INTERNATIONAL COMMISSION FOR PLANT-BEE RELATIONSHIPS

Programme and Abstracts of the 11th International Symposium of the ICPBR Bee Protection Group

HAZARDS OF PESTICIDES TO BEES

Wageningen, The Netherlands
November 2-4, 2011

Meeting place:
Hotel *De Nieuwe Wereld*
Marijkeweg 5, 6709 PG Wageningen
The Netherlands

Organising Committee

Dr. Pieter A. Oomen (ICPBR, NL), Chairman
Dr. Helen Thompson (FERA, UK), Secretary
Dr. Gavin Lewis (JSC International Ltd, UK), Vice-Chairman

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Pieter A. Oomen
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Sjef van der Steen (WUR)
Claudia Jilesen (nVWA)
Adindah Visser (Symposium assistant)

Symposium Hazards of Pesticides to Bees
Wageningen, 2-4 November 2011
Programme

Wednesday 2 November 2011 - Opening session

9:00 **Registration**
9:30 **Welcome** by Pieter A. Oomen, chairman ICPBR Bee Protection Group: About this symposium
9:45 **Official opening** by Dr. W.H. van Eck, Deputy Director of the *new Food and Consumer Product Safety Authority* / Netherlands' Plant Protection Service

10:00 **Pieter A. Oomen:** Practicalities
10:15 **Dr. Robert Luttik,** RIVM, chairman EFSA honey bee group: About the EFSA working group on honey bees

1. Session – Regulatory review: honey bee risk assessment for pesticides in Europe

10:30 – 11:00 Brief (6 minutes!) introductions to ICPBR working groups by their coordinators:

- Risks posed by dusts (Rolf Forster)
- Assessment of risks posed by guttation (Jens Pistorius)
- Acceptability of effects in field studies (Gavin Lewis)
- Acceptable levels of control and toxic reference mortality from in-cage and field tests (Christine Vergnet)
- Risk to honeybee larvae (Roland Becker)

11:00 – 11:30 Coffee break

9 presentations, 11 posters
11:30 – 13:00

- Anne Alix, Laurie Adams, Mike Brown, Peter Campbell, Ettore Capri, Amalia Kafka, Konstantinos Kasiotis, Kiki Machera, Christian Maus, Mark Miles, Petru Moraru, Lisa Navarro, Jens Pistorius, Helen Thompson, Alexandru Marchis: Bee health in Europe – Facts & figures
- Tjeerd Blacquière, Guy Smagghe, Cornelis A.M. van Gestel & Veerle Mommaerts: Neonicotinoids and bees: an overview on concentrations, side effects and risk assessment
- Anne Alix, M. Miles: Pollinators, pesticides and agriculture: developing regulatory tools for the future
- James Cresswell: Using sublethal effects in TER triggers: examples from neonicotinoids, honey bees and bumble bees

13:00 – 14:00 Lunch break

14:00 – 15:30

- Dermine M, Kievits, J., Lortsch JA, Mouret C., Simon-Delso N.: Risk assessment of pesticides on bees: evaluating risk coefficients for assessing acute and chronic toxicity and their methodologies
- Fabio Sgolastra, Teresa Renzi, Stefano Draghetti, Claudio Sciò, Piotr Medrzycki, Marco Lodesani, Stefano Maini, Claudio Porrini: Effects of neonicotinoid dust from maize seed-dressing on honeybees
- Dermine M, Kievits, J., Lortsch JA, Mouret C., Simon-Delso N.: Assessment of pesticides risk for bees: methods for PNEC measurements
- Rolf Forster: Risk mitigation measures for seed treatments using neonicotinoids
- Jacoba Wassenberg and Martine Lans: About the recent re-evaluation of neonicotinoids regarding bee risks in the Netherlands

15:30 – 16:00 Tea/coffee break

Related posters:

- A. Alix, M. Miles: Exposure of honey bees and other pollinating species to pesticides
- M.Miles, A.Alix: Assessing the comparative risk of plant protection products to honey bees, non-target arthropods and non-*Apis* bees
- Malte Frommberger, Jens Pistorius, Andreas Schier, Ina Joachimsmeier, Detlef Schenke: 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
- Ina Joachimsmeier, Malte Frommberger, Jens Pistorius, Udo Heimbach, Detlef Schenke, Peter Zwerger,

Wolfgang Kirchner: Guttation and the risk for honey bee colonies (*Apis mellifera L.*): a worst case field scenario in maize

- Ingrid Illies, Verena Gottschalch, Klaus Wallner, Bernhard Engelhard, Jens Pistorius, Gabriela Bischoff: *Thiamethoxam* in the cultivation of hop – does it pose a threat to honey bees?
- Ingrid Illies, Stefan Berg, Jens Pistorius, Gabriela Bischoff: Effects on honey bee colonies following a granular application of Santana containing the active ingredient *clothianidin* in maize in 2010 and 2011
- Ina Joachimsmeier, Jens Pistorius, Udo Heimbach, Detlef Schenke, Peter Zwerger, Wolfgang Kirchner: Are guttation drops a potential water source for honey bees?
- Ina Joachimsmeier, Jens Pistorius, Udo Heimbach, Detlef Schenke, Peter Zwerger, Wolfgang Kirchner: Details on occurrence and frequency of guttation in different crops in Germany
- M. Miles, A. Alix, V. J. Kramer: Review of higher tier methods for assessment of the risk of pesticides to honey bees
- Pistorius J. , Wallner K., Joachimsmeier I., Reetz J., Schenke D., von der Ohe W., Illies I. Maus C., Block T., Becker R.: Review on activities in Germany to assess the occurrence, residues and possible risk of guttation for honey bees
- Klaus Wallner, H.-M. Wied, J.E. Reetz: Orientating experiments on guttation fluid of seed treated maize (*Zea mays L.*) in relation to the water collecting behavior of honey bees (*Apis mellifera*)

2. Session - Bee health issues – country specific experiences including Varroa and varroacides

No presentations, 1 poster:

- W. Loucif-Ayad, N. Aribi, G. Smagghe & N. Soltani: Efficacy of selected acaricides on *Varroa destructor* and evaluation of their environmental risks on *Apis mellifera*

3. Session - Test methodology - laboratory, semi field, field, etc. ***Chair: Gavin Lewis.***

9 presentations, 2 posters

16:00-18:00 hrs

- Hervé Giffard: Improvement in the calculation of indices in brood tests
- Job P. van Praagh & Egbert Touw: Scanning & analysing individual bee behaviour using RFID
- Hervé Giffard: Comparison in EPPO and French field methodology
- Piotr Medrzycki, Jamie Ellis, Vincent Dietemann, Peter Neumann: Introduction to the BEEBOOK, a manual of honeybee research methods
- Lukas Jeker, Thomas Meschberger, Marco Candolfi, Josef P. Magyar: Digital image analysis as a tool to improve the assessment and evaluation of brood development in higher tier honeybee studies
- Piotr Medrzycki, Fabio Sgolastra, Gherardo Bogo, Simone Tosi, Simone Venturi: Influence of some experimental conditions on the results of laboratory toxicological tests on honeybees

18:00 End of first day presentations, session 3 to be continued on next day.

