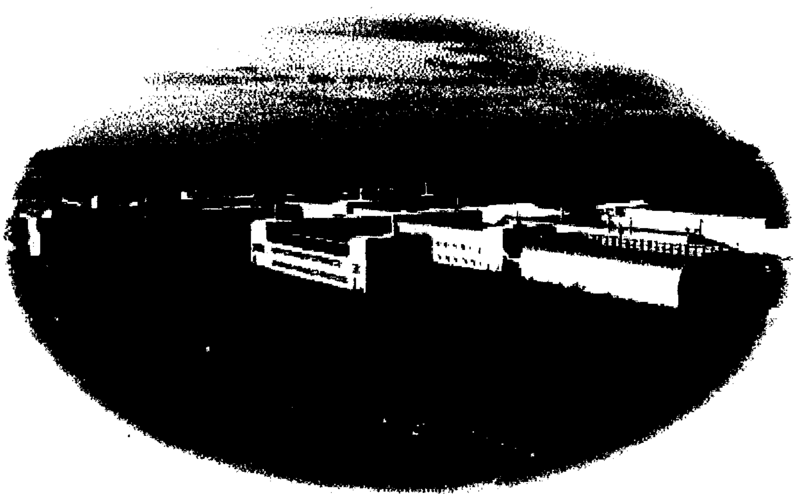
INTERNATIONAL COMMISSION FOR PLANT-BEE RELATIONSHIPS

Bee Protection Group

9th International Symposium

HAZARDS OF PESTICIDES TO BEES



October 12-14, 2005
York, UK

PROGRAMME AND ABSTRACTS

The organisers would like to thank the sponsors of this event:

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Scientific Committee

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Dr Helen Thompson (CSL)
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Coaches will pick up from the Novotel front entrance at 0830 followed by York Art Gallery each morning; the coaches will return to the Art Gallery and Novotel at 18:00 on the 12th , to the Castle Museum at 14:00 on the 13th and to York railway station at 16:30 on the 14th.

Wednesday 12 October
0930-0945 Welcome John Stevenson Chairman ICPBR Bee Protection Group
0945-1015 Introduction Prof Tony Hardy, Research Director CSL and Chair EFSA PPR Panel

1015-1230 (incl coffee break)

1. Bee health issues – country specific experiences including *Varroa* and varroacides (90 min)
    a. An introduction to the Bee Health working group (IAHAC) Gavin Lewis and Richard Rogers
    b. IAHAC Report on the status of Bee Health: Assessing the factors, Richard Rogers et al
    c. The health status of honey bee colonies in England and Wales Mike Brown and Selwyn Wilkins

1330 –1700 (incl tea break)

2. Honey bee poisoning incidents and monitoring schemes (180 mins)
    a. Survey on bee losses in Italy , Piotr Medrzycki et al
    b. Multifactorial prospective survey on bee troubles in France, M-P Chauzat
    c. A monitoring study confirming the safe use of STEWARD 30WDG (a.s. indoxacarb) for honeybees in flowering apple orchards Jozef van der steen, Axel Dinter
    d. Resume on the symposium on honeybee mortality in Germany during the winter of 2002/2003 Rolph Forster
    e. Incidents of honeybee poisoning with pesticides in the United Kingdom, 1994-2003.Libby Barnett et al
    f. Monitoring of bee colonies in Germany, Martina Wehling et al

1700-1800 Poster session

Thursday 13 October
0900-1030

3. Bumblebees and other bee species (90 mins)
    a. Effects of chitin synthesis inhibitors on growth and reproduction of Bombus terrestris Veerle Mommaerts et al
    b. Bioassay to test toxicity, reproductive and behavioural effects of insecticides against Bombus terrestris (Hymenoptera) Guido Sterk et al
    c. Bumble bee brood subgroup report – Jozef van der steen

1100-1300

4. Test methodology – laboratory, semi-field and field (120 min)
    a. A field method for testing toxicity of crop protection products to honey bee brood – Richard Rogers

b. A larval in vitro rearing method to assess effects of pesticides on honey bee brood P Aupinel et al
c. Learning of bees confined in a tunnel as an application to the evaluation of the toxicity of agropharmaceuticals Marc-Edouard Colin et al
d. Long term effects of IGRs on honeybee colonies Helen Thompson et al

1400 – excursion – Castle Museum, York
1900 Dinner –National Railway Museum, York (Station Hall, City entrance)

Friday 14 October
0900 - 1000

5. Regulatory review- honeybee risk assessment for pesticides in Europe (60 min)
    a. Hazards of insecticides to honeybees and bumble bees foraging on oilseed rape flowers, Reet Karise, Eneli Viik
    b. Risk assessment to bees – scheme developed in France for systemic compounds Anne Alix and Christine Vergnet

1030-1300
Regulatory subgroup
Sublethal effects- Christian Maus, Helen Thompson
Systemic scheme – Anne Alix, Ed Pilling
Brood effects – Jean-Noel Tasei, Roland Becker
Population effects – Chris Topping, Gavin Lewis

1400 –1600 General discussion

Posters:
Assessing the toxicity of adjuvants and compounds with a physical mode of action to bumble bees Inge Meurissen et al
Lethal and sublethal effects of neonicotinoids on worker bumble bees Sofie Reynders et al
Bumble bees can be used in combination with juvenile hormone analogues and ecdysone agonists at their MFRC Veerle Mommaerts et al
Foundation causing honey bee brood damage, Klaus Wallner,
Influence of sunflower on the development of bee colonies, Jean-Daniel Charrière et al
Effects of field margins management on pollen resources in different arable crops farms in France André Fougeroux and Hervé Giffard

1. BEE HEALTH ISSUES

An introduction to the Bee Health working group (IAHAC)

Gavin Lewis & Richard Rogers

Simpson House, Windsor Court, The last meeting of the Bee Protection Group, the 8th International Symposium "Hazards of Pesticides to Bees", was held in Bologna, Italy; 4-6 September 2002. One of the major issues that was discussed at this meeting related to the concerns over declining bee health in several EU countries (and also N. America). A considerable amount of work has been undertaken to try identify single factor causes e.g. pesticides, and much of this was presented during the symposium. The consensus view of the meeting was that no single factor cause had been shown to be responsible but that there was clearly a significant issue that needed to be addressed.

