LES COLLOQUES

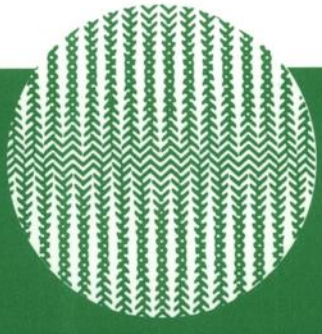
98

Hazards of pesticides to bees

Avignon (France)
September 07-09, 1999

L.P. BELZUNCES, C. PÉLISSIER
& G.B. LEWIS

Editors



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Avignon (France), September 07-09, 1999

7th International Symposium of the ICP-BR Bee Protection Group
co-organised by INRA and ACTA

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INTERNATIONAL COMMISSION FOR
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Foreword

I am delighted to learn of the success of the seventh Symposium of the ICP-BR Bee Protection Group and I congratulate everyone concerned with the organisation of a particularly important meeting and with the production of this excellent report.

We are most grateful to Institut National de la Recherche Agronomique (INRA) for financial help and for undertaking the organisation of the meeting. We also thank Association de Coordination Technique Agricole (ACTA) for their contribution to the organisation, the University of Avignon for the use of their facilities, and the following companies and organisations for generous support:

AgrEvo France	Novartis Agro SA
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The Bee Protection Group provides a forum where representatives of industry, National Regulatory Authorities and Government and University Research Departments come together to discuss the assessment of the hazards to bees of crop protection operations and to ensure that the farmer and the beekeeper can remain in harmony.

The Group has been working on the methodology for identifying and assessing these hazards since its first meeting in 1980, and it was a major achieivement that the final form of the EPPO:

Guideline for the efficacy evaluation of plant protection products
SIDE EFFECTS ON HONEYBEES

was agreed at the Symposium.

Ingrid H. Williams

Professor Ingrid H. Williams PhD
Chairman ICP-BR
October 1999

ICP-BR

The International Commission for Plant-Bee Relationships (ICP-BR) was founded in 1950 by the swiss scientist Anna MAURIZIO, whose outstanding work was mainly devoted to bees and their relationships with plants. Since 1980 this Commission - which is affiliated to the International Union of Biological Sciences (IUBS) - has regularly organised in Europe working sessions on the harmonisation of methods for testing the toxicity of pesticides to bees.

ICP-BR develops the scientific process preceding decisions from European administrative Authorities, EPPO (European and Mediterranean Organization for Plant Protection) and OECD (Organization for Economic Cooperation and Development). ICP-BR Bee Protection Group symposia are thus always expected with great interest since they represent the first step in the evolution of legislation concerning bee protection related to the use of plant protection products.

NOTE

The abstracts of the following communications have been published in *IOBC WPRS* Bulletin*, vol. 23, n° 3, 2000 (C. Pélissier & L.P. Belzunces, eds.)

*International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section.

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Preface

Over time, humans have developed a relationship of fear and respect with the honey bee. Although it stings, it offers valuable products that associate the image of the honey bee with sweetness and health. It is only recently that the role of the honey bee in pollination has been discovered and extended to other pollinating bees. Hence, this insect is beneficial not only for beekeepers but also for farmers, for whom it is a companion required to obtain beautiful and abundant fruits and vegetables. During the past twenty years, this insect has become a highly sensitive bio-indicator subject to different environmental pollutants, and the necessity to protect the honey bee emerged directly from agricultural, economic and environmental considerations. An efficient legislation was developed in many countries during the 80's, which resulted in international guidelines in the 90's, compiled in OECD and European directives. This legislation is primarily intended to protect the bees from pesticides as these products are intentionally spread in the environment to protect the cultures from pests and diseases. However, the dream of ecologists, beekeepers and farmers would be to protect the bees from all potent pollutants, a seemingly impossible dream at this time.

The beginning of bees' protection was somewhat difficult. The assessment of pesticide toxicity to bees was originally based on the determination of the median lethal dose (LD50), which rapidly proved relatively insufficient to manage the pesticide risk to honey bees. This notion has evolved to the hazard ratio, which takes into account the exposure of bees to the compounds, and more realistic toxicological tests such as tent, greenhouse and field tests, have been developed to yield more pertinent and relevant data.

