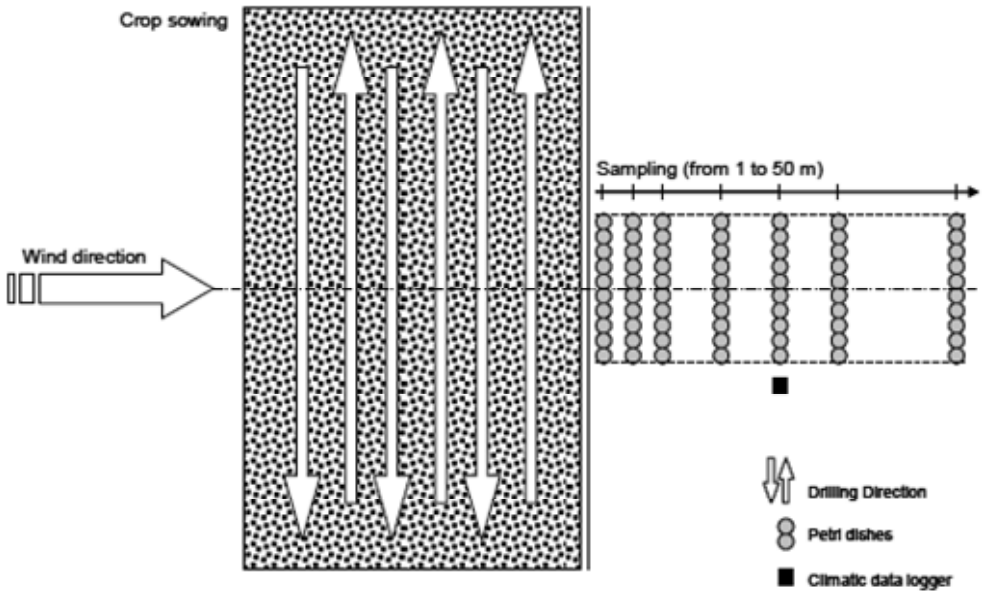

Fig. 1 Typical study design to investigate the drift of dust particles (Fent G, 2011, unpublished)

According to the findings of the JKI (Heimbach U and Stähler M, 2011, unpublished), sugar-beet pills were obviously very resistant to abrasion (Heubach-value of 0.03 g per 100000 seeds). This fact was approved by the analysis of dust drift for sugar-beet seeding, which showed dust drift values below the LOQ, with a few exceptions of single sampler-values only (Fent G, 2011, unpublished). It was concluded that ground dust deposition caused by mechanical seeding of pesticide coated sugar-beet pills is negligible.

The JKI also investigated ground deposition rates of dusts collected in Petri dishes adjacent to a drilled area with seed treated barley (Heubach-value 2.1 g per 180 kg seeds) in 2008, using a

mechanical and a pneumatic driller.[9] The mean deposits were about 0.14 % and 0.16 % of the field rate at 1 m to the edge of the field. For dust drift from several cereal seed drilling experiments the 90th percentile of the off-crop ground deposition data for pneumatic sowing technique was about 0.1 % of the field rate applied at the edge of the field (Fent G, 2011, unpublished). Deposition of dust resulting from mechanical seeding was lower compared to pneumatic seeding.

For OSR, the mean ground deposition measured with Petri dishes, which were placed on the open ground at 1 m distance to the edge of the drilled field was found to be about 0.2 % of the field rate applied (Heimbach U et al., 2011a, unpublished). However, no information was given concerning the Heubach-value. The 90th percentile for the off-crop ground deposition following pneumatic drilling of OSR was about 0.1 % (of the field rate applied) at the edge of the field (Fent G, 2011, unpublished). The dust deposition from mechanical seeding technique was significantly lower compared to pneumatic seeding technique.

The ground deposition for maize sown in 2009, using deflector technique in combination with batches of low quality seeds of 2008 (Heubach-value of 2.12 g per 100000 seeds) was up to about 0.5 % of the field rate applied at 1 m to the edge of the field (Heimbach U et al., 2011a, unpublished). When seeds of a higher quality were used (i.e. Heubach-value of 0.86 g per 100000 seeds) and the concentration of the active substance in the dust was reduced at the same time, ground deposition was reduced to about < 0.1 % of the field rate at 1 m to the edge of the field. The JKI also found different concentrations of the active substance in fractions of different particle size. These findings demonstrated a trend towards higher concentrations the smaller the fractions were (Heimbach U and Stähler M, 2011, unpublished). Further it was demonstrated that seeds treated with different treatment rates (in terms of g active substance per 100000 seeds) may generate dust with similar concentrations of active substance and lower treatment rates may even produce higher ground deposits and hence higher exposure than higher treatment rates (Heimbach U et al., 2011b, unpublished). It must therefore be concluded, that the treatment rate alone might have an uncertain impact on the deposition of actives in downwind drift samples, because the concentration between different samples of dust can vary. However, data also showed that within the same treatment facility using the same treatment procedure, concentrations of actives within the dust may correlate with the field application rate. The 90th percentile following the drilling of maize seeds (Heubach-values of < 0.75 g per 100000 seeds) by using deflected, vacuum-pneumatic drilling technique, was extrapolated to be about 0.13 % (of the field rate applied) at the edge of the field (Fent G, 2011, unpublished).

2.2.2 Studies on dust drift from treated seeds and subsequent deposition on vegetation

According to the findings of the JKI, deposits on adjacent flowering crops were higher than on ground level (Heimbach U et al., 2011a, unpublished). Accordingly it was questioned whether ground deposition data could reliably predict exposure of non-target arthropods (e.g. honeybees) to dust on vertical structures adjacent to the field (e.g. hedges or neighbouring flowering crops). Based on the studies available the ground deposition of dust was compared to the amount of dust that was collected by a vertical sampler (i.e. wetted gauze netting) (Neumann P et al., 2011, unpublished). The ratio of vertical deposition divided by horizontal deposition was up to 12.4 (90th percentile: 9.5) with no crop-specific pattern. A field study on maize conducted in 2010 by the JKI showed that deposition data at 0.15 m distance downwind in neighbouring OSR exceeded ground deposition in Petri dishes at 1 m distance by a factor 8.9 (Heimbach U et al., 2011a, unpublished). These data suggest the need to implement an extrapolation factor in the risk assessment in order to cover exposure of bees to dusts on vegetation adjacent to a particular field, if ground deposition data are used for the exposure assessment.

2.2.3 Effects of dust drift on honeybees

The JKI complemented its studies on the quality of treated seeds from different origins with additional investigations on dust drift during sowing, with special emphasis on the exposure of honeybees in adjacent vegetation and at very short distances (Heimbach U et al., 2011a, 2011b, unpublished). The seeds employed for this investigation had a Heubach-value of 0.86 g per 100000

seeds and a concentration of 11 % of clothianidin was determined. Seeding was conducted at an average wind speed of 2.3 m s^{-1} using deflection technique. At the same time the JKI investigated the effects of dust drift from the seeding operation of the treated maize seeds on honeybees in a field test (Pistorius J et al., 2011a, unpublished). The honeybee colonies of the treatment group were located directly adjacent to the maize sowing area, at the edge of a flowering OSR field, which ensured that the honeybees were foraging on the flowering OSR during and after the sowing operation. Honeybee mortality in the treatment group increased to about 200 dead bees per colony at the day of seeding and to about 250 dead bees at day 1 and 4 after seeding. The control colonies, placed at flowering OSR at the upwind border of the sowing area of the maize field, still showed a slight increase of honey bee mortalities (about 50 to 100 per colony day^{-1}), the remote controls at about 800 m distance to the sowing area of the treatment maize showed normal mortalities (about less than 50 per colony day^{-1}). Even though the deposits found in Petri dishes at 1 m distance were quantified below the supposed $NOEC_{field}$ for honeybees for the active substance used, honeybee mortality indicated a higher exposure starting at the day of sowing. The findings reported underline the need to implement an extrapolation factor in the risk assessment in order to cover exposure of honeybees to dusts on vegetation directly adjacent to fields, if ground deposition data are used for the exposure assessment. Furthermore the findings demonstrated that both the quality of seeds and the seeding technique needs to be improved.