Thursday 3 November 2011, continuation of session 3 – Test methodology

9:00-10:30 hrs

- Simone Venturi, Piotr Medrzycki, Gherardo Bogo, Simone Tosi, Fabio Sgolastra, Marco Lodesani: A new experimental method for studying trophallaxis as an additional determining factor in the effects of chemicals on foraging bees (*Apis mellifera*)
- M. Miles, C. Bourgouin, S. Schmitzer: Effects of spinosad on honey bees (*Apis mellifera*): Findings from over ten years of testing and commercial use
- J. Pistorius, R. Becker, J. Lückmann, A. Schur, M. Barth, L. Jeker, S. Schmitzer, W. von der Ohe: Effectiveness of method improvements to reduce variability of brood termination rate in honeybee brood studies under semi-field conditions

Related posters:

- R. Becker, S. Schmitzer, M. Barth, H. Bargen, H.-H. Kaatz, H. T. Ratte, A. Schur: Statistical evaluation of regulatory honey bee trials – a pragmatic approach. *This subject and poster will be discussed also in session 7*
- Simone Tosi, Piotr Medrzycki, Gherardo Bogo, Laura Bortolotti, Francesca Grillenzoni, Giuseppe Forlani: Role of food quality in bee susceptibility to fipronil and clothianidin

4. Session – Honey bee poisoning incidents and monitoring systems. Chair: Anne Alix

4 presentations, 3 posters

- Anne Alix, Aurelien Cocoraju, Jean-Michel Laporte, Christian Maus, Mark Miles, Noa Simon & Helen Thompson: Monitoring effects of pesticides on pollinators – a review of methods and outcomes - *(including introduction on work of ICPBR working group on monitoring)*

10:30 – 11:00 Coffee break

11:00 - 13:00

- Bert van der Geest: Bee poisoning incidents in the Pomurje region of Eastern Slovenia in 2011
- Daniela Laurino, Aulo Manino, Augusto Patetta, Andrea Romano, Marco Porporato: Quantitation of neonicotinoid insecticide residues in experimentally poisoned honey bees
- Marco Lodesani, Piotr Medrzycki, Laura Bortolotti: Project 'APENET: monitoring and research in apiculture'

Related posters:

- P.-Th. Georgiadis, J. Pistorius, U. Heimbach, M. Stähler, K. Schwabe: Dust drift during sowing of maize - effects on honey bees
- P.-Th. Georgiadis, J. Pistorius, U. Heimbach, M. Stähler, K. Schwabe: Dust drift during sowing of winter oil seed rape - effects on honey bees
- Adindah Visser & Tjeerd Blacquière: Do residues of imidacloprid in surface water cause honeybee colony losses?

6. Session - Biocontrol methodology using bees

2 presentations, no posters

- Heikki Hokkanen, Ingeborg Menzler-Hokkanen & Aino-Maija Mustalahti: Pesticide sprays compromise pollination and biocontrol services on strawberry
- Veerle Mommaerts, Kurt Put, Jessica Vandeven, Guy Smagghe: Laboratory miniature dispenser-bioassay to evaluate the compatibility of powder compounds in the entomovectoring with *Bombus terrestris*

13:00 End of second day presentations
13:00– 14:00 Lunch break, thereafter informal program:

Thursday afternoon & evening:

14:00 departure by bus from *Hotel de Nieuwe Wereld* (*RijnIJssel*) to Arnhem,
15:00 - 21:00 excursion & dinner at Burgers' Bush, Arnhem
21:00 departure by bus from Burger's Bush, arrival to Wageningen **22:00 hrs**

Friday 4 November 2011

7. Session - Plenary discussion on regulatory issues and revision of the EPPO guidelines/risk assessment scheme. Chair: Pieter A. Oomen

9:00 – 11:00 Actual developments in Europe:

- Definition of 'negligible exposure' and of 'sublethal effect', (New Regulation, Annex II, point 3) (introduced by Robert Luttik of EFSA)
- Statistical evaluation of regulatory honeybee trials (introduced by Roland Becker, AG Bienenschutz working group)
- Issues that we suggest to be addressed by EFSA working group
- Any other issue brought forward by the symposium participants

- Summary by secretary Helen Thompson
- Details and requirements about the publication of the proceedings (to be published again as a monograph in the Julius Kühn Archiv; editor Pieter A. Oomen, manuscripts to be submitted before 1 January 2012 to oomen.pieter @ gmail.com)

11:00 – 11:30 Coffee break

5. Session - Bumblebees and other pollinators. Chair: Irene Koomen

7 presentations, 4 posters
11:30 – 13:00

- Introduction by Irene Koomen
- Stephan Carvalho, Thaisa Roat, Andrigo Pereira, Elaine Silva-Zacarin, Roberta Nocelli and Osmar Malaspina: Brazilian bee loss
- Muo Kasina: Bees require protection for sustainable horticultural production in Kenya

13:00 – 14:00 Lunch break. Then continuation session 5
14:00 – 16:00

- Andrigo M. Pereira, Roberta C. F. Nocelli, Osmar Malaspina, Odair C. Bueno: Side-effect of acetamiprid in adult Africanized honeybee
- Tavares Lourenço, Clara; Malfitano Carvalho, Stephan; Malaspina, Osmar; Nocelli, Roberta Cornélio Ferreira: Determination of LD_{50} of fipronil for bees *Melipona scutellaris*
- Jozef van der Steen, Ivo Roessink, Muo Kasina, Mary Gikungu and Roberta Nocelli: Is the European honeybee (*Apis mellifera mellifera*) a good representative for other pollinator species?
- Harold van der Valk, Irene Koomen, Tjeerd Blacquière, Marcia de F. Ribeiro, Roberta C.F. Nocelli, Muo Kasina, Mary Gikungu, Jacoba Wassenberg, Sjef van der Steen: Aspects of determining pesticide risks to wild bees – implications for risk mitigation and risk assessment

Related posters:

- Siqueira, Kátia M.M.; Kiill, Lucia H.P.; Coelho, Márcia S.; Araújo, Diêgo C. S.; Gama, Diego R.S.; Lima Jr, Ivan O.; Ribeiro, Márcia F.: Effect of agrochemicals in the pattern of visitation of *Apis mellifera* in *Cucumis melo*
- Veerle Mommaerts, Linde Besard, Gamal Abdu-Alla, Guy Smagghe: Assessment of lethal and sublethal effects by spinetoram on *Bombus terrestris*
- Ivan Meeus, Dirk de Graaf, Kris Jans, Guy Smagghe: Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers
- Ivan Meeus, Dirk de Graaf, Guy Smagghe: Detection of viral replication in bees

8. Close of symposium, chair Pieter A. Oomen

16:00 – 16:15

- About the changes in the board of ICPBR Bee Protection Group
- About next symposia (principally three years from now (2014) in Ghent, Belgium, on kind invitation of Guy Smagghe !)

16:15 Tea and farewell drinks & end

Abstracts

Bee health in Europe – Facts & figures

A OPERA document

Anne Alix, Chair, Ministry of Agriculture, France
Laurie Adams, North American Pollinator Protection Campaign, USA
Mike Brown, National Bee Unit, UK
Peter Campbell, Syngenta
Ettore Capri, OPERA Research Centre, Italy
Amalia Kafka, OPERA Research Centre
Konstantinos Kasiotis, Benaki Phytopathological Institute, Greece
Kiki Machera, Benaki Phytopathological Institute, Greece
Christian Maus, Bayer Crop Science
Mark Miles, Dow Agrosciences
Petru Moraru, former Head of the Apiculture Research Institute, Romania
Lisa Navarro, Syngenta
Jens Pistorius, Julius Kühn-Institut, Germany
Helen Thompson, National Bee Unit, UK
and Alexandru Marchis, OPERA Research Centre

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, OPERA[1], 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.