Accordingly, a working group, called the International Apis Health Assessment Committee (IAHAC), was set up "To define the nature of bee health problems, to identify all possible causes and critical factors, and to develop protocols for assessing bee health status.". This working group comprises members from France, Italy, Germany, UK, Canada and Argentina. It has already met on several occasions to establish the principles of the approach it believes should be adopted:

1. Information gathering: paper-based questionnaires, web-based surveys and the development of a bee health database.
2. Stress testing of honey bee colonies to measure the response of colonies to varying degrees and combinations of stressors.
3. Development of standardized protocols for investigations, so that results are comparable on a global scale.
4. Development of improved tools for bee health diagnosis e.g. diagnostic matrices, population models etc.

Most importantly, it has established a Website (www.apishealth.org), which is designed to be a global, open communication tool allowing interactive exchange of information.

IAHAC Report on the Status of Bee Health: Assessing the factors

Richard E.L. Rogers, Piotr Medrzycki, Claudio Porrini, Anna Gloria Sabatini, Teodoro Stadler
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In recent years, beekeepers in many countries around the world have been reporting increased losses of honey bee colonies. The cause of the losses was not clear in many cases and pesticides soon became the focus of blame. Even though there was little, or no evidence to link many of the massive bee losses to field relevant doses of advanced new crop protection compounds, the idea that minute sublethal doses were infiltrating and killing colonies gained popularity. Investigations of dead colonies did little to improve our understanding of what was happening. In response to claims that crop protection products were involved, corporations, governments and other stakeholders initiated many studies. At the 8th International Symposium on the Hazards of Pesticides to Bees, it was unanimously agreed that a clear statement about the true state of bee health was needed. It was thought that such a statement might help guide research in the most appropriate directions. A working committee of ICP-BR Bee Protection Group, known as the International Apis Health Assessment Committee (IAHAC), was mandated to review bee losses on an international scale and develop an opinion and statement to describe the status of bee health.
To achieve this goal, IAHAC developed a comprehensive list of factors that can affect bee health. Next, the committee identified that a process was needed to assess the impact of each factor and how these factors might interact. This is an enormous task for any small group to undertake because the work is very complex and there is no single cause that is common to all countries. Therefore, the work of IAHAC is not complete. Nevertheless, the committee has made significant progress toward developing guidelines and a universal process for the assessment of bee health. It is hoped that the tools and recommendations that are put forth by IAHAC will create a path to improving our understanding of what factors are negatively impacting honey bees worldwide. This report highlights:

1. the factors that can be involved
2. the tools and processes that are being developed
3. recommendations for how to proceed
4. a summary statement about the status of honey bee health

The health status of honey bee colonies in England and Wales

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There are an estimated 274,000 colonies of honey bees in the UK kept by about 44,000 beekeepers. Some 250,000 colonies are managed by 37,000 beekeepers in England and Wales. Around 200 beekeepers manage bees on a professional basis and are members of the Bee Farmers' Association; collectively they manage around 40,000 colonies. The remainder are small-scale producers, many of whom are members of national and local beekeeping associations. Bees make an essential contribution to agriculture and the environment through pollination: they also produce honey and wax. The honey bee (*Apis mellifera*) plays a dominant role, currently being the major managed pollinator available to provide this service. Recent estimates for agricultural and horticultural crops grown commercially in the UK that benefit from bee pollination are in the region of £200m p.a. The national Bee Health Programme is run by the National Bee Unit (part of the Central Science Laboratory, a Defra executive science agency), on behalf of core Defra policy customers (Defra Horticulture Division), and the Welsh Assembly Government. The programme is funded to safeguard the bee population due to its importance in the pollination of wild plants and commercial crops, and is underpinned by a programme of research and development to provide up to date technical support to beekeepers. The work includes disease and pest diagnosis, development of contingency plans for emerging threats, import risk analysis, related extension work and consultancy services to both government and industry.

Honeybees as well being affected by contaminants and pesticides can be affected by a large range of diseases, pests and parasites that are of importance for the health of colonies and also from the point of view of regulation and the movement of bees in trade around the world. Pests and diseases can cause high levels of colony losses creating a vacuum of available pollinators for important crops. Recent experience with a lack of bees available for almond pollination in California demonstrates this.

The development of generic techniques that enable you to test for a full range of pests and pathogens using single types of assay in a centralised facility will allow you to provide complete health status check for bee hives. This approach allows you to deliver very rapid, comprehensive and large scale testing services both direct to beekeepers and in the case of survey work to regulatory bodies. Work at CSL has focused on real-time PCR (TaqMan®) systems to provide high-throughput diagnostic services in the laboratory and on field test kits where the assays are carried out by non-specialists at the point of inspection, and these need to be simple and rapid but speed up the decision making process. Lateral flow devices (LFDs) incorporating antibody coated latex particles for foul brood disease have been developed to allow detection of pathogens in the field in a 3 minute, single step. The development of these techniques has made a significant impact in these areas for Plant Health, and is now being developed for the National Bee Unit and bee inspection services.

Data will be presented on bee disease incidence in England and Wales. Examples will be presented of both aspects of testing from the survey of the UK for the detection of Kashmir bee virus (KBV) using TaqMan real-time PCR to the detection of foul brood using the lateral flow devices.

2. HONEYBEE POISONING INCIDENTS AND MONITORING SCHEMES

Survey on bee losses in Italy

Piotr Medrzycki, Donato Tesoriero, Fabio Sgolastra, Roberto Colombo, Giorgia Serra, Simona Rossi, Milena Rancan, Anna Gloria Sabatini, Claudio Porrini.
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In Italy, high mortality of bees with subsequent 60-70% honey production decrease was recorded since 2000, always in the period March-July. A questionnaire survey carried out in 2002 showed that most damages to the hives occurred in the maize-dedicated areas during and after sowing. Rolling, high aggressiveness, disorientation and other abnormal behaviours were often observed in honey bees.