2.2.4 Studies on the technical means for a reduction of drift

The JKI documented for pneumatic (vacuum) maize seeding machines a reduction of the emission of contaminated dusts on ground level by about 90 % by reconstructing the vents in the way that the waste air is discharged onto or into the soil (i.e. deflector technique) (Rautmann D et al., 2011, unpublished). The JKI holds a list of suitable drift reducing equipment.

3. Discussion and conclusions

3.1 Discussion

Studies of the JKI clearly demonstrated that the quantity of dust in seed bags and concentration of active substance within the dust may differ depending on seed quality, crop type, treatment rate, treatment recipe, particle size and even the facility where the treatment had been performed (Heimbach U et al., 2011a, unpublished). For the interpretation and standardization of the available data on drift this means that the ground deposition given as relative proportion of the field application rate (e.g. g active substance per 100000 seeds) is only acceptable if the quality of seeds (e.g. classified by the Heubach-value) and the concentration of the active substance within the dust are known and considered sufficiently representative. Furthermore, knowledge on the particle sizes is still lacking. It is therefore considered necessary to appropriately account for the remaining uncertainties, if the currently available drift data (e.g. Fent G, 2011, unpublished) are used for authorization purposes, e.g. by applying additional extrapolation factors. For mechanical seeding of sugar-beet, OSR and cereals the companies of the plant protection industry (Neumann P et al., 2011, unpublished) concluded that dust ground deposition seems to be negligible. However, while this assumption may be supported based on the data reported by JKI for sugar-beet, it is not supported for OSR and cereals (Heimbach U and Stähler M, 2011, unpublished, Heimbach U et al., 2011a, unpublished).

3.2 General conclusions

In general from the data available it can be concluded that dust drift from sowing treated seeds is a common phenomenon along with the deposition of dust particles on soil and on plant surfaces. Resistance to abrasion of treated seeds and the subsequent dust drift during the sowing operation differs significantly between crops, coating recipes and facilities and so do concentrations of active substances. Potentially depending on their size dust particles are filtered out by and may cumulate on neighbouring vegetation or deposit on bare ground (Heimbach U et al., 2011a, unpublished) but may also fly long distances (Höke S and Burghardt W, 1997, unpublished). The drift of dust depends on the

type of seeder and environmental conditions (e.g. particle size, wind speed, adjacent vegetation, soil humidity). Maximum ground deposition of dust drift is usually found at the edge of the field. Measurements on plants or on vertically mounted sampling devices show up to about 12 times higher deposits than measurements in Petri dishes on ground level (Neumann P et al., 2011, unpublished). In fact the findings indicate that for seeding operations of some crops, e.g. cereals, maize and OSR, treated with compounds highly toxic for honeybees, e.g. some neonicotinoids and fipronil, best seed treatment techniques with respect to effective dedusting measures (i.e. reducing free dust within the seed bags) and increased resistance of treated seeds to abrasion (i.e. reducing potential of dust generation during packaging to seeding operations) together with the best seeding techniques (i.e. reducing potential of dust emission e.g. by effective deflectors for maize seeds) need to be established (Pistorius J et al., 2011a, unpublished). For sugar-beet the present coating quality already seem to allow a safe seeding operation.

3.3 Recommendations for risk assessment and risk mitigation

In order to facilitate a scientifically sound risk assessment, the appropriate methods need to be elaborated. Because the commonly used HQ-approach has not been validated for the exposure of honeybees to contaminated dust, the TER approach, commonly used in ecological risk assessments, might be a better alternative. This in turn would create the need for establishing and validating higher tier toxicity studies, e.g. in order to assess the $NOEC_{field}$ in terms of g active substance ha^{-1}, as well as the need to establish a risk assessment paradigm. In principle dust drift should be considered in risk assessment for all actives substances respectively pesticides which are toxic to honeybees and which are applied on seeds or as granules and where the mode of application is suspected to generate the emission of contaminated dust into the environment. The risk assessment should be performed especially for critical GAPs (i.e. intended uses) taking into consideration the type of crop, the field rate, the season of application as well as the mode of application (e.g. type of seeder). However, before a risk assessment can be made, also methods for an appropriate exposure assessment need to be elaborated and agreed (i.e. field testing method for drift), e.g. including 3D extrapolation factors for the exposure of honeybees on plants. For all types of seeds and granules potentially toxic to honeybees, the abrasion resistance should be investigated (e.g. by the Heubach-method) and further investigations regarding the concentration of active substance in the abraded dust should be conducted. Finally, appropriate risk mitigation measures as well as appropriate label phrases need to be worked out. In case risk mitigation measures are mandatory, these should be covered by the exposure assessment. All these aspects should be addressed by the relevant Guidance Document which is currently being prepared, lead-managed by the Netherlands, the potential impact on other non-target organisms included.

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Assessment of risks to honey bees posed by guttation

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Abstract

Background: Besides their nectar and pollen collecting activities, honey bees also forage water. Guttation droplets may be used as a water source. Measurements of high residue levels of some intrinsically highly toxic, systemic insecticides in guttation droplets triggered research activities on the potential risk for honey bees. Since 2009, a large number of studies have been conducted on the environmental conditions and factors favoring guttation, foraging of guttation, the occurrence of guttation in different crops, the frequency of guttation events and residue measurements in guttation droplets in different crops, at different growth stages and with different active ingredients. Different approaches of laboratory, semi-field and field studies were set up to address the potential risk of guttation to bees and to gain clarification whether and how this concern would need to be specifically addressed in the risk assessment for bees.

Results: Occasionally increased mortalities of worker bees were reported from single events in some trials, when colonies were placed directly next to the sown maize crop treated with a systemic insecticide. However, there were no long-term colony effects (e.g. on colony strength and brood development) reported from any of the realistic worst case exposure trials conducted by either public research institutes or industry. Conclusion: The potential risk for bees is in the first instance dependent on the distance of the colonies to treated crops. Maize is considered as the worst case crop in terms of frequency, duration and intensity of guttation and of residue level of compounds found in guttation liquid. Though increased worker bee mortality on individual days was seen in some of the field studies where hives were placed directly at guttating maize fields, adverse effects to colony vitality, colony and brood development were never observed.

Keywords: Guttation, risk assessment, pesticides, honey bees.

1. Introduction

Guttation is a physiological process by which many vascular plants can secrete water by an active process under certain environmental conditions, in contrast to transpiration which is a passive process. The secreted water forms droplets which usually occur on tips or edges of leaves. The content of dissolved substances like salts, sugars in guttation liquid is very low, usually below 1%. In recent years, attention has been focused on guttation of systemic pesticides as a possible exposure pathway for water-collecting bees to systemic pesticides, in particular soil-systemic applications (e.g. seed treatment, granular or drench applications). Measurements of high residue levels of some intrinsically highly toxic, systemic insecticides in guttation droplets from different crops were

reported by different researchers[4,13,14] and triggered significant interest on the possible risks posed by the presence of residues of systemic pesticides in guttation fluid to water-collecting honey bees[20].