[1] 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)

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

Tjeerd Blacquière*, Guy Smagghe, Cornelis A.M. van Gestel*** & Veerle Mommaerts****

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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).

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

A. Alix- Ministry of Agriculture, Paris, France
M. Miles-Dow AgroSciences UK Ltd., Abingdon UK

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.

Using sublethal effects in TER triggers: examples from neonicotinoids, honey bees and bumble bees

James Cresswell

Biosciences, College of Life & Environmental Sciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, United Kingdom

In the process of pesticide approval, regulatory agencies often impose a 'first tier' screening process that evaluates the product's 'toxicity-exposure ratio', or TER, for a specified non-target organism. In the case of honey bees, *Apis mellifera*, the TER is calculated based on dose-dependent mortality and compared against a threshold value, or 'trigger'. However, there is an increasing recognition that sub-lethal effects may need also to be taken into account. Here, I investigate how the principle of the TER can be applied to sublethal effects. To test the implications of this, I use new data that describes a range of dose-dependent sub-lethal responses of honey bees and bumble bees (*Bombus terrestris*) to a dietary neonicotinoid, imidacloprid. I explore the relative sensitivity of TER triggers based on lethal vs. sublethal effects and compare the environmental relevance of the two approaches.

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

Dermine M[1], Kievits, J.[1], Lortsch JA[1], Mouret C.[1], Simon-Delso N.[2]

[1] European Beekeeping Coordination
[2] Centre apicole de recherche et d'information, 4 place Croix du Sud, B-1348 Louvain-la-Neuve, Belgique

Risk coefficients fundamentally orientate the way the assessed pesticide active substance or formulated product will follow in the risk assessment scheme dichotomy. Defining them is thus one of the main challenges of a risk assessment scheme elaboration. In the light of the scientific publications on the subject, the existing risk coefficients and methodologies used for the evaluation of toxicity under European Directive and Regulation, OECD and EPPO guidelines result questionable. On the one hand, mortalities occurring more than 2 days after an acute contact can be observed when measuring the acute toxicity. On the other hand, the toxicity derived from the exposure to substances that are continuously available for bees through ingested matrices like pollen, nectar or water needs to be evaluated separately, given the wide differences between acute and chronic lethal doses. As a result the risk coefficient currently used in the assessment scheme needs to be reconsidered. The objectives of this article are (1) to review in the scientific literature the data mentioned regarding acute and chronic toxicity, (2) to draw consequences on the risk coefficients to be applied in the evaluation of these substances and (3) to propose alternative methodologies for the measurements and calculations of toxicity and risk coefficients.

Effects of neonicotinoid dust from maize seed-dressing on honeybees

Fabio Sgolastra*, Teresa Renzi, Stefano Draghetti, Claudio Sciò, Piotr Medrzycki, Marco Lodesani, Stefano Maini, Claudio Porrini.

Department of Agroenvironmental Sciences and Technologies – Entomology. University of Bologna. Viale G. Fanin 42, 40127 Bologna, Italy.

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.

Assessment of pesticides risk for bees: methods for PNEC measurements

Dermine M[1], Kievits, J.[1], Lortsch JA[1], Mouret C.[1], Simon-Delso N.[2]

[1] European Beekeeping Coordination
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The individual honeybee shows a complex behavioural structure. On its own, each bee takes part in the collective behavioural set up that ensures the bee colony survival and development. Apart from behavioural 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 sub-lethal dosses on bees. Such tests can be developed both in laboratory conditions (e.g. PER tests to evaluate impact on learning capacity and memory of bees) or as part of the semi-field and field tests that are currently required as higher tier tests of risk assessment schemes (e.g. homing flight or foraging behaviour). Risk coefficients based on PEC/PNEC calculations can help classifying the category of risk expected for the way of application and toxicity of the substance. Peer-reviewed scientific literature provides a wide range of methods used for testing honeybees' behaviours. Nevertheless, none of these methods have been ring-tested. Therefore, the purpose of this paper 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 ring-testing.

Risk mitigation measures for seed treatments using neonicotinoids

Rolf Forster
Federal Office of Consumer Protection and Food Safety, 38104 Braunschweig, Germany

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 of 12th March 2010 laid down the following general recommendations for mitigating risk arising from the emission of dusts:

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,
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,
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,
4. the conditions of the authorisation, (…), include, where appropriate, risk mitigation measures to protect honey bees.

According to these provisions the German authorities decided to apply the following risk mitigation measures via labelling of neonicotinoid products or seed bags of treated seeds:

1. RMM on the PPP for the application in professional facilities
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-Institute (visit the homepage of the Julius Kühn-Institute <http://www.jki.bund.de)/>). Justification: It has been established for maize, that the emission of contaminated dusts can be reduced by about 90 % by improving the treatment of seeds.

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-Institute (this can be seen on the Julius Kühn-Institut's website at <http://www.jki.bund.de/geraete)/>). Justification: 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.

3. RMM on the seed package to avoid dispersion of dusts
The following label must be printed on the seed package: Do not sew treated seeds at wind speeds of more than 5 m/s. Justification: A threshold of > 6 to 8 m/s wind velocity for wind erosion has been suggested for German conditions (ZALF 2005).
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. Justification: The spillage of seeds and dust has been regularly reported.

4. RMM on the seed package to protect honey bees
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. Justification: It has been clearly established, that honey bees may forage on guttation drops of plants near-by to their colony. In addition, the direct exposure of honeybee colonies to dusts from sowing can be omitted for further risk reduction.

About the recent re-evaluationof neonicotinoids regarding bee risks in the Netherlands

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The decline of bees is a worrying phenomenon in many areas of the world and 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.

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 substance 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. 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. In these meetings, the newly submitted studies, information from public literature and new options for risk mitigation were discussed. Label mitigation was ensured to be enforcable and manageable in practice.

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. During the project, many (more or less generic) issues were identified:

- Definition of ‘flowering crop’ needs clarification for bee relevance.
- A list with crops attractive to bees needs to be easily available online.
- Risk mitigation for non-professional users should be formulated in a simple way.
- Risks from succeeding crops and risks from re-sowing after crop failure for persistent substances: a minimum waiting period for bee-attractive succeeding crops may be necessary; NB for some crops the user of the land may change from year to year.
- Ensure that restrictions are seen by the right person. Restrictions are mentioned on the product label, but for seed treatment 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.
- Honeydew formation may need to be avoided
- Dipping treatment of flower bulbs – exposure in flower unknown - avoid flowering.
- Spray drift restrictions to protect bees in the off-field area may be necessary.

Poster

Exposure of honey bees and other pollinating species to pesticides

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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.
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.
Many PPP are typically applied either by spray to the above ground part of plants or to soil either directly (by spray or granules) or into the soil as treated seed. A third method of trunk injection is important for the protection of trees. The method and timing application will affect if bees are exposure and if so by what routes. The physico-chemical properties can also impact exposure which may affect the distribution of the substance within plant and also the duration of exposure. In this paper, different exposure scenarios are developed with respect to method and timing of application also in relation to the properties of the substance. Various scenarios are described where bees and other pollinators can be exposed along with an indication of possible residues levels present in matrices of relevance to bees. Explanations of the exposure scenarios due to spray applications and soil/seed treatment uses are given and way in which these can be used and link to risk assessment presented. By establishing clear guidance for exposure of pollinators to PPP a more informed risk assessment process can be developed and utilized.