Following topics were studied in Italy in order to investigate on bee loss phenomena: dispersion of imidacloprid during sowing of pre-treated corn seeds; treatments against *Scaphoideus titanus* Ball, vector of Grapevine yellows; effects of microencapsulated pesticides on honey bees and effects of IGRs on queen larva development.

Imidacloprid dispersion during sowing was studied on five different commercial corn hybrid seeds. Paper filters, installed on the output of the drill fan, captured dispersed imidacloprid from each kind of Gaucho® dressed seed. Even the new adjuvant glue did not eliminate the loss of active ingredient, and the difficulty of washing the drill contaminated with imidacloprid is pointed out.

Grapevine yellows (Flavescence dorée) is an infective grape disease caused by a phytoplasm. Treatments against *S. titanus*, the vector of the phytoplasm, often cause high bee mortality. In 2002 and 2003 a monitoring study was conducted in various vineyard areas of the Emilia-Romagna Region. In 2002 fenitrothion was the only active ingredient found in the dead bee samples. In 2003 fenitrothion was found in 94.1% of the dead bees, followed by chlorpyrifos-ethyl in 17.6%, and dimethoate and methyl-parathion both in 11.8% of the samples. Surveys conducted by the Regional Plant Protection Service showed that winegrowers progressed from a large-scale use of microencapsulated pesticides in 2002 to a more diversified use of agrochemicals in 2003. The palynological analyses revealed the presence of grapevine pollen on dead bees, suggesting that chemical treatments were applied before grapevine bloom completely finished.

Experiments carried out in Italy by other research groups, showed that microencapsulated pesticides often don't cause damages on bees in the field, but the insecticide action takes place inside the hive. On the contrary, the corresponding EC pesticide may affect bees strongly and quickly, whereas there is no long-term damage to bee colonies due to toxic effects inside the bee hive. The microencapsulates developed with the newest technologies seem to cause toxic effects on bees, but with no long-term damage for bee colonies.

In the last years, many queen breeders of Emilia-Romagna Region, observed a dramatic presence of malformed queen bees (up to 30%) from first week of June to first week of July. The beekeepers suspect that the reason of this phenomenon are IGRs used mainly in peach orchards and vineyards. An *in vivo* experiment was projected in order to test, directly in the hive, the influence on queen larva development of four mainly used IGR insecticides: Alsystin® (Triflumuron), Match® (Lufenuron), Mimic® (Tebufenazide) and Cascade® (Flufenoxuron). Basing on the first results it seems that Mimic is the pesticide that, if assimilated in larval state, provokes bee malformations and mobility problems. Nevertheless, the low number of repetitions executable in field and the hypothesized drift phenomenon impose to repeat the tests in vitro as soon as a laboratory test procedure will be available.

Multifactorial prospective survey on bee troubles in France

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In 2002 a field survey has been initiated on French apiaries in order to monitor weakness of honey bee colonies (*Apis mellifera* L.).
Studied apiaries were evenly distributed in five counties located on continental France. In each county five apiaries were chosen to represent different environments. Five colonies were randomly selected in each apiary leading to a total of 125 studied honey bees colonies. During a three years (starting in automn 2002) colonies were visited four times per year : after wintering, before summer, during summer and before wintering. At each visit, adult and larval bee population was estimated as well as adult bee mortality and colony mortality. Any clinical symptom or abnormality was noted. Brood showing disease symptoms was sampled for further analyses. Different matrix samples were collected depending on the season: pollen from traps, adult bees, foundation wax or honey. Any medical treatment reported by the apiarist was also noticed.
Surveyed pathologies were acarapisosis, nosemosis, varroosis, American foolbrood, European foolbrood and chalkbrood. Brood and adult bees were also analysed for six virus during the first year of sampling. Studied virus were the Chronic Bee Paralysis Virus (CBPV), the Acute Bee Paraysis Virus (ABPV), the Deformed Wing Virus (DWV), the Sacbrood Bee Virus (SBV), the Kachmir Bee Virus (KBV) and the Black Queen Cell Virus (BQCV). Specific and multiresidue analyses were performed on foundation wax, adult bees, pollen loads and honey to research residue of 43 different molecules. Fipronil and metabolites together with imidaclopride and metabolites were searched in pollen and honey during the first year of sampling, and in adult bees during the second year of survey.
An environmental survey was also conducted on studied apiaries. Every agricultural parcels within a 1.5 km radius around the apiary were monitored. The type of crop cultivated on parcels (time of sowing, varieties) and chemicals treatments were recorded and mapped. These data will be correlated with results from palynological analysis of pollen from traps.

A monitoring study confirming the safe use of STEWARD 30WDG™ (a.s. indoxacarb) for honeybees in flowering apple orchards

Jozef J.M. van der Steen[1] & Axel Dinter[2]
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DuPont de Nemours (Deutschland) GmbH, DuPont Str. 1, 61352 Bad Homburg v.d.H., Germany[2]

In 2003 in the South East part of the Netherlands, honeybee mortality was recorded during the apple flowering period. As STEWARD 30WDG™ (30% a.s. indoxacarb) was applied for the first time, this mortality was linked to STEWARD. Based on general data of STEWARD application, on pesticide application and on mortality recordings in the particular orchards, this linkage could not be proven. In order to clarify the role of STEWARD, DuPont de Nemours has commissioned PPO Bijen to conduct a monitoring study.
In spring 2004 this monitoring field study was conducted. Spread over six Dutch regions 39 orchard sites were selected. Before flowering began, in each orchard two honeybee colonies were placed. The hives were provided with Münster dead bee traps to collect dead honeybees. The traps were emptied every 3 to 4 days pre- and post-treatment. During the flowering period, STEWARD was applied once. The amount STEWARD applied varied from 170 to 260 g per hectare. Spray applications were carried out by the fruit growers according to local practice. Besides STEWARD other plant protection products (PPP) approved in orchard crops and nutrients were applied. The application of STEWARD caused no effects on honeybee mortality. At one test site, STEWARD was mixed with several other PPP's plus nutrients. In this orchard a slight increase of acute mortality was recorded after application of the mixture. All honeybee colonies used in this study showed a normal development during the test. The average daily mortality before and after STEWARD application was 11 and 13 bees per colony (n = 52 colonies). In the control orchards the average mortality during the flowering period was 8 (n = 26).
The applications of 170 to 260 g STEWARD 30WDG™ per ha (equivalent to 51 - 78 g active substance, indoxacarb, per ha) did not cause acute lethal effects on honeybees.