Studies have since been conducted on the environmental conditions and factors favoring guttation, collection of guttation liquid, the occurrence of guttation in different crops, the frequency of guttation events and residue measurements in guttation droplets in different crops with different active ingredients in different growth stages. Different approaches of studies with bees in lower and higher tier tests were set up to gain clarification about collection of guttation liquids by bees and possible effects on bees and whether and how this concern would need to be specifically addressed in the risk assessment for honey bees. So far, consideration of guttation has not been specifically required in the risk assessment by SANCO/10329/2002, but it has nevertheless been addressed in the risk assessment of a few active substances. However, future European legislation could include the risk assessment for pesticides residues in water, including guttation for systemic products**.** Meanwhile, there is more information available from laboratory studies, semi-field and field studies as well as post-registration monitoring from both industry and public research institutes.

2. Results

2.1 Guttation- different factors influence the potential risk

2.1.1 Water need of bee colonies

Honey bees need water for different tasks in the hive, such as the regulation of air humidity and temperature (cooling) in the hive[8], and the production of larval food which has high water content. Water foraging activity is regulated by demand as it is not stored in the hive[9,17]. As water collecting bees will most likely choose water sources in the proximity of the hive[19] and long distance flights are avoided due to energetic reasons, the position of the bee hive in relation to the treated crop and the availability of alternative water sources, e.g. rivers, ponds, dew, condensed water in the hive, nectar flow with high water content, determine the potential risk of uptake of guttation droplets from treated crops to satisfy water requirements. Guttation may also occur in untreated plants like grasses and weeds. The possible risk from guttation water may be highly variable and is determined by, e.g. climate conditions, meteorological conditions, soil nature, time of overlapping of bee activity and guttation, the distance to treated and untreated crops and other plants, seasonal activity and seasonal water needs of colonies and the occurrence of guttation droplets with high residue levels. In general, the water need of a colony is highest during spring and summer. Plants offering nectar and pollen will attract bees from larger distances, whereas water is usually collected closer to the hive[19]. Therefore, collection of guttation liquid does not appear to be a regular exposure scenario like nectar and pollen. Usually, guttation droplets are one out of several possible water sources in the surroundings of a colony and mostly only available at a limited time period in the morning and evening and not every day.

2.1.2 Occurrence of guttation

Several crop species such as sugar beet, winter oilseed rape, maize, barley, potatoes, oat, sunflower, onions, carrots, peas and cucumber and also weeds were investigated and an assessment of the occurrence, frequency and intensity of guttation (size/number of guttation drops, number of guttating plants per culture) in the tested crop species was conducted (Fig. 1). Different crops varied in the intensity and frequency of guttation events. Some crops showed guttation more frequently than others, also the intensity of guttation varied. Some major crops like winter oilseed rape, cereals and maize showed guttation frequently. Some crops showed very low guttation probability and very small droplets, e.g. sugar beet (Fig. 1, top). Whereas some crops produced guttation throughout a large part of their growing season, others showed guttation only for a short period (Fig. 1, left). Finally, while some crops showed guttation only in younger growth stages, some may show guttation up to inflorescence (Fig. 1, right) (Joachimsmeier et al. 2011).

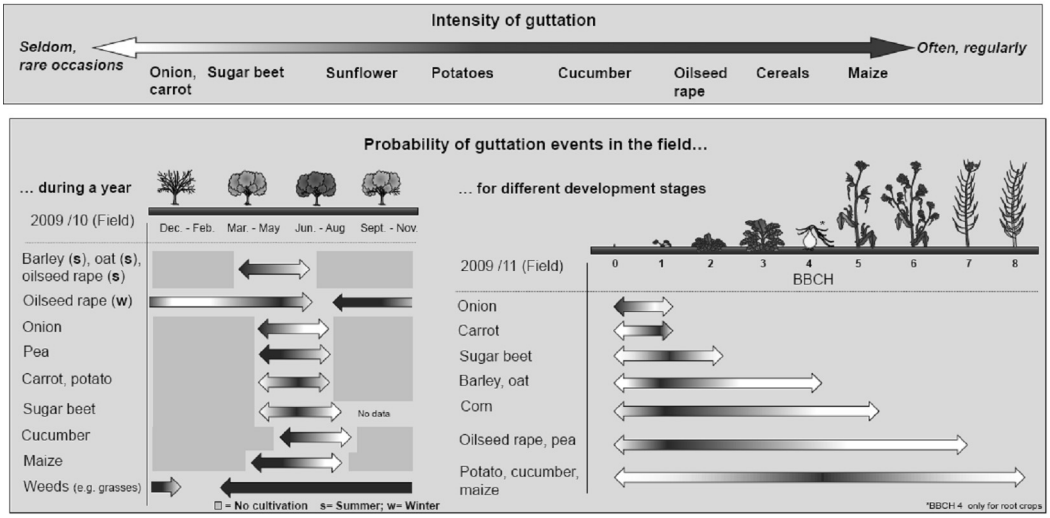

Fig. 1 Intensity and probability of guttation in differenct crops in field conditions (Joachimsmeier et al., 2011)

2.1.3 Residues in guttation droplets and potential risk

Residues of systemic fungicides, herbicides and insecticides may be found in guttation droplets. For all tested crops, peak residue levels occurred at the onset of guttation activity after emergence and declined with time (Fig. 2). Depending on the residue levels, the period of concern may vary from crop to crop. Depending on the toxicity of the active substance, concern for honey bees may be triggered, e.g. in maize high residue levels of some intrinsically highly toxic, systemic insecticides in guttation droplets were found.

The concentrations in guttation droplets tend to be slightly lower for granules than for seed treatments in young growth stages, nevertheless the potential risk is likely to be comparable. (Fig. 2, left).

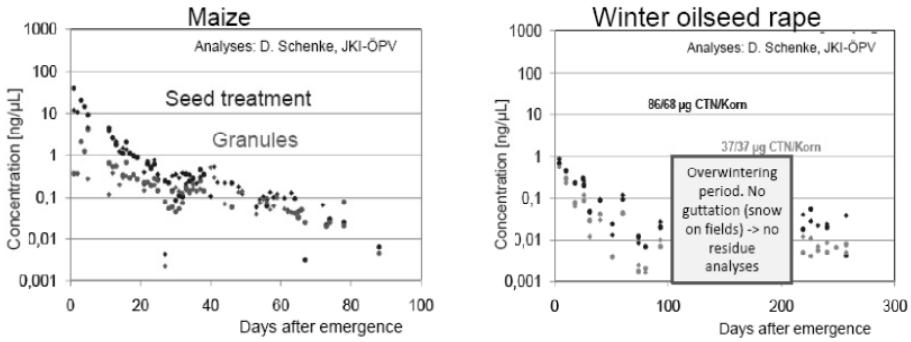

Fig. 2 Residues of a systemic pesticide (clothianidin as an example) in guttation droplets after seed treatment and granular treatment for maize (left) and seed treatment for winter oilseed rape (right)[14]

Highest residues were found in all crops at younger growth stages, showing decline with increasing plant age and growth stage[13,14]. The amount of residues in guttation droplets depends on the crop and its growth stage, the properties of the active substance, the amount of active substance per seed and other factors[15]. (In some trials during sampling at two different times (morning and midday), increased residue concentrations were measured in the midday samples due to the evaporation of the water content with increasing solar radiation[1].