Poster

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

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Risk assessments are conducted for plant protection products (PPP) with respect to potential impacts on non-target species. These include pollinators such as the honey bee but also to other non-target arthropods (NTA). In common with other areas of ecotoxicological risk assessment sentinel species are employed aiming at ensuring a high level of protection/conservatism. Tier I screening risk assessments are 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 European Union a Hazard Quotient (HQ) approach is used to assess the risk to both honey bees non-target arthropods at Tier I. This is calculated by dividing application rate by the LR50 (Lethal Rate 50). In other regions such as North America, a contact toxicity trigger of 11 µg active substance/bee is currently employed.

At the Pellston workshop it was suggested that for a risk assessment for sprayed products the honey bee could be a suitable surrogate 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.

On the basis of ecotoxicological data of the European data sets for honey bees and NTA, the relative risk of PPP to NTA, honey bees and non-*Apis* bees, as depicted by HQ values is compared and where possible data on NTA pollinators (e.g. Syrphids) and non-*Apis* bees (e.g. *Bombus* sp.) are also included. At tier I using HQ trigger of 50 for honey bees, 2 for NTA, in accordance with the current EU legislation, and 5 (i.e. 50 divided by the extra factor of 10 abovementioned) for non-*Apis* bees, the NTA scheme identified more compounds and uses to move forward for further evaluation. The suggest non-Apis HQ trigger of 5 gave a similar pass / fail rate to the NTA scheme but was slightly confounded by a lack of determined end points. However, even taking this into consideration the most restrictive tier I assessment was that for NTAs.

This poster examines the potential for a tier I risk assessment to cover NTA, honey bees, non-*Apis* bees and other arthropod pollinators as part of a tiered risk assessment scheme and compares the empirical HQ approach with methods using more specific species exposure estimates.

Poster

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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In a semi-field study with maize treated with clothianidin the possible risk of guttation for bees were investigated and in a worst- case scenario effects on adult and brood mortality of bees with special consideration of the brood development of the honey bee (*Apis mellifera L.*) assessed.

The study was performed under semi-field conditions in Lucklum (Braunschweig, north Germany), in treated and untreated maize. The tents (4 treatment and 2 control) had an area of 96m^2 (16 x 6m) each and were covered with a gauze permeable for wind and rain but impenetrable for bees. The study was repeated twice, in the first run (BBCH 13-15) with two variants, one with and one without artificial water source containing uncontaminated tap water. In the second run (BBCH 15-19) all variants had an artificial 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 nectar and pollen stores in the hives and were allowed to forage on additional sugar feeding paste and pollen sources in the tents. The mortality of bees was assessed in dead bee traps and on linen sheets in the crop. The flight activity and behavior of bees at the entrance of the hives and in three flight squares in the crop were determined once daily. The observation time in the tents were 11 (1st run) and 10 (2nd run) days, the observation of brood development for 100 brood cells per hive was conducted nearly for four weeks following the protocol of Schur et al., 2003. During the whole observation period the occurrence of guttation was documented and, if guttation occurred, guttation droplets were sampled daily for residue analyses.

Due to the weather conditions in the first repetition guttation occurred only once, which caused a high mortality in the waterless variation as well as a brood-termination rate of up to 100%. In the second run guttation of the corn occurred on 6 of 11 days. The mortality in treated and control variants 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%.
In the first run of the study, in the artificial extreme situation without any additional water supplies 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 effects on adult mortality and Brood were observed. The results of the second run are also with no effects on adult mortality or brood development. The comparison of a parallel open field study with same conditions (poster Joachimsmeier et al., 2011), also confirmed the results of no effects on mortality.

Poster

Guttation and the risk for honey bee colonies (*Apis mellifera L.*): a worst case field scenario in maize

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Aim of this experiment was to investigate whether bee colonies that are moved to a new location and are not familiar with their surrounding landscape are more at risk by guttation drops from seed-treated plants than honey bees which already know every site of water source in their surroundings. The experiment was conducted nearly at the same time (18th of May until 28th of June 2011) and in the same area (surrounding of Lucklum, Lower Saxony/Germany) as the semi-field studies on guttation and its risk for honey bees presented by Frommberger et al. in 2011.

In order to assess the research question, the number of dead bees was compared in bee colonies that a) had the possibility to get familiar with all sites of water sources in a field with seed-treated maize and b) bee colonies that were recently moved to the field.
The experimental field consisted of one plot planted with maize seed-treated with clothianidin and one plot with untreated maize. The set up of the experimental bee hives was identical for both field plots. The trial started on a day with guttation events in both maize plots approximately 4 weeks after emergence of the plants when high residues are present in guttation droplets. The bee colonies were located directly at the field border with the hive entrance pointing towards the treated maize crop.

In total, 18 comparable bee hives (approximately 10.000 bees/hive) were used with three bee hives for each of the three variants employed in the experiment.
The first variant (V1) was located permanently in the experimental field, the second variant (V2) was allowed to get familiar with the experimental field and then were located at various locations (at least 6 km away from the field) and finally moved back (at the start of the trial) to the experimental field again and the third variant (V3) was primarily located at the same various locations as V2 and placed (at the start of the trial) for the first time in the experimental field. Aim of this procedure was to get information about the mortality of bees which a) know their surrounding landscape (V1, V2) and were permanently potentially exposed to guttation droplets from seed treated plants, containing high residues of active ingredients (V1), b) occurs after transport of beehives (V2) and c) do not know their surrounding landscape and were never before potentially exposed to guttation droplets from seed treated crops, containing high residues of a.i. (V3).

The mortality of bees which occurs by moving from the beehives only slightly increased from 6 (-1 day before moving) to 21 (1 day after moving) dead bees per day. In the main study was no difference in the number of dead bees between the bees which had the possibility to get familiar with all sites of water sources in the experimental field and bee colonies that were recently moved to the field.

Poster

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

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One serious problem in the growing of hop is the feeding damage caused by different soil insects (e.g. *Curculionidae, Alticinae*) during springtime. 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 out and prepared for further examinations. Also the pollen loads were analysed for residues.

The used agent and the known metabolite clothianidin were neither detectable (LOQ 0,001 mg/kg) in the pollen loads from single bees (n=26), in the honey sacs (n=2000), 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.

Poster

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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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.

Poster

Are guttation drops a potential water source for honey bees?

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Aim of this experiment was to investigate whether guttation drops may be a potential water source for honey bees and to investigate in which distance from the bee colony bees may take up guttation fluid. Background for the studies on the interest of bees on guttation drops were reports from Germany and Italy. These reports suggested that guttation drops from seed treated plants can contain systemic active chemical substance e. g. neonicotinoids that have been applied as insecticide seed treatments and that these substances may be found in the guttation fluid at concentrations toxic for bees.

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 experimental fields consisted of two plots; one planted with winter wheat and one adjacent plot planted with oilseed rape. The development stage of winter wheat ate the beginning of the experiment was BBCH 29 (end of tillering), that of oilseed rape BBCH 53 (inflorescence emergence). The experiment was terminated at flowering of the oilseed rape crop (BBCH 65). During this period both crops often shown guttation drops.