Behavioral and social aspects of bee biology also complicate risk assessment, but in the past fifteen years, knowledge incorporating these notions into tests of adverse and sublethal effects has made great advances. The 1999 International Symposium of the ICP-BR Bee Protection Group was held in Avignon, France, an historical city in an area of intense agriculture and beekeeping. The focus was sublethal effects of pesticides on honey bees and developing new methods to study the impact of pesticides on the bee colony. Most of the methods presented in this symposium will continue to develop, and they represent the basis of a new bee toxicology in which the adverse effects of very low doses will be studied.

Luc P. Belzunces, Coordinator

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Avignon (France), September 07-09, 1999
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Report of the meeting

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1 - OPENING SESSION

J. STEVENSON, Chairman of the ICP-BR Bee Protection Group, opened the meeting expressing thanks to the Institut National de la Recherche Agronomique (INRA) and the Association de Coordination Technique Agricole (ACTA) for hosting the 7th International Symposium on the Hazards of Pesticides to Bees. In particular, he thanked **Dr.Luc Belzunces** and **Colette Pélissier** for the excellent organisation that had gone into the meeting.

Dr Stevenson conveyed greetings and best wishes to the meeting from **Prof. Ingrid Williams** (ICP-BR President). He then introduced the Bee Protection Group vice-chairmen, **Dr Dietrich Brasse** (who together with himself had attended all 7 meetings of the group since its formation in 1980) and **Dr Pieter Oomen**, as well as the group secretary, **Dr Gavin Lewis**. Thanks were given to the meeting sponsors (see introductory statement from **Prof. Ingrid Williams**).

In 1980 at the first symposium of the Bee Protection Group there had been 35 participants from 9 countries while at the current 7th symposium over 80 people were attending from 11 countries. At the first meeting a lot of test methodology had been considered, from measurement of toxicity in the laboratory to an assessment of hazard in the field. This had formed the basis of a risk assessment scheme, developed over successive meetings. This incorporated the concept of the hazard ratio, combining toxicity (LD_{50}) and exposure (application rate) to give an indication of the risk to bees that would be experienced under field conditions. Much of this work has formed the basis for the assessment of pesticide risk to other groups of non-target organisms and in particular the hazard ratio, in the form of the Toxicity Exposure Ratio (TER), has been widely adopted e.g. with birds, aquatic organisms and earthworms.

Dr Stevenson noted that at this meeting, unlike many of the previous ones, there were no papers on varroacides and he speculated whether this was due to a lack of problem or answers. He finished by asking for suggestions for the venue for the next meeting and indicated that a proposal from southern Europe would be particularly welcome, in order to encourage wider participation.

Dr J.N. TASEI, the ICP-BR secretary, gave a brief overview of the International Commission for Plant-Bee Relationships (ICP-BR). The ICP-BR is one of 82 scientific Commissions within the International Union of Biological Sciences (IUBS), one of a number of scientific unions joined under the International Commission of Scientific Unions (IUSU) which is affiliated to UNESCO. The ICP-BR was founded in 1950 by **Anna Maurizio** as the International Commission for Bee Botany (ICBB). The current officers are: **I. Williams** (chairman), **K. Richards** (vice-chairman), **J.-N. Tasei** (secretary). There are three working groups within the ICP-BR: pollination (chairman – **A. de Ruijter**), nectar (chairman – **A. Davies**) and the Bee Protection Group dealing with the effects of pesticides (chairman – **J. Stevenson**).

Membership of the ICP-BR is free (contact **Jean-Noël Tasei** for details) but needs to be renewed each year. A general assembly is held every 4-5 years while the working groups are organised according to their own requirements. Activities take a variety of forms e.g. symposia, publication of proceedings, literature reviews etc. The role of the council is to provide co-ordination, stimulation of new initiatives, decision making, dissemination of information (reporting to the IUBS and producing an annual circular and membership directory) and providing for communication between members (in closed forums, by e-mail etc).

Dr L.P. BELZUNCES then welcomed everyone on behalf of INRA and ACTA and gave his thanks to the organising committee and to the meeting sponsors. He informed the meeting that it was intended to publish the proceedings of the meeting (for the first time in the history of the Bee Protection Group) in order to improve their availability. He asked all presenters to submit their manuscripts by the end of September.