In comparison to other crops studied here, potential risk via guttation is in general higher for maize, which can be assumed to be the 'worst-case' crop, as residues of soil-systemic treatments at emergence and young growth stages are much higher compared to other crops and guttation occurs frequently at time of high water needs of colonies.

To assess the potential risk, in a first step oral toxicity data e.g. LD_{50} values can be used for a calculation of the amount of liquid that would lead to an uptake of a lethal dose e.g. the acute LD_{50}. Other values e.g. NOEC or LC50 values could also be used for a refined calculation both for acute or chronic toxicity. In this case, the LD_{50} is only used to demonstrate a potential risk. In Table 1 such an example of a calculation is given. For a substance with a LD_{50} of 100 ng/bee 100 µl water would need to be consumed at a concentration of 1 ng a.s./µl in guttation droplets. The data e.g. for clothianidin show that at a residue in guttation droplets of 1 ng/µl, a value found in seed treated maize or granular applications for approximately 4 weeks after emergence, only 3.7 µl of water would need to be consumed to achieve the LD_{50} of 3.7 ng/bee. Thus, concern was particularly raised for systemic insecticides with high toxicity for adult bees and/or bee larvae, especially for highly toxic systemic neonicotinoids, e.g. imidacloprid, thiamethoxam and clothianidin.

Tab. 1 Calculation of the amount of guttation water that, if consumed would lead to an uptake of a lethal dose for different active substances

Thiamet-hoxam		Clothia-nidin		Substance A		Substance B	
LD_{50} in ng/bee	**5**		**3,7**		**50**		**100**
Guttation droplets residues ng/µl	**Consump-tion µl/bee**	**Guttation droplets ng/µl**	**Consump-tion µl/bee**	**Guttation droplets ng/µl**	**Consump-tion µl/bee**	**Guttation droplets ng/µl**	**Consump-tion µl/bee**
0,01	500	0,01	370	0,01	5000	0,01	10000
0,05	100	0,05	74	0,05	1000	0,05	2000
0,1	50	0,1	37	0,1	500	0,1	1000
0,5	10	0,5	7,4	0,5	100	0,5	200
1	5	1	3,7	1	50	1	100
1,5	3,33	1,5	2,47	1,5	33,33	1,5	66,67
2	2,5	2	1,85	2	25	2	50
3	1,67	3	1,23	3	16,67	3	33,33

2.2 Risk evaluation

2.2.1 Methodology for risk evaluation studies

In laboratory studies it is not possible to stimulate the uptake of guttation liquid or pure water by honeybees without adding sugar. Such guttation liquid artificially spiked with sucrose is then used by bees as a carbohydrate source. Thus such laboratory feeding studies constitute a very unrealistic exposure scenario and provide only limited information for risk assessment to assess the risk for honeybees. Such laboratory studies have been used as a fast screening of guttation with feeding tests in cages. The outcome of such tests have shown to be of comparable outcome with OECD 213/214

laboratory toxicity data, resulting in high mortality after feeding sugar-enriched guttation droplets of maize treated with a systemic insecticide[4].

In semi-field studies, controlled conditions in tents or tunnels offer the possibility to simulate water collection from guttation droplets and other water sources, and to study honeybees' reaction to known residue levels in water. Alternative water sources can be excluded to ensure a maximum exposure. Effects on foragers, hive bees, and different brood stages can be measured in worst-case exposure scenarios. Nevertheless, semi-field studies have a limited potential for extrapolation of the findings to field conditions.

In field and monitoring studies honey bees can freely choose water sources. Field and monitoring studies can be designed to cover different scenarios from realistic field conditions to artificially aggravated exposure. In both, it is difficult to conclude on the activity of water foraging bees in the surroundings and to estimate the portion of water foragers using guttation droplets or other sources, and there is no control about the intensity of use of focused water sources. Likewise, the assessments are very labor intensive. Behaviour of foragers, effects on foragers, hive bees and different brood stages, brood development and colony development under realistic worst-case exposure conditions can be investigated. Residue analysis of dead honeybees and guttation fluid can be done for verification of a cause-and-effect chain.

Monitoring studies offer a wide range of possible designs under which presence or absence of the effects on honeybee colonies are determined in different environmental conditions. The significance of the results depends on the design of the study and environmental conditions. As the colonies show individual water foraging behavior and the environmental conditions of the study sites may be variable, the intrinsic variability of the systems can be compensated by appropriate replicate (e.g. colony and field) numbers.

2.2.2 Findings from semi-field, field trials and monitoring

Not surprisingly, when bees were fed with sugar-enriched guttation droplets of maize high mortality or total mortality was observed. In semi-field trials it was clearly demonstrated that increased mortality of worker bees may occur when bees are thirsty and no other water source is available (Fig. 3, left). On the other hand, when an alternative water source was available, no clear increase of mortality was observed (Fig. 3, right). In field conditions at the same site, no increase of mortality was observed for free flying colonies set up at the field border [5].

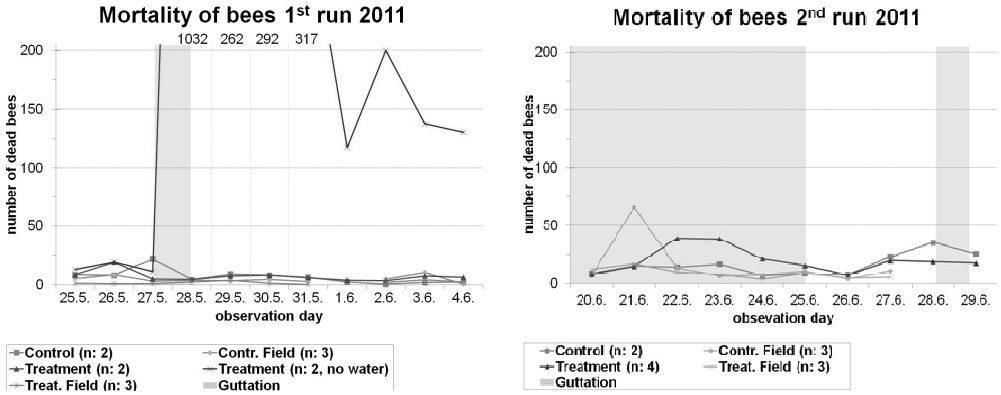

Fig. 3 Mortality of bees following exposure to maize guttation droplets in semi-field and field conditions, semi-field: 2 colonies with, and 2 without alternative water (left) or 4 with additional water (right) [5]

A number of studies with realistic worst case exposure were done by public research and industry. For granular application in maize with the active substance clothianidin, honey bee monitorings were

conducted in 2010 and 2011 in different regions of Germany by public research institutes, the Apicultural State Institute LWG Veitshöchheim (Bavaria), the Bee Institute LAVES, Celle (Lower Saxony) and the DLR (Rhineland Palatinate). Colonies were set up at the field border before emergence of the maize crops. At the location in Veitshöchsheim, in both years 2010 and 2011[6] and also in Rhineland Palatinate[16] no noticeable mortality peaks were seen, and it was concluded that mortality, brood and colony development were on a normal level during the whole study and no treatment related effects were seen. However, in dead bee samples from days with no increased mortality, residues of clothianidin were found, indicating that single bees came in contact with the active substance which, however, was not leading to an overall increase of mortality[6].