Per field a total of three small bee colonies and two *full size colonies* were set up. The bee hives were placed in the winter wheat plot with distance of 50m from each other and a distance of 0m, 10m, 20m, 30m and 50m to the adjacent oilseed rape field. The flight openings of the bee colonies pointed toward the oilseed rape plot. Flight observations on flying honey bees were conducted several times per day at fixed observation points that were located next to the bee hives and at the transition zone between winter wheat and oilseed rape plots. In addition, the population development of the bee colonies was documented during the trial.

The majority of flying bees was observed adjacent to the bee hives; in the transition zone between the plots smaller numbers, approximately two thirds of that number could be observed. In the majority of cases, bees were spotted while scanning the leaf area of winter wheat plants. Only single bees could be observed that actually took up guttation fluid from crop plants growing at a distance of 50 m from the bee hive in the transition zone of the plots. No abnormalities regarding the population development of the employed bee colonies were observed. Single bees could be observed taking up guttation fluid. With increased distance from the bee hives, fewer bees were observed. No adverse effect on the development of bee colonies was detected, the development and mortality of both the organically and the conventionally managed fields was on a comparable level.

Poster

Details on occurrence and frequency of guttation in different crops in Germany

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In crop species of economic relevance in Germany, several glasshouse and field trials were conducted from 2009 to 2011 by the Julius Kühn-Institut in Germany and by cooperating research partners (DWD, IfZ, BDP and UFOP) to determine the frequency and occurrence of guttation. Several crop species such as sugar beet, winter oilseed rape, maize, barley, potatoes, oat, onions, carrots, peas and cucumber were investigated and an assessment of the occurrence, frequency and intensity of guttation (size/number of guttation drops, number of guttating plants) in the tested crop species was conducted

Observations were conducted from the beginning of early plant emergence until at least the flowering stage. During this period, the climatic conditions (relative air humidity, air and soil temperature) were recorded. In the glasshouse trials, the size of guttation drops was determined by weighing of the guttation drops. In the field trials, sky cover and soil humidity were estimated. In the field, the guttation frequency of adjacent vegetation was also assessed in order to facilitate a comparison to the investigated crop species. Daily assessments were conducted in the glasshouse while assessments in the field trials were only conduced under climatic conditions suitable for guttation.

Guttation occurred mainly at young growth stages in the tested crop species. Only maize, cucumber and potatoes produced guttation droplets up to emergence of inflorescence. The released amounts of fluids were comparable for maize and oilseed rape but considerably lower for sugar beets and barley.

Monocotyledonous crop species such as maize and cereals showed a higher guttation frequency than dicotyledonous crops such as sugar beets. While maize showed large guttation drops even under low relative air humidity, guttation drops in sugar beets were comparably smaller and only observed under very high humidity conditions. Other dicotyledonous crops such cruciferous species (oilseed rape) and potatoes showed a much higher guttation frequency than sugar beets. Even under climatic conditions suitable for guttation, guttation could only be observed on 50 % of the preselected observation days for crop species that showed frequent guttation events in the glasshouse and for the adjacent vegetation. Thus it is not possible to predict the occurrence of guttation on climatic conditions like temperature, relative air humidity (>90%) or soil humidity, yet.

Poster

Review of higher tier methods for assessment of the risk of pesticides to honey bees

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Risk assessment procedures have been developed for domesticated honeybees (*Apis mellifera*) potentially exposed to residues of insecticides. After initial tier I screening risk assessments further studies may be conducted for those substances for which a potential risk cannot be excluded. Higher tier studies are often performed in cages or tunnels containing colonies of honey bees which can forage on treated crops. Cage tests are typically smaller in size than tunnels but allow for the testing of more treatments often with more replications than can be offered by the larger scale tunnel tests. Field tests can be conducted to establish the effects of the product under more realistic conditions but due to use of free flying colonies there is less control than in the tunnel and cage studies. Finally, post-registration monitoring can offer additional information on specified uses of the product under commercial conditions and can be used to give feedback on the outcome of the risk assessment and the effectiveness of any risk management practices put in place to protect bees.

This paper will review the various higher tier methods with respect to their ability to accurately predict the potential effects of pesticides to honey bees and how they can be employed in a robust risk assessment. The range of parameters investigated typically include; mortality, foraging, behavior, brood and colony development. The ability of higher tier studies to predict potential adverse effects will be evaluated and how these parameters can be employed in a risk assessment with clear decision making linked to honey bee and pollinator protection goals.

Poster

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

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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.

Poster

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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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.

Poster

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

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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.

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

Improvement in the calculation of indices in brood tests

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Over the laboratory conditions brood tests are managed according to the OECD document n°75 "GUIDANCE DOCUMENT ON THE HONEY BEE (*Apis mellifera L.*) 'Brood test under semi-field conditions'.
Data provide the brood evolution of growth stages over 22 days on at least 100 marked cells.
In the evaluation the test results allow calculations such as the brood termination rate, the brood index and the compensation index. However when managed in field conditions and under semi-field conditions results can differ because of the colony behavior or 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 that are usual in beekeeping practice but in discordance with the indices calculation, mainly under semi-field conditions.
Hopefully the OECD document n°75 precise "Specific statistical analysis…are still under development".

The queen laying eggs is supposed to evolve in larvae then pupae and honey bee emerge in a precise temporality. For the evaluation of the different brood stages of single marked cells, the recorded growth stages are transferred into values counting from 0 to 5.

The timing refers to the common theory case and calculations do not allow using results in deviation to this theory. If a cell does not contain the expected brood stage during BFD+5 to BFD+16 the cell has to be counted 0 at the assessment day and also on the following days. Most of the time when the brood development is abnormal this justification fits the elimination of a cell in 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 achieved. Apiarist observations confirm that honeybees take liberty with the theoretical framework.
Adapted formulas allow considering this normal brood development when conditions are required.
In order to increase the data significance it is reasonable to use adapted formulas from an apiarist point of view.

Scanning & analysing individual bee behaviour using RFID

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At the Floriade horticultural world expo Venlo NL (5th April -7th October 2012) a complete system will be active. The system will consist of:

1. **RFID reader**; covering the flight entrance of a colony in a standard hive (25 cm wide). Each passing tagged bee is detected; it's ID, day, time is stored in the database and shown via the web-interface.

2. **ApiScan** covering the same entrance width; giving in and out flight-activity on an individual basis.

3. **Camera** allowing a permanent remote observation and registration of behaviour at the flight entrance.

4. **Weather station** registering air temp., air humidity, wind velocety and direction, rain.

All these data will be shown in real-time together on a web interface (46" monitor).

The equipment and the data storage allows an in depth analysis of the flight behaviour of the worker bees during the measuring period or the lifespan of a worker bee.
Changes in behaviour eventually due to the use of 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 which is as part of a whole normal sized colony (up to 50.000 individuals).

Comparison of EPPO and French field methodology

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The French working group about bee trial guidelines worked during 2 years under official supervision on the redaction of a methodology adapted to field studies with honeybees. This working group composed of members from research, industry, beekeeping and service society presented the issue in March 2011 to the Ministry of Agriculture. This field methodology became an official guideline under CEB recognition, compliant with the EPPO guideline.

However field studies were not used in France for a long time because of poor realistic conditions, this kind of a test became interesting in post homologation of plant protection products. The working group attaches to look at simple parameters in order to manage field studies with honeybees that could be used in a European way of registration.