Résumé on the Symposium on Honeybee Mortality in Germany During the Winter of 2002/2003

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From 31 March to 1 April 2004, a symposium was held at the BVL in Braunschweig on the subject of bee mortality in Germany in the winter of 2002/2003. During this particular winter, there were worrying losses of bee colonies in Germany. In scores of press publications, it was speculated that this was due to seed treatment products containing the active substance, imidacloprid.

The Federal Office of Consumer Protection and Food Safety (BVL) as the competent authority for the authorisation of plant protection products in Germany decided to examine this hypothesis in a scientific symposium. In addition to the authorities involved in the authorisation procedure for plant protection products, participants from universities, industry, professional beekeepers, bee scientists, representatives from the plant protection service of the federal states, apiculture associations (DIB, DBIB), nature and environmental associations (NABU), the German Farmers Association (DBV) and the organisation for organic food production (BÖLW) were also invited to this meeting. 50 persons in all took part in the event, during which 14 lectures on apiculture, plant protection products, bee diseases, forage supplies in the agrarian landscape and potential interactions were given.

As a result, it was found that due to all scientific findings, including the results from tests carried out during the authorisation procedure for plant protection products, the claimed plant protection product effects could not be traced back to bee mortality in the winter of 2002/2003.

In former times, there have also been very high winter losses of over 30 % on average at irregular intervals, locally or regionally even up to total losses. Moreover, in Germany, none of the bee samples submitted were tested positively for imidacloprid residues. In fact, in the specialist lectures, and the intensive discussions, the entire complex of stress factors that could influence the honeybee was given central significance. In addition to plant protection products, which, in light of the current knowledge, are not considered of prime importance with regards to bee mortality in 2002/2003, this includes in particular the influence of weather, forage supplies, selection and timing of the application of veterinary drugs for controlling varroa, the *Varroa destructor*, the diseases transmitted by it and other bee diseases caused by bacteria, fungi, viruses or microsporidia. Even incorrect colony management and not observing the principal rules of good bee-keeping practice could have led to drastic bee losses.

It remains to be investigated, to what extent additional factors of stress need to be introduced into the assessment and evaluation of the effects of plant protection products on honeybees (e.g., nutrition, veterinary drugs, diseases). In the end, only a long-term monitoring can provide data, which might facilitate a forecasting of a coincidence of several unfavourable factors, in order to avoid high honeybee mortality in the future.

Incidents of honeybee poisoning with pesticides in the United Kingdom, 1994-2003.

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For over twenty years, the UK Agriculture Departments have monitored the direct effects of pesticides on beneficial insects, mainly honeybees and bumblebees, as part of the Wildlife Incident Investigation Scheme (WIIS). The Central Science Laboratory contributes to WIIS by providing the required laboratory skills for the determination of bee diseases and the expert analytical experience necessary to determine low-level pesticide residues and interpret these results. The results from WIIS are part of the pesticide regulatory process co-ordinated by the Pesticides Safety Directorate and are published each year. Accumulated data for the last ten years of WIIS will be reviewed (1994-2003). The overall trend is that suspected poisoning incidents, reported by beekeepers and the general public, have declined from over 50 incidents per year, to less than 30 incidents per year. The number of these incidents that have been attributed to pesticide poisoning has also declined. This decline may be attributed to the successful consideration of the environmental effects of pesticides on bees during the approvals process for a product. It also provides some assurance that where pesticides are used in accordance with their statutory conditions of approval, including any restrictions in respect of bees, they do not pose an unacceptable risk to non-target species or the wider environment. Also, the evidence from WIIS may be used to amend these statutory conditions of use for a product, which may prevent the occurrence of further bee poisoning incidents. However, the source of the pesticide in bee poisoning incidents is often uncertain and the likely cause of these incidents and any trends over time will also be discussed.

Monitoring of bee colonies in Germany

Martina Wehling, Peter Rosenkranz, Werner von der Ohe
Arbeitsgemeinschaft der Institute für Bienenforschung

In autumn and winter 2002 /2003 there has been a tremendous loss of bee colonies in Germany. Beside Varroosis many other causes like other bee diseases, nutrition supply, weather as well as effects of pesticides have been discussed. A monitoring of bee colonies in Germany is the result of several common meetings with beekeeping associations, bee biologists, chemical industry and agricultural organisations.
Data of 150 apiaries and 1500 bee colonies will be captured by the bee institutes. A wide range of criterions in the subject fields' location of the apiaries, apicultural practice, development of bee colonies, residues of pesticides and diagnoses of bee diseases should be monitored and recorded several times in the next years.