2 – SUMMARY OF THE MEETING

The papers presented at the meeting were divided into a series of sessions covering a range of distinct areas :

2.1 Test methodology *

B. LEYMANN described ***a semi-field test to evaluate side-effects of pesticides on honey bee brood*** (page 61). Currently, there are no standard methods for quantitatively assessing the effects of pesticides on honey bee brood in cage or field tests (e.g. in EPPO 170). In particular, tests conducted with full colonies result in unacceptably high brood losses due to handling of the combs, disturbing the nurse bees etc. Laboratory tests, on the other hand, are too far removed from field conditions in the hive i.e. it is not possible to simulate field conditions in the laboratory. The aim of this work therefore, was to develop a semi-field test for honey bee brood assessment. Cages large enough for small colonies were used (4 x 12 x 2 m) and two combs were replaced with capped honey and empty cells. Daily observations were conducted of brood development in individually marked brood cells, using acetate sheets attached to the window of an observation hive, up to emergence. In addition, mortality was assessed, using dead bee traps and counts from the cage floor, while bee activity was assessed using bee counters (BIOSCAN) on the hive. NeemAzal (active ingredient – Azadiradectin) was tested at a rate of 6.0 L in 400 L/ha, with Alsystin (active ingredient –) as a reference product and with a water-treated control. There was no adult mortality after treatment (no acute toxicity) and no effect on flight activity in any of the treatments: on this basis NeemAzal was classified as 'harmless to bees'. Bee brood development in two replicates was: 86.9 and 78.6% (NeemAzal); 9.6 and 0.6% (Alsystin); 85.7 and 85.2% (control). NeemAzal was therefore also classified as 'harmless to bees' on the basis of brood mortality. It was concluded that the larger cages allowed normal foraging

* The name of each new speaker is in capital letters.

activity without any areas of 'trapping' and that the observation of the brood through the window of the observation hive allowed the assessment of brood development without any disturbance.

It was pointed out that in the method of Oomen ***et al.*** there was 10-15% replacement of brood in the control (**H.M. Thompson**). **B Leymann** replied that in the Oomen method the brood is only assessed weekly and it is therefore not easy to track individual cells whereas this method is very precise and allows lots of cells to be followed. **P.A. Oomen** added that his method is closer to an initial laboratory test whereas this is a complementary semi-field test. **J. Stevenson** encouraged **B. Leymann** and his colleagues to contact the larval testing sub-group with their experiences. The question of the problem of brood disturbance resulting in dead larvae being replaced was raised. As the relationship of this disturbance to the impact on the colony is a complex one e.g. it depends on the strength on the colony, this is a potentially confounding variable in trying to measure the effect of a treatment on the colony (**H.W. Schmidt**). **W. Mühlen** replied that there are two factors: one is the effect of a pesticide application on the brood, which this method is assessing, the other is will disturbed brood effect the colony which is not considered here.

L.P. BELZUNCES presented a paper addressing ***the effects of pyrethroid insecticides on honey bee thermoregulation*** in combination with the synergistic effects of pyrethroids and some fungicides (page 297). A synergisitic effect has been found with pyrethroids and azole fungicides (imidazoles and triazoles) such that mortality may be found at normally sub-lethal doses. In addition, pyrethroids show a negative temperature coefficient such that toxicity increases with decreasing temperature. Infra-red thermography was used to study honey bee thermogenesis (concentrating on the thorax). Seven pyrethroids were applied at a dose level of 5 µg/bee: in all cases there was no effect on mortality and two did not effect thermoregulation but the other five did have an effect. In a more detailed test, deltamethrin was applied at dose levels of 0.5, 1.5, 2.5 and 4.5 µg/bee: effects on thermoregulation were seen at levels of 2.5 µg/bee and above. In the case of prochloraz a weak effect only was seen at a high dose level of 1250 µg/bee. However, when prochloraz was applied at a dose level of 850 ng/bee with deltamethrin at levels of 1.5 and 2.5 µg/bee, a synergistic effect on thermoregulation was seen. A similar result was seen with difenaconazole. It was concluded that azole fungicides potentiate pyrethroid-induced hypothermia: the mechanism is not clear but it was postulated that the effect could occur at the mitochondrial level.