The objective of this method is to assess the effect of foliar application of phyto-pharmaceutical products on honeybee colonies. Main characteristics are:
- General conditions of agriculture with sufficient surfaces for the foraging activity
- Strong bee colonies such as professional use. No experimental stress, on surfaces dedicated to a normal bee activity

By extension these studies allow to realize mid-term observations and quantitative samples of different matrices for residue analysis (worker honeybees, brood, flowers, honey and pollen).

In comparison with EPPO guideline n°170 about field studies, some differences are significant:
- Surfaces of at least 2 ha per modality
- 2 colonies among 6 are equipped with pollen catch traps
- Applications are realized during flowering and after bee flight. Other modalities (before flowering, during bee flight…) can be added to the schedule.
- Mortality and foraging are observed on similar timing
- Apiarist visits cover at least a brood cycle

This CEB guideline would like to bring an improvement to the EPPO guideline considering more realistic conditions that could be useful for the validation of field trials with honeybees.

Introduction to the BEEBOOK, a manual of honeybee research methods

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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.

Digital image analysis as a tool to improve the assessment and evaluation of brood development in higher tier honeybee studies

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The potential impact of plant protection products on honeybee brood development is of increasing concern. Therefore, regulatory authorities request studies monitoring the potential adverse effects on honeybee brood development. 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 the brood development in honeybee combs will be presented. It is possible to systematically evaluate brood development on the basis of high definition pictures of brood frames taken during semi-field or field honeybees trials with this tool. The developed 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 described method and software has been designed and compiled with the intention to provide a tool for a 100% traceable analysis of bee brood studies which is gaplessly and systematically documented. This is of utmost importance when working under Good Laboratory Conditions (GLP). The method minimizes the impact on the 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 (acetate-sheet technique). As a result, data reliability and quality have been significantly improved.

Keywords: *Apis mellifera*, method, honeybee brood, risk assessment, acetate-sheet technique, digital evaluation, image analysis, OECD guidance document 75, OEPP/EPPO guideline No. 170

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

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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.

Role of food quality in bee susceptibility to fipronil and clothianidin

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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 evidenced 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. Moreover, 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 other 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 pollens. 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 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.

To conclude, 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 evidenced. 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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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. Spinosad controls many caterpillar pests in vines, pome fruit and vegetables, thrips, particularly in tomatoes, peppers, strawberries, peach trees plus ornamental cultivation, and dipterous leafminers in vegetables and ornamentals.

This review will summarise the impact of spinosad on bees with regard to effects on mortality and foraging, from a range of studies conducted in semi-field studies (cages and tunnels) over the past 10 years in France, UK, Germany, USA and Japan. Effects observed were standardised by comparing the level of mortality and foraging in control replicates with those of the treatment to allow for a comparison between trials. Findings indicated that although applications to flowering crops during bee flight can cause an intermediate level of mortality, when applied out of bee flight, limited and acceptable levels of effect of spinosad were noted.

Further more detailed studies have been conducted to investigate the potential effects on brood and longer term colony survival using nucleus hives. The findings from these studies clearly demonstrate that the use of spinosad at 76 and 96 g a.s./ha applied out of bee flight has no unacceptable adverse effects on honey bee mortality, foraging, behaviour or longer term colony survival and development.

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

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One recently used methodology to investigate the bee brood development under realistic exposure conditions are semi-field studies according to Schur *et al.* (2003) (Bulletin of Insectology 56(1), 1-7) superseded by the OECD Guidance Document No. 75 (Guidance Document on the honey bee (*Apis mellifera* L.) brood test under semi-field conditions). In the course of the last few years it became obvious that the brood termination rate (= mortality of bee brood in marked cells on combs) was subject to a certain degree of variation, e.g. resulting in replicates with increased rates in the control and reduced rates in the reference item group. As variability complicates the interpretation of results regarding potential brood effects of the test items, in a first step a consortium of German and Swiss testing facilities discussed potential improvements (e.g. timing of the experiment, crop area, size and composition of bee colonies, digital comb vs. acetate sheet assessment of brood cells) of the method in spring 2011 (Pistorius 2011: AG Bienenschutz: Protocol of the meeting at Julius Kuehn Institute (JKI) on February 23th, 2011; Becker & Lückmann 2011: Variability of brood termination rate in honeybee brood studies under semi-field conditions. 58. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung e.V., Berlin-Dahlem 29. - 31.3.2011). The effectiveness of some of these factors were investigated in 2011 and first improvements in the experimental procedure were identified which may result in a proposal for an addendum of the existing OECD Guidance Document.

Poster

Statistical evaluation of regulatory honeybee trials - a pragmatic approach

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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). 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) constituted a working group with the remit to develop a recommendation how to analyse data of regulatory honey bee trials statistically.

Whereas the choice of statistical parameters is better established in standard laboratory trials (OECD 213/214), the appropriate evaluation of the data for semi-field and field tests is more challenging. The aim of this working group is to provide recommendations for experimenters and practical guidance for the assessment of more complex honey bee data. The present poster will show a scheme how the data of honey bee trials can be statistically analysed as one possible option.

Depending on the data base, dose response relations (LDx) can be calculated for standard laboratory tests (OECD 213/214) via *e.g.* probit analysis.
During higher tier bee testing, comprehensive data on mortality, foraging activity or generic brood assessment are recorded that can be evaluated at several levels.
<u>Pre-treatment level:</u> 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 or multiple t-test, two-sided).
<u>Post-treatment level:</u> effects of the treatment can be analyzed 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).

However, it should be generally acknowledged that statistical evaluation, in particular for field trials, is sometimes limited and thus not fully adequate. Hence these limits should be recognized and interpretation should also 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 assessing the brood development (according to OECD GD 75) or the combined data of field trials. Also, new approaches like evaluating the time dependency of data or the use of a conceptual model will be considered.

Poster

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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Foraging bees perform daily in average 10 trips, 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 quite much 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 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 must be necessarily foragers, while receivers are younger bees. Finally, the mortalities of donors and autonomous 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 (lower) fed with the two active ingredients.

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

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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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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 clodithianin 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 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 clodithinidin 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.

Quantitation of neonicotinoid insecticide residues in experimental poisoned honey bees

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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 and their analysis. In former times the determination of the Subsequent Residue Level (SRL) was proposed considering these losses.

Since honey bee mortalities due to neonicotinoid insecticides were recently reported, it seemed appropriate to verify if also for this class of active ingredients the phenomena were similar.

Thiametoxam, clothianidin, imidacloprid were tested in the laboratory on *Apis mellifera ligustica* to research the presence and quantity of the poisoning a.i. used.

Oral and indirect contact trials were carried out for each pesticide, using commercial formulations, dispersed in sugar syrup and water, respectively, at the highest dose level reported on the label. The three a.i. caused a higher mortality than the untreated controls and were therefore tested at decreasing concentrations until the mortality was statistically insignificant in comparison with that of the control; the acute oral LD_{50} - and the related HQ - and the acute indirect contact LC_{50} were calculated for them.

Honey bees that died during the trials were stored at -18 °C and analyzed through a LC-MS/MS analytical procedure adapted from A.O.A.C. methods; the quantity of residues of insecticides were detected. These quantities resulted much lower than the administered ones.
In particular, considering oral toxicity tests, the quantity found for clothianidin was from 8.15 to 0.90% of the administered doses. For imidacloprid it was from 2.86 to 0.71% and for thiametoxam from 57.14 to 2.28%.