3. BUMBLE BEES AND OTHER BEE SPECIES

Effects of chitin synthesis inhibitors on growth and reproduction of *Bombus terrestris*

Veerle Mommaerts,[a] Guido Sterk,[b] Kurt Put,[b] Kris Jans,[b] Jan Vermeulen,[b] Guy Smagghe[a,c]
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This research project examined the toxicity of a major class of insect growth regulators (IGRs) against survival, reproduction and larval growth of bumblebees *Bombus terrestris*. In total eight chitin synthesis inhibitors (CSIs) were tested: diflubenzuron, teflubenzuron, lufenuron, novaluron, flufenoxuron, flucycloxuron, buprofezin and cyromazin. The different IGRs that are important in the control of pest insects in greenhouses were tested via three different routes of exposure under laboratory conditions : dermal contact, and orally via the drinking sugar water and via pollen. The compounds were tested at their respective MFRC and also in dose-response assays to calculate LC_{50} values. In general, none of the CSIs showed acute worker toxicity. However, there was a dramatic reduction on brood production especially with treated pollen. Conspicuously egg fertility was reduced with a total inhibition with diflubenzuron and teflubenzuron. In addition to egg mortality, the worker bumblebees removed larvae from the nest, and in most cases these individuals were dead first-second instars. Under the binocular such larvae showed an abnormally formed cuticle leading to mechanical weakness and death. Overall our results suggest that CSIs are to be applied with caution in combination with bumblebees. The compatibility of each compound to be used in combination with *B. terrestris* is discussed in relation to calculated LC_{50} values, routes of uptake and effects.

Bioassay to test toxicity, reproductive and behavioural effects of insecticides against *Bombus terrestris* (Hymenoptera)

Guido Sterk,[a] Sofie Reynders,[b] Veerle Mommaerts,[b] Inge Maurissen,[b] Jana Boulet,[b] Xavier Cuvelier,[b] Kurt Put,[a] Kris Jans,[a] Jan Vermeulen,[a] Guy Smagghe[b,c]
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Bombus terrestris is one of the most prevalent bumblebee species in Western Europe and an excellent pollinator of e.g. sweet pepper and tomato in greenhouses and fruit trees. The current development of new families of pesticides and the awareness of the importance of pollinators for crop pollination have stimulated interest to evaluate potential effects of novel pesticides on bumblebees. In the present study we describe the development of a bioassay in which *B. terrestris* workers are treated via three different routes of worst case field scenario exposure: dermal contact, and orally via the drinking sugar water and/or via pollen. The design of our bioassay is realistic to practice and ease in use. After optimisation assays with different worker densities and pollen from different origins, per treatment we started 4 nests each with 5 workers and used commercial Mediterranean pollen. After dominancy, worker mortality and brood production were followed for 10 weeks. The workers were selected from colonies that are to be used for commercial pollination. In the different tests, the pesticides are tested at their respective maximum field recommended concentration (MFRC). Here, worker bee mortality and effects on brood production are scored as biological endpoints. Then a dose-response assay of the pesticides is to be executed to evaluate sublethal effects. Sublethal effects of pesticides may have significant impacts on the bees and pollination in addition to the more easily observable mortality, disrupting foraging and causing decreased pollination and bee reproduction. In this paper we will exemplify the different tests and their usefulness using representative pesticides. In conclusion, we believe that inclusion of the sublethal bioassays that we report here to detect detrimental effects of pesticides against pollinators help in the development of environmentally responsible pest management strategies.

4. TEST METHODOLOGY – LABORATORY, SEMI-FIELD AND FIELD

A field method for testing toxicity of crop protection products to honey bee brood

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There are various laboratory and semi-field methods for studying the toxicity of pesticides to adult honey bees and honey bee brood. Both approaches are necessary and provide valuable data. However, there is interest in knowing how the brood is affected under field conditions. Laboratory methods involve individual bees or small groups of bees in highly unnatural and stressful conditions. Semi-field methods attempt to be more field relevant by exposing adult bees to treatments by allowing them to forage on a treated crop under a screened tent. The limitations of these approaches and the relevance to field conditions have been questioned. The goal of the method reported here is to develop a system for administering accurate treatments and to monitor the effects on brood development while allowing the bees to function normally under field conditions.

Groups of honey bee nucleus colonies in two types of hives (i.e. single standard Langstroth super and half-frame observation hive) were fed various treatments in syrup provided in modified top feeders. The doses used were intentionally chosen to be similar to field relevant residues in an effort to address current questions about sub-lethal effects on brood. Brood development was monitored by mapping egg locations and following development until emergence. Two brood cycles were followed during the course of the study. The first brood cycle occurred during an intentional eight-day period of confinement where the front entrance and rear ventilation opening where closed off with cluster screens. After this period the bees were allowed to free-fly and the test was continued through to the end of a second brood cycle.

Based on the results of this study, it was determined that the nucleus colony in the larger hive type worked best in this case. Also, the method of feeding was simple and effective. Treatment results suggest that none of the field relevant doses prevented brood from developing normally in the absence of the confinement stressor. Although subjective, no behavioral changes were noted in adult bees. Foraging, defense of the colony, care of brood, and general adult bee behavior all appeared normal. However, confinement stress appeared to be responsible for a disease outbreak and there may be a link between confinement and one of the treatments. Numerous recommendations for using this method in future studies are provided.

A Larval *In Vitro* Rearing Method To Assess Effects Of Pesticides On Honey Bee Brood

Pierrick Aupinel, D. Fortini, B. Michaud, F. Marolleau, J.N. Tasei, J.F. Odoux
INRA, Unité expérimentale d'entomologie Le Magneraud BP 52, 17700, Surgeres, France

A new *in vitro* rearing method of honey bee larvae was devised to assess the effect of pesticides on brood. This method could be used in the risk assessment process since it meets with the following characteristics:

- low mortality rate when no treatment is applied,
- standardisation of the test,
- easiness of carrying out,
- sensitiveness to treatment,
- precise control of the ingested doses of diet and pesticide,
- control of larval mortality for each larval instar,
- control of pre pupae weight,
- measurement of adult emergence rate.

The method enables the study of 1/ lethal effects (calculation of LD50) 2/ sublethal effects (prepupal weight, duration of development, adult morphology and behaviour).The method can be used either to study acute effects by applying contaminated diet to one particular instar, or to investigate chronic effects by providing each day the larvae with the test insecticide . These objectives were reached after testing different rearing protocols and by improving the feeding method.