In the trials conducted in 2010 by LAVES events of clearly increased worker bee mortality were observed, and residues of clothianidin were found in the dead bees (Fig. 4). It was concluded that the mortality was caused by uptake of guttation fluid. Although guttation occurred frequently during this trial, use of guttation fluids leading to increased mortality did not occur regularly but only on single events. Adverse effects on brood and colony development were not observed[21]. In the monitoring done by LAVES in 2011 no noticeable mortality peaks were seen and it was concluded that mortality, brood and colony development were on a normal level and no treatment related effects were observed during the whole study in 2011 (Von der Ohe, pers.com.). As no mortality peaks were seen in the other maize monitoring trials although guttation frequently occurred, it can be concluded that a use of larger amounts of guttation fluids by a larger number of bees only occurs in very specific circumstances. The high variability of effects observed under practical conditions is due to the individual location, climate conditions, water availability and water need. Also in a monitoring trial with seed treated maize in 2011 and also in winter oilseed rape 2010 and 2011 by the JKI (Pistorius, unpublished) no treatment related mortality peaks were observed.

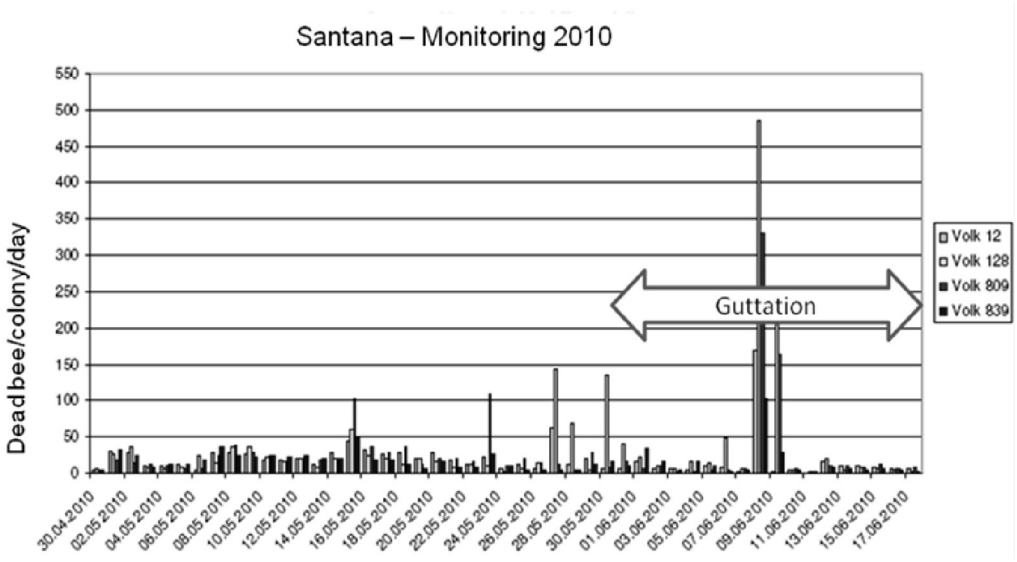

Fig. 4 Daily bee mortality, monitoring trial with clothianidin soil granular application in 2010 [21]

During guttation period in maize and wheat fields, no honeybees were observed collecting guttation drops[18,13]. Nevertheless, as noted from available data and practical experience it seems very difficult to observe bees taking up guttation fluid, even if guttation-related mortality occurred (as shown by residue analysis); mortality assessments seem to provide the more reliable information; however, if not conducted along with residue analysis of dead bees, they cannot differentiate between mortality related to guttation and other causes of mortality.

In the years 2009 and 2010, manufacturers of systemic insecticides conducted a series of field studies on guttation with soil-systemic applications of insecticides in maize, the crop that has been identified as worst case crop with regard to guttation.

Study setups varied with regard to methodological approaches and exposure conditions (e.g. availability of alternative water and food sources) but were nevertheless basically consistent in their experimental approaches. In all studies, exposed colonies were followed up for several weeks or even months in order to account for potential chronic or delayed effects.

A majority of studies employed realistic exposure conditions prevailing during normal agricultural practice. In others, worst-case exposure conditions were tested in terms of availability of alternative water and food sources. A few studies investigated the influence of additional provision of water sources.

Overall, data for more than 170 bee hives exposed to guttating maize have been considered. About two thirds of the hives have been exposed to treated fields, and one third to control fields (no systemic insecticidal seed or soil treatment)[2]. Due to the long exposure periods (most studies from emergence until flowering), the number of 'assessment days' (number of observation days per study x number of observed hives) sums up to more than 10.000 assessment days. On the vast majority of the assessment days and sites in the described studies, no increased worker bee mortality was recorded. Nevertheless, an increased number of dead bees could occasionally be observed for some hives. These events were limited to one or very few days which coincided with the guttation period. Results of analytical investigations suggest that honeybees occasionally use guttation droplets as water source. Causality between individual mortality peaks and colony strength, health or survival could not be concluded for any of these studies. Some details of studies conducted in maize by the manufacturers of systemic insecticides are summarized in table 2.

In their key findings, studies of the manufacturers of systemic insecticides are consistent with the results of comparable studies that were conducted by independent research institutes as described above. Each of the company-owned studies was or will be evaluated and assessed individually by the competent authorities; at least most if not all of these data were available to the JKI before the elaboration of this publication.

2.2.3 Potential risk under field conditions

Shawki et al. [19] assumed honey bees might collect water at distances up to 50 m. Thus, it is likely that at a certain distance between crops and colonies a potential risk is usually reduced to a very low level. Therefore the potential risk of guttation is in the first instance depending on the distance of the colonies to treated crops, because uptake of guttation droplets is mainly determined by the distance between colony and crop and the availability of other water sources. The risk of uptake of contaminated water is higher if the colonies are located in closer proximity to the crop, and lower with increasing distance. If the crop is showing regular guttation activity and seeds are treated with a systemic active substance with high intrinsic bee toxicity and findings of residue levels of high concern in guttation droplets occur, then guttation is a potential risk for individual bees if hives are located near such fields.

For a number of other crops in some countries, e.g. winter oilseed rape or sugar beet crops in Germany and the UK, insecticides for seed treatment containing neonicotinoids have been registered for more than 10 years with no link to honey bee poisoning incidents based on the national investigation schemes (Germany: Pistorius J, 2011, pers. comm., United Kingdom: Thompson H, 2011, pers. comm.).

[2] Experimental colonies with initial strengths that do not reflect realistic apicultural conditions (i.e. < 5,000 bees) were not considered for this evaluation.