Honey bees that did not die within six hours from the trial start were also analyzed and the quantity of residues of insecticides detected; these quantities resulted much lower than those found in the dead honey bees.

On the basis of these results, it is opportune that the determination of SRL is required during the normal procedures of authorization for the use of pesticides.

Project 'APENET: monitoring and research in apiculture'

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'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: 1) to give explanation about the colony losses and high bee mortalities reported in the recent years in many countries; 2) 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.

Poster

Dust drift during sowing of maize - effects on honey bees

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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.
Two large-scale drift experiments (2010: Wendhausen; 2011: Lucklum) were conducted during the sowing of maize seeds treated with clothianidin (Heubach - Value 2010: 0,86 g / 100000 seed with 10,6 % clothianidin in dust; Heubach - Value 2011: 0,45 g / 100000 seed with 19,2 % clothianidin in dust).
In both drift experiments two different approaches were used: 2010 the experimental area (flowering oil seed rape) was in the middle of two areas reserved for maize drilling depending on wind direction, in contrast 2011, the drill area was surrounded by two areas with flowering oil seed rape. The maize was sown by a pneumatic vacuum operated precision air planter with at least 90 % drift reduction due to a deflector.
In 2010, on both sides directly along the edge of the oil seed rape (distances to the drilling area: 0 and 90 m) 4 hives for the field exposure as well as three gauze-covered tunnel tents (16 x 6 m) with bee hives for the semi-field experiment were exposed, with the side exposed in opposite to the wind direction used as control. Before sowing the bee hives in the tents were closed and the gauzes from the tunnels at the distance of 0 m to the drilling area were removed. Immediately after sowing, which lasted about 1 h, the tunnels were covered again and the hives reopened.
The bee hives in the field trial were left open during the drilling process, so that they were continuously exposed to the contaminated dust. Besides the outdoor bee hives directly bordering the exposed area, other hives were exposed in about 90 and 800 m distance from the exposed oil seed rape.
In 2011 a similar approach was used with tunnels located in oil seed rape in wind direction and opposite to this and outdoor bee hives in distances of 0, 50 and 500 m to the exposed oil seed rape. Here before sowing the gauze from all tents was removed.
The contamination of adjacent flowering oil seed rape 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. Dead bees were documented, collected, frozen and analyzed for residues. In both trials bee mortality was clearly increased. Though the experiments show 'worst – case' scenarios, further improvement in terms of seed dressing quality in maize or in drilling technology is needed.

Acknowledgement: The work was financed through the Diabrotica research program funded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV).

Keywords: honeybee (*Apis mellifera* L.), bee poisoning, clothianidin, dust abrasion, dust drift

Poster

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

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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 in 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 bee hives 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 bee hives 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.

Acknowledgement: The work was financed through the *Diabrotica* research program funded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV).

Keywords: honeybee (*Apis mellifera* L.), bee poisoning, clothianidin, dust abrasion, dust drift

Poster

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

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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.

Brazilian bee loss

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The neotropical region which Brazil belong, have a great and rich diversity of natives bees, reaching a total of 3.000 species including the allochtone genre *Apis* that by natural crossing among European and African races, originated a hybrid called Africanized honeybee. Moreover, beekeeping enjoys a spectacular moment with good production mainly of honey and propolis from *Apis mellifera*, as well as the recognition of the world as a great potential country. On the other hand, comparing with countries of the north hemisphere where several reports showed that the vanishing of honeybee was associated with diseases caused by *Varroa*, *Nosema* and virus or pesticides, the Brazilian bees loss remains a question! In this sense and especially for *A. mellifera*, we can suggest different points of the Brazilian situation that are directly linked and can modulate the honeybee population. Given the extension of the territory and a rich vegetal fauna, it might be considered that all food resource and nidification sites exist for the correct development of bees.

However, we verified that the annual bee losses in southeast can reach 20-30%, which is mainly due the genetic mechanisms of swarming or abandon. Many times the major factor of abandon is the lack of food, than may be viewed mistakenly by the untrained beekeeper as death of hive caused by diseases or pesticides. Although in Brazil diseases does not represent an important problem for Africanized honeybee, some cases of presence of *Nosema ceranae* and *Varroa desctructor* led the specialists to precaution and monitoring the colonies. Despite this, the Brazilian beekeeping is managed without use of any acaricide or antibiotic, thus obtaining contaminant-free products.

In relation to the pesticides, we have a particular edaphoclimatic condition that might affect differently the risk of exposure of xenobiotics to bee. For example, comparing between Brazil and Europe the dynamics of carbamate pesticides in soil, it was found that in our condition it is necessary to obtain ten-fold more times of the metabolites sulfone and sulfoxide, both more soluble and toxic than it precursor.

Comparing the pesticides consumption, currently Brazil became the world leader followed by USA, being the total spending was 44.9% with herbicide, 28.5% insecticide and 22.1% fungicide. For other way, even with this consumption Brazil still belongs to the group that uses a 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, also are those where the use of pesticides is needed for the conventional breeding.

Thus, the Brazilian bee losses are still a query! What is really happening: pesticides? toxic plants? diseases? genetic improvement? beekeeping management? starvation? or interactions among them? Therefore, the local group on ecotoxicological assessment has done efforts to increase the knowledge on the pesticides hazard to bees (*Apis* and non-*Apis*) beyond protect them.

Key-words: Brazilian bees, losses, diseases, pesticides, management, protection.

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Bees require protection for sustainable horticultural production in Kenya

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Horticulture is currently the third most important sector in Kenya after tea and tourism. Horticultural production has been on the rise with export market accounting for less than 10% of the production while the rest ends in the domestic market.

However, horticulture leads in the consumption of pesticides owing to wide range of pest problems. Some farmers reportedly use more than 10 spray application of insecticides within a season. Existence of strong regulation from the export market through good agricultural practice standards has ensured reduced use of pesticides that are less toxic. Production for domestic market remains uncontrolled. Farmers reportedly use high toxic pest control products because these are less expensive.

Most horticultural flowering crops require to be pollinated for fruit and seed set. Both honey bees (which are different races from the European honey bees) and non-*Apis* bees are important for wide range of crops. Interestingly, most farmers do not manage pollinators and do not comprehend their role in production. Thus their crop management system does consider pollinators.

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. In addition, the size of the greenhouses are small for honey bees foraging needs.

Bees will be protected only if farmers understand their value. Currently, only honey bees are known by farmers but only as provider of honey. This bee, similar to others, is not protected in crop production since its flower visit is not related to crop yields by farmers. Preferred protection would ensure bees are utilized in crop production and their presence in the agro-ecosystems is assured. Already there are steps being undertaken towards ensuring sustainable use of bees in agriculture.