The use of our rearing method is illustrated with two insecticides:

- Dimethoate considered as a reference insecticide in toxicological tests on adults,
- Fenoxicarb which belongs to the IGR group.

Learning of bees confined in a tunnel as an application to the evaluation of the toxicity of agropharmaceuticals

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The methodology to evaluate the risk of agropharmaceuticals requires more realistic simulations of field conditions than those deduced from laboratory tests. Because the presence of sublethal doses may alter the behavior of foraging bees, we propose a protocol for evidencing various alterations in free–flying bees behavior confined in insect-proof tunnels. The confinement of small colonies allows (i) to observe and quantify the foraging activity of one colony without disturbance or competition due to foreign foragers, (ii) to control the pesticide exposure (dose or concentration, administration mode).
Bees are trained to a feeder placed in the tunnel at a distance of about ten meters from the hive (Moore *et al.*, 1989, Nigg *et al.*, 1991) at one defined time of the day (Beling, 1929). After learning, the foraging bees (Vandame *et al.*, 1995) or the feeder surfaces (Rieth and Levin, 1989) or the sucrose solution of the feeder (Cox and Wilson, 1984; Colin *et al.*, 2004) is contaminated with the pesticide. The observation criteria can be related to the general activity and/or the individual behavior if the foragers are marked,
(i) at the feeder itself (Rieth and Levin, 1989; Colin *et al.*, 2001; Colin *et al.*, 2004)
(ii) between the hive and the feeder (Vandame *et al.*, 1995)
(iii) at the hive entrance (Cox and Wilson, 1984)
(iv) inside the hive (Schricker, 1970, Cox and Wilson, 1984).
Clinical signs of intoxication such as trembling dance, leg rubbing, rotation, abdomen tucking, etc ... (see Cox and Wilson, 1984), have to be noticed if present.
Results from one colony are expressed in terms of evolution of the quantified criteria before and during the pesticide exposure of the bees. Afterwards, the colony evolutions of the experimental and control groups can be analysed and compared in shape and in value. The simplicity of the experiment allows the appropriate number of repetitions to determine the No Observable Effect Levels of pesticides for instance.

Long Term effects of IGRs on honeybee colonies

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It is well established that effects on invertebrates can occur through interference with the endocrine system. This study aimed to assess the effects of exposure to a juvenile hormone analogue, fenoxycarb, a chitin synthesis inhibitor, diflubenzuron, an ecdysteroid synthesis inhibitor, azadirachtin and an ecdysteroid analogue, tebufenozide which are used as insecticides on:

1. the long term development of the honeybee colony
2. viability of queens exposed immediately post-emergence
3. sperm production in drones

and to integrate the data into a honeybee population model to assess the effects of the timing and level of exposure on the impact in honeybee colonies

The study showed significant effects of some IGRs on the longer-term viability of honeybee colonies and on the ability of emerged queens to mate and lay eggs. Short-term effects on brood mortality may appear to recover within 4-6 weeks but there may also be longer-term sublethal effects on emerging adults, e.g. precocious foraging.

The model showed that even if only those bees reared within two weeks of the IGR being applied are subject to premature ageing, such relatively short-term persistence of IGRs might nevertheless significantly reduce the size of over-wintering colonies. Such effects may explain the delays in development of the bee population as occurred in fenoxycarb-treated colonies or dwindling and dying in late winter or early spring observed in the azadirachtin-treated colonies.

5. REGULATORY REVIEW- HONEYBEE RISK ASSESSMENT FOR PESTICIDES IN EUROPE

Hazards of insecticides to honey bees and bumble bees foraging on oilseed rape flowers

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Cruciferous oil-bearing crops are gaining importance in the entire world. Oilseed rape is extremely attractive to bees (Cook *et al.*, 2003) and gives high honey yield. The expansion of growing area of cruciferous plants has caused an increase in the abundance of pests. Exposure to organophosphate, carbamate, and pyrethroid insecticides has been associated with bee poisonings in food crops. The intoxication can occur through direct contact, exposure to residues, or spray contamination of nectar and pollen. Certain systemic organophosphates and carbamates also have the potential to contaminate nectar and pollen in low, sub-lethal doses (Gels *et al.*, 2002). We tested residual effects of a pyrethroid insecticide, alphacypermethrin, on the behaviour of honey bees and bumble bees foraging on oilseed rape fields.

Field experiments were carried out in 2003 and 2004 at the experimental field station of Estonian Agricultural University in Estonia. The purpose of the experiment was to compare the density of bees (Hymenoptera: Apidae) on the oilseed rape fields untreated and treated with alphacypermethrin. Two different oilseed rape fields were under observation. (i) The experimental design of the first field was a randomised block. A total of 12 plots of 10 m^2 (10 x 1 m) were established. The trial variants were: untreated, treated once (in early spring) and treated twice (in early spring and before flowering) with alphacypermethrin. Each treatment was conducted in four replications. (ii) The second field experiment consisted of two fields (each 2 ha), one untreated and the other treated with alphacypermethrin. Ten 10 m^2 (10 x 1 m) observation plots were measured on each field to count the number of bees. Flower number was counted on 1 m^2 from each plot.

The most important pollinators of spring oilseed rape were honeybees and bumble bees, collecting both pollen and nectar. The number of bees was higher on alphacypermethrin treated fields because the number of flowers was larger there. Optimal foraging theory says that foragers should distribute between different areas so that the average recourse amount per individual would become equal. We calculated the number of bees per 1000 flowers and got the same results: in spite of optimal foraging theory bees preferred treated fields. Foraging behaviour of bees is dependent on the food patch density: dense food patches attract them more than sparse patches. Because the fields treated with insecticides are not repellent to bees it could endanger honey bee colonies and natural bee species as well as the honey they produce.