Tab. 2 Field studies on guttation in maize with insecticidal seed or soil treatment

Country	Year/Season	Field Studies on Guttation in Maize with Insecticidal Seed or Soil Treatment					Bee mortality assessment	Assessment of colony development	Assessment of guttation occurence	Assessment of bee exposure	Occurence of guttation	Treatment effects on mortality	Treatment related effects on colony levels
		Treatment Sites	Control Sites	No of treatment group hives	No of conrol treatment hives	Setup of hives (time, location)							
Austria	2009 (Spring)	30	0	60 (small colonies)	0	Directly at or in treated fields; before crop emer-gence	yes (every second day)	yes (three-week inter-vals)	yes (every second day)	yes (every second day)	Fre-quent	Single days and hives with mortality peaks that coincide with the guttation period and detected bee residues	No[1]
France (North / South)	2009 (Spring to Sum-mer)	4	4	24 (full size colonies)	24	Directly at treated fields; before drilling	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	frequ ent	Mortality peaks but no difference in the number of peaks between control and treatment.	No
France	2010	19	3	4 per site (full size colonies)	4 per site (full size colo-nies)	Prior to drilling directly in field	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	frequ ent	Single days and hives with mortality peaks that coincide with the guttation period, bee residues still to be confirmed	No
France	2010	1	1	6 (full size colonies)	6 (full size colo-nies)	Prior to drilling directly in field	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	fre-quent more so in treat-ment plots	Single days and hives with mortality peaks that coincide with the guttation period and detected bee residues	No
France	2009	1	1	6 (full size colonies)	6 (full size colo-nies)	Prior to drilling, directly in field	yes (daily to once every 4 days)	yes (weekly)	yes (daily)	yes (daily)	Fre-quent	No treatment related mortality effects during guttation period	No
France	2010	1	1	6 (full size colonies)	6 (full size colo-nies)	Prior to drilling directly in field	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	frequ ent	Single days and hives with mortality peaks that coincide with the guttation period and detected bee residues	No
France (South)	2010 (early Sum-mer)	1	1	6 (full size colonies)	6	Directly at treated fields; before crop emer-gence	yes (daily)	yes (weekly)	yes (daily)	yes (every 2-3 days)	frequ ent	Low mortality throughout the study. Mortality peaks in treatment and control coincided in most cases.	No
Germa ny	2010	1	1	6 (full size colonies)	6 (full size colo-nies)	Prior to drilling directly in field	yes (daily)	yes (weekly)	yes (daily)	yes (daily)	frequ ent	Single days and hives with mortality peaks that coincide with the guttation period and detected bee residues	No

As many different systemic active substances of low to moderate toxicity to bees have also been used for seed treatments and soil applications in the past, it can be assumed that in many cases honey bee colonies would have been exposed to guttation water. Due to the fact that no effects on bees had been observed, it can be concluded that in these cases unacceptable effects, e.g. increased mortality, might not occur, e.g. for fungicidal seed treatments (Pistorius J, 2011, pers. comm.).

2.2.4 Implications for the registration of pesticides

Data from experiments with intrinsically highly toxic, systemic insecticides indicate that further studies beyond standard laboratory toxicity data might be needed for a limited number of highly toxic active substances in a worst case crop. Criteria for active substances that may trigger further consideration may be

- systemic properties of active substance (xylem mobility),
- persistence,
- intrinsic toxicity for bees and
- mode of action
- crop

Regulatory decisions need to be made on a case by case basis. While exposure of honey bees to contaminated guttation water will be regularly addressed if the above mentioned criteria are met, specific testing does not need to be a standard regulatory requirement for all substances.

In order to assess the potential risk from guttation, commonly used study designs can principally be used. Nevertheless, some adaptations for semi-field trials and field trials are needed depending on study aim and these should be carefully considered for the study set up (e.g. the location directly at field edge, the set up of colonies at the field to cover crop stages with high residues, absence or availability of alternative water sources). For guttation studies prolonged assessment periods, e.g. on mortality and colony development, are necessary.

3. Conclusions

A large number of studies were conducted by both public research labs and industry to address the potential risk of guttation to bees. From available studies it can be concluded that different crops vary in the intensity and frequency of guttation events, residue levels in guttation liquid depend on the properties of the active substance, the amount of active substance per seed and other factors. Peak residue levels of systemic insecticides in guttation droplets have been measured soon after emergence and in young growth stages. Guttation droplets are one of several possible water sources in the surroundings of a colony and usually are only available at a limited time. The collection of guttation liquid is not an exposure scenario comparable to exposure to nectar and pollen, and the risk is likely to decrease rapidly with distance of the colonies to treated crops and the availability of alternative water sources nearby. In both field trials and monitoring from research institutes and industry, occasional increased mortalities of worker bees were reported from single events in such trials, where colonies were placed directly next to the sown maize crop. However, data indicate that even when such mortalities occurred no long-term effects on colony strength and brood development were seen. The potential risk from guttation seems to depend in the first instance of the distance of the colonies to treated crops. As guttation issues with particular focus on honeybees have been investigated for a few years only, the conclusions represent the current state of knowledge. Further basic research on mechanisms of water collection of the bees and use of water in the hive are recommended.

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Working Group – Acceptability of effects in the field

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Aims of the working group

The aim of this working group is to continue the work of field and semi-field group that had reported at the last Bee Protection Group meeting in Bucharest (2008). This had resulted in the latest revision of the EPPO 170 guidelines (2010)[1], with particular attention being paid to the higher tier (cage and field) studies. One of the primary considerations in the revision exercise had been to maintain a balance between providing sufficient information to enable suitable studies to be conducted but maintaining flexibility (not too prescriptive) to ensure that the specific requirements of all studies could be addressed.

During the revision of the EPPO 170 guidelines, a number of specific issues were identified that went beyond the scope of the current exercise but were considered to need further work. These could generally be characterised as the 'acceptability of effects in the field'.

Specific issues identified

In the latest version of the EPPO 170 guidelines (2010)[1] it states that "Tests (cage and field) should be repeated where control mortality is excessively high or where effects in the toxic standard treatment are low". It was considered that more precise definitions of excessively high control or low toxic standard mortality would be appropriate. This issue formed the basis of another working group, led by Christine Vergnet ('Acceptable level of control and toxic reference mortality from in-cage and field tests'). Accordingly, to avoid duplication of effort, this aspect of cage and field testing was not addressed by the 'Acceptability of effects in the field' working group.

Another issue identified was the need for further guidance to be provided for the assessment of any effects seen in test colonies during cage or field tests i.e. as a result of the test item treatment.

The current requirements for cage and field testing identify the need to assess all factors e.g. mortality, behavior (including foraging activity) and colony assessments. In terms of the interpretation of the data collected, consideration is given to a statistical evaluation, particularly for cage studies (although the limitation on replication in field studies is recognised) but no specific guidance is provided. The main emphasis is given to an assessment of the biological significance of any effects seen, but again little specific guidance is given and reference is made to the need for 'expert judgement'.

These aspects therefore formed the basis of the work of this group.

Current work

A key aspect identified for the approach of the working group is the need to avoid duplication of effort. Accordingly, it was considered important to identify existing guidance available as well as other ongoing initiatives that might be useful for its deliberations. A number of useful sources have been identified.

An assessment of field trial methodology has been undertaken by a working group in France to consider the use of field testing for national requirements. A comparison of the recommendations for the French field methodology with that provided by the EPPO 170 guideline[1] was presented at the meeting (see Hervé Giffard, this volume[2]). One specific aspect of the assessment of field effects relates to colony development and this is being addressed by the 'Bee brood' working group (see Becker *et al.*, this volume[3]). In addition, a number of papers were presented at the meeting that also provided useful information with regards to bee brood for the working group: 'Non-treatment related

variability of termination rates in honey bee brood studies and possibilities for further improvements of existing guidance (see Jens Pistorius *et al.*, this volume[4]); 'Improvement in the calculation of indices in brood tests' (see Hervé Giffard, this volume[5]).