Side-effect of acetamiprid in adult Africanized honeybee

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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 LD_{50} of fipronil for bees *Melipona scutellaris*

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Pollination is one of the most important environmental services and the bees are major pollinators, responsible for pollinating more than 70% of angiosperms and about a third of agricultural crops. Bees are pollinators that occur naturally in agroecosystems and susceptible to pesticides used in these environments. They are mainly exposed to insecticides, wich can intoxicate them by contact or ingestion that depending on the dose and exposure time, can cause death. There are over 30.000 species of bees in the world and it is believed that the number of species in Brazil could reach five thousand. Among the species already described can highlight Meliponinae family, popularly known as stingless bees. Bees of eusocial habits and with more than 400 species, of which about 100 are in danger of extinction occur in most Neotropical America with Brazil being one of the main places of occurrence of these bees, with more than 200 species of stingless bees, essential pollinators of Brazilian native plants. The genus *Melipona* has more than 50 species of bees, many with potential for pollination in greenhouses. The species *Melipona scutellaris* (Latreille, 1811) is one of the most important due to its easy domestication and the high productivity of honey. It is endemic to Brazilian northeastern and in size similar to the honey bee.
The insecticide fipronil is used in about 70 countries and widely used in Brazil. It is a broad-spectrum systemic insecticide that acts on the nervous system of the insect acting as a noncompetitive blocker on the gamma-aminobutyric acid (GABA) receptor, inhibiting the opening of chloride channels causing hyperarousal, paralysis and death. The aim of the study was to determine the topical LD_{50} of the insecticide fipronil for foragers of *M. scutellaris* under laboratory conditions. Three colonies of the CEIS/UNESP meliponary were used, each colony is considered one repetition. Toxicity tests were performed according to OECD, 1998. Bees were collected at the exit of the hive, packed in disposable plastic cages of 250 mL and kept in chamber B.O.D. at 29ºC±1ºC and relative humidity of the 70%±5%. Each treatment group consisted of three cages with ten bees each. According to the LD_{50} of fipronil established by others authors for others species of bees, the doses used in this bioassay were 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0 ng of fipronil solved in acetone. Bees were anesthetized with CO_2 and 1.0 µL of solution was applied with an automatic micropipette repetitive on the pronotum of each bee. Sucrose solution (50% invert sugar, 50% water) was offered as food for all groups during the course of the experiment. Mortality was assessed every 24 hours for 72 hours.
The result shows that fipronil is highly toxic to foragers of *M. scutellaris*, with a LD_{50} for 48 hours from 0.41ng/bee. Comparing these results with others authors the *M. scutellaris* bees seem to be more sensitive to fipronil than Africanized *A. mellifera*, *A. mellifera*, *Nomia melanderi* and *Scaptotrigona postica*. Considering the average weight of foragers of *M. scutellaris* (0.1g) and transforming this data into LD_{50} ng/g we have had a LD_{50} for 48 hours of 4.1ng/g. This result will be used to evaluate the behavioral changes that sublethal doses of insecticide fipronil cause to foragers of *M. scutellaris*, in order to better understand what are the effects of this insecticide for these bees in the environment.

Is the European honeybee (*Apis mellifera mellifera*) a good representative for other pollinator species?

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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 an 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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Aspects of determining pesticide risks to wild bees – implications for risk mitigation and risk assessment

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To be able to carry out an appropriate risk assessment of pesticides to wild bees, or to non-*Apis* managed bees, information is needed on the toxicity of the pesticide to the bee as well as the probability and magnitude of exposure of the bee to that pesticide.

Pesticide toxicity data have been mainly generated for European honey bees (*Apis mellifera mellifera*), but much less so for other *Apis* species and non-*Apis* bees (both wild and managed). However, increasingly toxicity tests are being conducted with non-*Apis* species, although not all of these have found their way into the international published literature.

The probability and degree of exposure to pesticides depends on cropping and pesticide application practices, certain pesticide properties, attractiveness of the crop to bees, and 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.

A preliminary assessment has been carried out, based on inputs from several countries, to determine availability of data on the above mentioned aspects for specific pollinator–crop combinations. This evaluation indicated considerable data gaps, in particular with respect to aspects of bee biology and behaviour that may influence pesticide exposure.

Results of the evaluation were also used to assess the potential constraints, effectiveness and feasibility of specific risk mitigation measures aimed at non-*Apis* bees.

The availability of data on aspects determining pesticide risks to wild bees, on the one hand, and the feasibility of risk mitigation measures, on the other, may have important implications for the development of pesticide risk assessment methods for wild bees.

Poster

Effect of agrochemicals in the pattern of visitation of *Apis mellifera* in *Cucumis melo*

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Lately pollinators of the main crops have presented decline with considerable economic impacts. Among the probable causes of this problem is the inadequate use of pesticides, mainly in monocultures. The impact of pesticides on pollinators, in special bees, besides the lethal effects, easily noticeable, can cause more subtle changes that culminate with the rupture of labor division and the social exclusion of contaminated bees, even bringing severe damages for the colony. The culture of melon (*Cucumis melo* L. – Cucurbitaceae) is dependent on pollination services done by honey bees (*Apis mellifera* L.). However, there is no care in relation to agrochemicals application concerning the visitation of these bees in the cultivated areas in the irrigation Pole of Petrolina-Juazeiro, located at Brazilian Northeast. Therefore, the objective of this study was to evaluate the effect of pulverization of agrochemicals at the pattern of visitation of *A. mellifera* at the flowers of melon.

This study was carried out in a commercial area, at the irrigate perimeter of Mandacaru, in Juazeiro, state of Bahia (09º24'S 40º26'W), in July 2010 (dry season) and March 2011 (wet season). For the observations it was used a melon cultivar of yellow type (10/00) in conventional system, with drop irrigation, without the introduction of honey bee hives. For the registration of honeybees visiting the flowers, simultaneous observations were done in recently open hermaphrodite and male flowers. The chronogram of pesticides application was different in the different seasons, following climatic and phytosanitary needs. At the dry season 10 flowers were observed (n= 5 male and n=5 hermaphrodite), and at the wet season, 32 flowers (n=16 male e n=16 hermaphrodite). The frequency and the behavior of the visitors were observed continuously, in the period of 5 a.m. to 6 p.m. The average number of visits was calculated by hour and kind of flower.

In the dry season the visits occurred since 6 a.m., at both flower types, with amplitudes of 1.5-17.65, for the hermaphrodite flowers, and 1.6- 12.8, for the male flowers. In the wet season, in general there was a negative effect of the application of agrochemicals in the frequency of visitation of *A. mellifera*, after the 1st, 2nd and 5th day of pulverization. This effect was more intense in the 1st day, when there were registered average values smaller than one visit, independently of the flower type and hour. Moreover, the first visits were observed only from 8 a.m. At the 2nd day the visits were registered from 7 a.m., being the frequency of visitation larger in both flower types (0.25-7.00, hermaphrodite flowers, and 0.25-3.00, male flowers).

At the 5th day, there was no difference in relation to the time of the first visits, but the frequencies were larger both in hermaphrodite (1.00-12.67) and male (1.00- 9.33) flowers. Comparing both seasons, we found the frequency of visitation observed in wet season was lower than that found in the dry season indicating that the productivity of the melon in the first period can be damaged by the excessive application of agrochemicals, since there will be a reduction in the pollination services.

Poster

Assessment of lethal and sublethal effects by spinetoram on *Bombus terrestris*

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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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Poster

Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers

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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.

Poster

Detection of viral replication in bees

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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.

Poster

Pesticide sprays compromise pollination and biocontrol services on strawberry

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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.

Poster

Laboratory miniature dispenser-bioassay to evaluate the compatibility of powder compounds in the entomovectoring with *Bombus terrestris*

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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.

Mommaerts, V. & Smagghe, G. (2011). Entomovectoring in plant protection. Arthropod-Plant Interactions, in press. pp. 1-15. doi:10.1007/s11829-011-9123-x

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