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Risk assessment to bees - scheme developed in France for systemic compounds

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Directive 91/414/EC envisages that the systemic properties of active substances, if any, are taken into account in evaluating the risk posed by plant protection products to the environment. In particular, any systemic properties may confer to a substance the capacity to be consumed by terrestrial organisms even when applied to soil or as seed coating. Among others, honeybees may be exposed to substances via this route, which may pose problem when substances with low threshold for toxic effects are ingested through pollen or nectar. In addition, this poses the question of the exposure of the bees of the hive since contaminated plant products may be brought back to the hive.

In this context, the guidance documents in support of the risk assessment to non target organisms in the frame of Directive 91/414/EC (Sanco documents) do not provide detailed technical guidance on how to proceed to assess the risks posed by substances with systemic properties. An approach was then developed in France with the aim to assess specifically the risk posed by systemic substances to bees for National registration. This approach was developed by the French CST[1], and was then discussed and adopted by the National Commission d'Etude de la Toxicité[2].

The approach is based on the principle that for systemic substances, the behaviour in the growing plant has to be taken into account into the risk assessment. As a consequence, the contamination of pollen and nectar has to be informed. Then exposure estimates are calculated for the different categories of bees susceptible to be exposed. The models according to which the exposure estimates are calculated are based on the available data on bee diet found in the literature. Exposure estimates may be calculated for foraging bees (nectar foragers and pollen foragers), hive bees (nurses, wax-producing bees, brood attending bees and winter bees) and larvae (workers and drones). They are estimated as PEC and expressed as g or µg a.s./bee. These PEC may then be compared to toxicological endpoints (LD50 or NOEC) or to a PNEC, in which case the safety factor is included into the effect threshold calculation. Safety factor values are established on a case-by-case basis, deduced from the amount and quality of the ecotoxicological data. These calculation models have been published by Rorstrais *et al.* (2005)[3].

In the National risk assessment process, the above PEC/PNEC approach applies when the recommendations of the relevant guidance documents and directive do not propose any detailed guidance for the risk assessment, *i.e.* for systemic substances.

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[1] Comité Scientifique et Technique (Scientific and Technical Working group) in charge of a multi-factorial study of bee disorders. Among the firsts tasks of this group was the evaluation of the role of imidacloprid as a seed treatment in bee disorders.

[2] Commission d'étude de la toxicité, des produits anti parasitaires à usage agricole et des produits assimilés, des matières fertilisantes et des supports de culture, in charge of the risk assessment of plant protection products for agricultural use.

[3] Rortrais A., Arnold G., Halm M.P. and Touffet-Briens F., 2005. Apidologie, 36, 71-83.

POSTERS

Assessing the toxicity of adjuvants and compounds with a physical mode of action to bumblebees

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The objective of this study was to assess the potential risks by agrochemical compounds for crop pollination by bumblebees. *Bombus terrestris* is one of the most prevalent bumblebee species in Western Europe and an excellent pollinator of *e.g.* sweet pepper and tomato in greenhouses and fruit trees. We tested here in this study several adjuvants and oils, that are added as wetting agents to pesticide formulations to improve toxicity and/or they can be used on their own, and also some compounds that have a physical mode of action. The latter compounds possess an insecticide action by contact based on a detrimental activity against the insect cuticle, and can be used in the control of aphids, whitefly, thrips, cicades and spider mites. The 10 different compounds were treated in *B. terrestris* workers via three different routes of worst case field scenario exposure: dermal contact, and orally via the drinking sugar water and/or via pollen. The assessment consisted of 2 types of tests. In a first test, compounds were applied at their respective maximum field recommended concentration (MFRC). In second, different concentrations were prepared and tested for evaluating sublethal effects. In both tests, worker bee mortality and effects on brood production were scored as biological endpoints. The results of this study will be presented and discussed in relation to type of compound, route of exposure, impact of active ingredient and bumblebee coverage/uptake.

Lethal and sublethal effects of neonicotinoids on worker bumblebees

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The objective of this study was to assess the potential risks of different neonicotinoid insecticides against the bumblebee, *Bombus terrestris*, that is one of the most prevalent bumblebee species in Western Europe and an excellent pollinator of *e.g.* sweet pepper and tomato in greenhouses and fruit trees. This presentation describes the toxic effects of 4 neonicotinoids, imidacloprid, thiacloprid, acetamiprid and thiamethoxam, that are used commercially against pest Lepidoptera, Hemiptera (whiteflies, aphids), Thysanoptera. Most members of this new class of chloronicotinyls that work through the insect nACh receptor have systemic properties and good contact activity. In this work we assessed traditionally the toxicity that involved the determination of mortality in acute tests, and also examined the effects of ecologically relevant sublethal exposure on aspects of bumblebee behaviour. Sublethal effects of pesticides may have significant impacts on the bumblebees and pollination in addition to the more easily observable mortality, disrupting foraging and causing decreased pollination and bumblebee reproduction. The different compounds were administrated in the laboratory via three different routes of worst case field scenario exposure: dermal contact, and orally via the drinking sugar water and via pollen. The compounds were tested at their respective MFRC and also in dose-response assays to calculate LC_{50} values. The results of this study will be presented, and in general they revealed large differences in toxicity. The different approaches for a suitable toxicity test for bumblebees are discussed and compared. However, it would be necessary to conduct further work on the dose-response relations and the sublethal effects of different pesticides before concluding about the hazards of neonicotinoids on bees.

Bumblebees can be used in combination with juvenile hormone analogues and ecdysone agonists at their MFRC

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This study examined the lethal and sublethal effects of two classes of insect growth regulators (IGRs) on the beneficial insect *Bombus terrestris*. Three juvenile hormones analogues (JHAs) (pyriproxyfen, fenoxycarb and kinoprene) and two ecdysone agonists (methoxyfenozide and tebufenozide) were tested. The bumblebee workers were exposed to the insecticides via three different routes: topical application and orally via the drinking sugar water and the pollen. In the first series of experiments the IGRs were applied at their MFRC. These tests showed that the tested IGRs caused no acute toxicity on the workers, and any compound had an adverse effect on reproduction (production of males). In addition, larval development was followed in the treated nests compared with the controls. After application of the two ecdysone agonists and the JHA fenoxycarb no adverse effects were observed on larval development. However, in the nests where the workers were exposed to the JHAs pyriproxyfen and kinoprene higher numbers of dead larvae were removed. These larvae were third and fourth instars, implying a blockage before metamorphosis. In a second test a series of dilutions was made for kinoprene. The results of this experiment revealed that only the MFRC caused a toxic effect on the larval development. On the other hand, kinoprene at only low concentrations (0.0650 mg AI/l) had a stimulatory effect on brood production. It was intriguing that ovaries of such treated dominant workers were longer and contained more eggs than in the controls. Overall, the obtained results indicate that the tested IGRs are safe to be used in combination with *B. terrestris* in an IPM system.