H.M. Thompson asked if reports of loss of bees (foraging workers) could be due to this effect on thermoregulation. It was thought that this effect could be manifested under field conditions e.g. with a negative temperature coefficient occurring below 28 °C, then with Spring temperatures of 15 °C mortality could occur due to this mechanism. In addition, the effects seen in the laboratory were at rates similar to those used in the field. **D. Brasse** asked if effects had been seen in the field from 'non-hazardous' products. **L.P. Belzunces** replied that this was the case, especially in the south of France. While it was not clear if pyrethroids were involved analysis had showed that they were often present, together with carbamates, organophosphates and lindane. He went on to say that it would be difficult to measure the temperature of bees in the field, when asked if this effect had been investigated in controlled cage experiments by **R. Schmuck**. However, it was pointed out that pyrethroid mortality was usually related to direct spraying and that residual exposure would be required for a synergistic effect resulting in increased mortality over several days.

2.2 - Effects of imidacloprid on honey bees

A series of three papers were then presented looking at the effects of the neonicotinoid insecticide, imidacloprid, on honey bees. In the first of these, **S. SUCHAIL** looked at ***the acute oral toxicity of imidacloprid and six of its metabolites on honey bees*** (page 121). Dose levels were in the range 1-1000 ng/bee, control bees were fed 50% sucrose solution with 0.1% DMSO and there were three experiments with three replicates of 20 bees per treatment. Imidicloprid and two of the metabolites, olefin and 5-OH imidacloprid, were very toxic (96-hour LD_{50} values of 50, 10 and 50 ng/bee, respectively) but the other four metabolites were less toxic (>1000 ng/bee). Chronic toxicity was also assessed, with exposure to concentrations of 0.1, 1.0 and 10 µg/L over 10 days. A similar increase in mortality over time was seen with imidacloprid and all the metabolites with all being identified as toxic to bees. A number of areas were identified for future investigation: (1) a study of imidacloprid metabolism in honey bees; (2) a comparison of the affinity of parent and metabolite compounds for imidacloprid receptors.

In response to a question about the relationship between the dose levels tested and field application rates, **R. Schmuck** replied that residues in seed treatments were less than 5 ppm which equated to a field rate of 105 g ai/ha. He then asked about the strain tested and **S. Suchail** said that it was *Apis mellifera mellifera*. He went on to say that in a tunnel test no effects had been seen at a dose level of 100 ng/bee and he wondered why there was this

marked difference compared to the laboratory, where effects were seen as low as 0.1 ng/bee. One proposal was that in the laboratory tests the bees only consume the active ingredient whereas under field conditions it is taken to the hive and ultimately consumed via the honey so it may be diluted and transformed.

In the second paper dealing with imidacloprid, **D. GUEZ** discussed ***the sublethal effects of imidacloprid on learning and memory in honey bees*** (page 297). Imidacloprid, the first of the new family of neonicotinoid insecticides, acts as an agonist (the opposite of antagonist) on two receptors in the cholinergic system producing hyperactivity. As this system is involved in memory and learning it could be affected by exposure to imidacloprid. Laboratory and field approaches were used. In the laboratory, habituation was examined (the waning of a reflex response to repeated stimulation). Bees were fixed and starved for 4 hours then given sucrose solution at intervals before treatment (4 and 1 hours and 15 minutes). After treatment, three stimulations without proboscis extension was deemed to be habituation. The learning in 7-day old bees was altered after treatment with imidacloprid (e.g. after 1 hour at 10 ng/bee) while in the case of 8-day old bees the learning ability was increased indicating a greater affinity for the receptors. Learning was also altered 4 hours after treatment in the case of the 7-day old bees and it was postulated that this might be due to metabolites. In the absence of imidacloprid, 7 and 8-day old bees present different habituation responses which indicates that there is a critical period in brain maturation at this time (this is the age that bees learn to fly).

It was concluded that under field conditions, imidacloprid has no detrimental effect on foraging behaviour at 1 ppm and no effect at 0.1 ppm over 6 days. In the laboratory, there is an age-dependent effect with a switch occurring between 6 and 7 days. It was suggested that the two cholinergic receptors have low and high affinity: at 7-days old a metabolite receptor predominates but at 8 days there is only the high affinity receptor facilitating rapid learning.

It was asked if anything was known about this difference at 7 and 8 days and whether it was related to any significant transition in development. The effect on learning had only been identified in this work although there it could be related to the start of flying at 8 days. It was considered strange that such an effect should occur over one day although it was pointed out that the data presented was a mean of three experiments. In response to a question from **D. Brasse** it was agreed that in practical terms this effect could be affected by the distance over which bees have to fly. Given effects were seen at 1 ppm and not 0.1 ppm, **H.W. Schmidt** asked if bees appear to tolerate levels of 0.1 ppm in sunflowers and it was confirmed