In addition, a SETAC Pellston workshop, 'Pesticide risk assessment for pollinators' has been held in the US (15-21 January 2011)[6]. This identified key outputs from cage and field studies (largely based on the EPPO 170 guidelines) and then considered the interpretation of effects linked to identified protection goals e.g. pollination, honey production, etc. As a result of this a need was identified for additional statistical input into the existing study designs and appropriate statistical analyses of the results obtained. Accordingly, a steering committee has been set up to address advice and guidance on appropriate statistical approaches to analyses of study data. The field effects working group considered it appropriate to wait and see the output from this exercise and evaluate its use in developing the EPPO 170 guidance (proceedings from the SETAC Pellston workshop are due in spring 2012).

Additional work identified

In preliminary discussions, a number of issues had been identified for further consideration. An important first question is how much further guidance is needed or to put it another way, how much of the evaluation of cage and field testing should be left to 'expert judgement'? One concern about this is that appropriate expert judgement may not always be available, as the guidance has to be capable of being used throughout the EU for regulatory purposes. This involves consideration of the potential audience for the guidance, which may comprise a range of backgrounds e.g. experienced assessors, regulators (non-specialist), beekeepers, extension services etc.

In terms of specific issues, it was considered important to provide a clear definition of the protection goals that are being addressed when assessing the significance of any effects seen in field testing. These could comprise a number of factors including biodiversity, honey production, pollination as well as other aspects. It was also important to define the relative role of statistical and biological significance in any overall assessment i.e. when would an effect be considered biologically important and when would statistical significance be considered necessary. It might be possible to identify stable background levels that provide a reference point for treatment effects under trial conditions (taking into account between-colony variability). This can provide the basis for an assessment of effects on the basis of percentage increases/decreases or ratios (comparing pre- and post-treatment levels). However, it was also recognised that it is necessary to define limitations for these approaches as if background levels are low the outcome can be very misleading (e.g. high relative changes but low in absolute terms). This could also involve considering a difference between cage and field studies. Should cage studies just be a trigger for field studies (above a certain threshold level of effects) or can they also be used for direct assessment of the significance of effects, taking into account the increased severity of exposure and differences in assessment?

Approaches to assessment

Preliminary consideration has been given as to whether it might be possible to identify specific thresholds of concern for different assessment parameters. For example, mortality: is there a level that is considered to have a significant impact on colony viability e.g. >50 bees/day (above background levels) for >2 days? Foraging activity: can we consider an acceptable level of effects on colony viability and pollination efficiency e.g. >50% reduction for >3 days. It is important to realise that these kinds of proposals are designed to provide a starting point for discussion to assess the feasibility of this approach. In reality the situation is more complicated and we also have to consider other factors e.g. crop, seasonal effects, size of hives and so on.

In the case of other sub-lethal effects (e.g. behavioural), we need to ask how do we determine the impact on colony viability. Firstly, we need to identify which sub-lethal effects might be important in this context e.g. disorientation, repellency and so on. However, this list can become extensive and we need to consider what *can* be assessed under field conditions (i.e. what is possible from a practical point of view). We then need to consider what *should* be assessed i.e. for the purposes of regulatory

risk assessment (significance in relation to protection goals). It is important that we provide information that is of value in relation to the assessment and not simply because it might be of interest or is being addressed by what we are already doing.

Additional considerations

In the first instance, consideration of colony development is to be addressed by the 'Bee brood' working group, as previously explained. A number of additional questions were also identified in preliminary discussions. It was felt that the over-wintering survival of colonies need only be included in specific circumstances e.g. late season application (according to GAP) or where there is the potential for residue carry-over. With regards to the assessment of interactive effects e.g. effects of treatment together with disease, climate etc, it was considered that this was beyond the scope of the regulatory risk assessment scheme but would be useful when considering other factors in post-registration evaluations. Finally, it was felt that the incorporation of risk management practices into the test guidance would be useful (to indicate what might be possible) although it was recognised that this would ultimately be addressed at a national level.

Future work

At the Bee Protection Group meeting in Wageningen, it was decided that the two working groups 'Acceptable level of control and toxic reference mortality from in-cage and field tests' and 'Acceptability of effects in the field' should be combined into a single 'Semi-field and field testing' working group, under the joint co-ordination of Christine Vergnet and Gavin Lewis. A call was made for any additional participants and following the meeting a number of people expressed an interest so that the working group now comprises 21 members from academia, regulatory authorities and industry (in order to maintain the viability of the group membership is now closed). An initial meeting of the working group has been held in January 2012 (at ANSES, France). In order to facilitate the work of the group, three subgroups have been identified: Acceptability criteria for the control and the toxic reference results (Chair: Jens Pistorius, JKI, Germany); Study design factors (Chair: Franck Marolleau, ANSES, France); Treatment effects (Chair: Gavin Lewis, JSC, UK). It was agreed to hold annual meetings (2013 at the Norwegian Food Safety Authority and 2014 at JSC, UK) until the next full meeting of the Bee Protection Group in Ghent, 2014. The sub-groups will carry out their specific work in the intervening periods with full discussion of all issues at the annual meetings of the working group.

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Acceptable levels of control and toxic reference mortality from semi-field and field tests - working group 2011 report summary

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Abstract

The EPPO guideline 170 (2010) recommends that semi-field or field tests should be repeated where control mortality is excessively high or where effects in the toxic standard treatment (if included) are low. Therefore, the working group was in charge of giving a better definition of:

- what is an excessively high control mortality, and:
- what are low effects in the toxic standard, focusing on mortality?

Preliminary outputs were presented:

- Control mortality needs to be considered in the context that natural (background) mortality in colonies can be highly variable.
- Also, if mortality in individual colonies is excessive, e.g. due to diseases or other non-treatment related factors, these may be excluded from the analysis rather than compromising a particular test group, where this can be justified.
- While there should be a statistically significant increase in effects with the toxic standard compared with the untreated control (as appropriate to the mode of action of the compound), the actual level will depend on the trial conditions (e.g. the attractiveness of the test crop) and so it is not always appropriate to set a required level.

The actual remit of the working group was questioned ('Formulate sharp cut-off criteria for mortality, or more broadly define validity criteria'). It was wondered whether there is a need for a formal quantitative approach (statistician) or whether qualitative criteria could suffice.

Several issues were identified (data collection: how to handle confidentiality issues, membership, geographical bias, overlap with field effects group).

The main outputs at Wageningen symposium are:

- Sharp cut-off criteria are neither desirable nor feasible. Mortality should be regarded in a broader context of factors affecting study validity.
- At the same time, clear guidance is needed to help regulators to objectively distinguish between biologically significant and deviant effects.

Given the overlap of issues addressed, the working group asks to liaise with the other field effects working group on 'acceptability of effects in field studies'.

Working Group - Risk to honey bee larvae

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Remit of the working group

The remit of this working group was to evaluate recent methodological developments on honey bee brood testing and risk assessment and to integrate these into the current risk assessment where appropriate.