Foundation causing honey bee brood damage

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A Polish professional beekeeper reported enormous brood losses after using beeswax foundation from a specialised dealer. He found masses of emerging worker bee dead in the cells or with their heads out of the cappings and lost many of his 120 colonies. In his opinion a plant protection pesticide accumulated in the basic beeswax, which was reworked to produce foundation could be the reason for this problem.

Searching for assistance, he contacted the National Beekeeping Institute in Hohenheim and sent some of the foundation he was using in the past. The wax was analysed in the residue laboratory in Hohenheim and a private institution, Labor Ceralyse in Celle/Germany specialised on beeswax control. Different groups of substances were analysed (varroacides, plant protection pesticides, pollutants) and the degree of adulteration with synthetic waxes was determined. In addition a field test with this foundation in experimental colonies in Hohenheim was initiated in 2005 to verify the reports from Poland.

Results of the laboratory studies:

colour/smell	inconspicuous	grinded, light yellow beeswax foundation
cleanness	dirty	not percolated
purity	adulterated with paraffine 50%	detection limit: 1%
wax components	increased value of benzoic acid und benzyl-benzoate	both substances are common in different levels in beeswax samples
varroacides	bromopropylate 0,5 ppm tau-fluvalinate 0,9 ppm	11 substances, including thymole and amitraz + metabolites (LOQ: 0,1 ppm)
waxmoth pesticide	not detectable	1,4-dichlorobenzene (LOQ: 0,05 ppm)
plant protection pesticides	not detectable	180 analysed substances (LOQ: 0,05-0,1 ppm)
pollutants	inconspicuous values	common in beeswax samples

Field experiment:

Several foundations were fixed into marked frames and offered to four experimental colonies. Foundations from the local market were used as control in the same hives. The bees accepted the Polish wax with slight delay and finished the combs faultlessly. The queens layed eggs rapidly and completely. The development of eggs to larvae was found to be normal. Capping was started as normal. The pupation and time of emergence was normal. The full developed adults had problems in opening the cappings and in leaving the cells. This was mainly due to the incapability to cut and open the cocoon. The bees were attached by the elastic remains of the cocoon between their head and thorax. There they died probably by hunger. Only few bees were able to emerge completely. A loss up to 90% was found. The dead bees were rapidly removed by other bees. Few appeared in front of the hives. The cells were inspected and corrected by the hive bees and soon new eggs could be found in the cells. Development and emergence in the control combs was undisturbed.

The development from egg to larvae and adult bee was found to be normal. Only the last step in the bees life, the emergence, was faulty. Several reasons could be discussed. Coordination problems or insufficient power in the mandibles of the young bees should be considered as well as an extreme tough and elastic cocoon due to the high paraffin adulteration, whereas this was never reported in the past.

Up to now, we were not able to identify the responsible substances. According to the present results, residues of varroacides, plant protection pesticides and pollutants seem not to be responsible for the observed problem. But results of analysis of more hydrophilic substances are still missing. The help of other laboratories would be very welcome.

Influence of sunflower on the development of bee colonies.

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Since a few years beekeepers complain about colony losses when the hives are placed close to fields of blooming sunflower. In different European countries, the beekeepers suspect that the problem is caused by insecticides (imidacloprid, fipronil) used for the seed dressing. Although these insecticides are not registered in Switzerland for this use, Swiss beekeepers also report bee colony losses due to sunflower. We carried out a two years trial, in order to find out if sunflower by itself is detrimental for bees and if it causes colony damages.
The colonies of one apiary were divided in two groups with comparable colony strength. During the flowering period of sunflower, we moved one group for three weeks to sunflower fields measuring at least 2 hectares. The other group had no access to sunflower (control). The colony strength, the bee mortality at the hive entrance, the foraging activity on sunflower and the harvest of pollen were determined in regular periods. Two trial repetitions per year in different regions of Switzerland were carried out.
We measured an intensive foraging activity on sunflower but only few of the collected pollens had sunflower origin (2003: 3-4%; 2004: 11-38%). No increase of colony weight during the 3 weeks flowering period was observed, which means that the bees did not collect sunflower nectar. Also, no detrimental effect of sunflower was seen as the mortality at the hive entrance and the colonies strength did not change during the flowering time and until the end of October. We also measured the wintering of bee colonies to assess an eventual delayed effect on the bee colonies. We observed no difference between the two trial groups. Our results show that there was no negative effect of sunflower on bee colony health. On the other side, we observed that during the two trial years the sunflower had no melliferous potential and represents only a minor pollen source.

Effects of field margins management on pollen resources in different arable crops farms in France

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This study is carried on 4 farms where arable crops are grown. During the first year, 6 beehives are implemented on each site in order to monitor pollen resources, available for bees during the growing season (from March to October). All beehives are equipped with pollen traps and pollen collection is determined twice a month.
Main results show the lack of pollen resource in summertime and collection by bees of poor quality pollen during this period. Based on first year results, field margin and set aside management are implemented in order to improve the pollen resources on each farm.
Through collection of pollen during the second and third year, effects of management are assessed in quantity and quality. Results and further improvements are discussed. Through this approach, recommendations to implement set aside for improvement of the development of bee colonies and other pollinators can be proposed to growers in agricultural areas.

9th International Symposium of Hazards of Pesticides to Bees

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