The new Regulation 1107/2009 has new data requirements for honeybees under Annex II and Annex III: „An active substance, safener or synergist shall be approved only if it

- will result in a neglegible expsoure of honeybees, or
- has no has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on *honeybee larvae* and honeybee behaviour"

Detailed data requirements for effects on honeybees

Study type	Requirements following 91/414	Requirements following 1107/2009
Annex II		
Bee brood feeding test	Required Guideline: EPPO 170 (Oomen et al. 1992) Report: not specified, in fact brood termination rate, brood index and brood compensation index are reported	New requirements (see below)
Annex III		
Effects on honeybee development and other honeybee life stages	Not required	Required • Guideline: validated tests for different life stages (e.g. eggs, larvae, immature adults) are not in place • Report: EC_{10}, EC_{20}, EC_{50}, NOEC for larvae and adults sub-lethal effects, if observed • Comment: currently covered by semi-field brood studies

Bee larvae/brood:

- no classification lab / semi-field / field (tiered system?)
- no specification of methods / endpoints
- no trigger values
- risk assessment to be revised

Established test systems

Tier I	In vitro bee larva laboratory test	No validated test system available yet
Tier I/II	Oomen & de Ruijter Bee brood feeding test	Test method established (Screening of products, so far IGRs)
Tier II	Semi-field test (OECD Guidance Document 75)	Test method established
Tier III	Field test comprising detailed brood evaluation	Test method established

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Summary of discussions regarding the Working Group reports

Helen Thompson
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On the basis of the discussions at the 2008 meeting six Working Groups were formed prior to the 2011 meeting:

- Risks posed by dusts
- Assessment of risks posed by guttation
- Acceptability of effects in field studies
- Acceptable levels of control and toxic reference mortality from in-cage and field tests
- Design of post-registration monitoring studies for systemic pesticides, and the
- Working group on brood studies continued its work

Presentations from each of these working groups were included in the 2011 meeting and there was a brief discussion following these. These views of the participants, summarised here, .have been incorporated into the further considerations of each of the working groups and outputs will be reviewed at the next meeting in Ghent in 2014.

Risks posed by dusts

There was concern raised by any 'black-box' approach to predicting dust dispersal. There are theoretical approaches in the literature for distribution of dust in the atmosphere, e.g. soil particles - how relevant are these?

The APENET data from Italy show differing dust distributions from all other studies - are there differences in methodology in these studies and is it possible to see the detailed methodology and data from the Italian studies to assess this?

The issue was raised that if all mitigation specified on labels is in place on products and on seed bags, can it be foreseen that dust from seeds will be considered a manageable issue, i.e. will there be a return to market?

The size of the plot is a critical factor in the interpretation of field studies on dust drift - it is important to include a correction factor to account for differing sizes of plots in dust drift studies.

Assessment of risks posed by guttation

It is important to determine if the risk is lower for some crops, e.g. sugar beet, and therefore there is no need to generate data from field studies whereas maize is a worst-case crop. However, even maize studies have shown that the effects may only occur on single days in one in 20 field studies and there is a need to determine the acceptability of these infrequent events.

Mitigation in terms of the distance of the colony from the crop is only appropriate for honeybees, guttation poses a risk to the wider environment. We should consider risk to other species separately, e.g. bumble bees.

In identifying an acceptable distance from a potentially guttating crop, who is responsible for maintaining the distance, the farmer or the beekeeper? - in some cases the apiary may be a permanent site

Acceptability of effects in field studies

How does the mortality of individuals affect the colony – we need to model at the colony level to identify impacts. A population model may be a useful tool to extrapolate the impact of effects but with caveats that we are dealing with the real world and there is a need for funding to link appropriate models, e.g. disease and population models, with pesticide effects.

Acceptable levels of control and toxic reference mortality from in-cage and field tests

Recommendation to combine this working group with the field effects group and divide into two subgroups on semi-field and field effects.

Brood studies

There was discussion on the current status of the validation of the Aupinel larval test method – is there anything we can do to progress this further to ensure acceptance?

Design of post-registration monitoring studies for systemic pesticides

There is a need for stewardship and ensure compliance with the label which requires farmer education.

Monitoring can provide essential information on both unforeseen effects under realistic use conditions and confirmation that risk assessments are protective.

VIII. General

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Glossary

Abbreviation	Meaning
a.i. / a.s.	Active Ingredient / Active Substance
AFSSA	French Agency on the Safety of Food
AOAC	Association of Official Chemists
AOT	Acute Oral Toxicity
BBCH	Standardized coding for growth stages of different crops
BFD	Brood area Fixing Day
BTR	Brood termination rate
BVL	Federal Office of Consumer Protection and Food Safety, Germany
CCD	Colony Collapse Disorder
CEB	Commission des Essais Biologiques, France
DAR	Draft Assessment Reports
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency, United States
EPPO	European and Mediterranean Plant Protection Organisation
ESCORT	European Standard Characteristics Of non-target arthropod Regulatory Testing workshop
FAO	Food and Agriculture Organisation
GAP	Good Agricultural Practice
HQ	Hazard Quotient
ICPBR	International Commission of Plant-Bee Relationships. Since 2012 renamed ICPPR
ICPPR	International Commission of Plant-Pollinator Relationships
ICT	Indirect Contact Toxicity
IGR	Insect Growth Regulator
IPM	Integrated Pest Management
IUBS	International Union of Biological Sciences
JKI	Julius Kühn Institute, Germany
LC-MS	Liquid Chromatography – Mass Spectrometry
LD50	Lethal Dose for 50% of the organisms
LOD	Level of Detection
LOQ	Level of Quantification
LR50	Lethal Rate for 50% of the organisms
MRL	Maximum Residue Limit
NOEC	No Observed Effect Level
NOED	No Observed Effect Dose
NTA	Non-Target Arthropods
OECD	Organisation of Economic Cooperation and Development
OPERA	European Observatory on Sustainable Agriculture
OSR	Oil Seed Rape
PER	Proboscis Extension Reflex
PPP	Plant Protection Product
QS	Quality Control System
RFID	Radio frequency identification
RMM	Risk Mitigation Measures
SANCO	Directorate General for Health and Consumers, EU
SETAC	Society of Environmental Toxicology and Chemistry
SRL	Subsequent Residue Level
TER	Toxicity Exposure Ratio
WIIS	Wildlife Incident Investigation Scheme, UK

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Für die Allgemeinheit sind vor allem die Faltblätter gedacht, die über Nützlinge im Garten, aber auch über spezielles wie den Asiatischen Laubholzbockkäfer informieren. Außerdem ist der regelmäßig erscheinende Jahresbericht allgemein interessant, vor allem mit den umfassenden Artikeln zu besonderen Themen, die Sie aber auch im Internet auf den thematisch dazugehörigen Seiten finden.

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Hazards of pesticides to bees

Honey bees, environment, risks and hazards, pesticides and neonicotinoïds, bee decline and colony collapse disorder, bumble bees and wild bees, pollinators… There is an intense public interest into these matters in Europe and outside, with many publications on television, in newspapers and in scientific papers. It is good to note so much interest in the subject of our discussions.

Indeed, nearly everybody is involved in some way. The gardens, the flowers and the apples touch everybody. Even Mr. Einstein is being cited.

The protection of bees and other pollinators is important, while protection from pesticide effects are a first priority. But how? It needs, more than anything else, scientific understanding and then understanding-based measures. Scientific considerations and arguments are at the base of all protection.

It is here that the ICPBR symposia are important. It is here that nearly all experts on bee protection from pesticides in Europe meet and exchange their views and discuss. It is here that new developments and suggestions come up, to be elaborated further into legislation and official regulations by national authorities, by OECD and EPPO, by EFSA and the European Commission and European Parliament.

Developing shared understanding and shared protection measures is not easy. Good scientific studies and arguments are the only way. The Bee Protection Group of the ICPBR is eager to promote and enable this way in its series of symposia. This Wageningen symposium was the 11th symposium of its kind since the start in 1980, and it will continue with a 12th symposium in 2014 in Ghent, Belgium.

The ICPBR has been renamed in 2012 to ICPPR: International Commission for Plant – Pollinator Relationships, in order to cover its widened scope.

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