Comparing the toxicity and the exposure value as outlined above, the resulting TER-value is 6. From this TER figure, it can be concluded that it is unlikely that there is an unacceptable risk for honey bees from abraded clothianidin deposits associated with the aforementioned seed-coating quality and machinery parameters. The margin of safety can be further improved by an enhanced seed-coating quality.

Final conclusions and outlook

Substantial work has been undertaken to investigate the causal factors that constituted the bee incident in the Upper Rhine Valley in 2008. Intensive activities were dedicated to develop optimizations in the areas which were identified as key factors for appropriate risk mitigation for seed treatments, i.e. seed-dressing quality and drilling technology. It was demonstrated in comprehensive field studies under realistic conditions that the developed mitigations measures work efficiently.

Therefore it can be concluded that by implementation of the outlined optimizations, the exposure of bees to dusts from seed-coating products during the drilling process can be minimized by orders of magnitude, and that a bee-safe use of insecticidal seed-dressing products can be ensured.

Acknowledgements

We thank all those who co-operated with us in the investigation of the incident and in the development of risk mitigation measures and technical improvements. In particular, we would like to mention:
Dr. G. Liebig, Dr. P. Rosenkranz and Dr. K. Wallner (University of Hohenheim), Dr. U. Heimbach and J. Pistorius (Julius-Kühn-Institut, Braunschweig), Dr. M. Glas and Dr. A. Trenkle (LTZ Augustenberg), F. Maass (Regierungspräsidium Freiburg), Dr. A. Maier (Regierungspräsidium Karlsruhe), Dr. J. Lückmann and T. Städtler (RIFCon GmbH, Heidelberg), and Ch. Laubert (Bayer CropScience, Monheim).

References

1. Liebig G., Kustermann Th. & de Craigher D., Bienenmonitoring während und nach der Maisblüte im Rheintal 2008. Unpublished report (2008)
2. Schmuck R, Keppler J., Clothianidin – Ecotoxicological profile and risk assessment. Pflanzenschutz-Nachrichten Bayer **56**: 26-58 (2003)

Spring honey bee losses in Italy

Laura Bortolotti[1], Anna Gloria Sabatini[1], Franco Mutinelli[2], Mario Astuti[3], Antonio Lavazza[4], Roberto Piro[4], Donato Tesoriero[1], Piotr Medrzycki[1], Fabio Sgolastra[5], Claudio Porrini[5]

[1]CRA–Unità di Ricerca di Apicoltura e Bachicoltura, Via di Saliceto 180, Bologna (Italy), Tel. +39 051 353103, Fax +39 051 356361, e-mail: laura.bortolotti@entecra.it;
[2]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD) (Italy)
[3]Direzione Generale Sanità Regione Lombardia, Milano (Italy)
[4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia (Italy) [5]Dipartimento di Scienze e Tecnologie Agroambientali (DiSTA), University of Bologna (Italy)

Abstract

Background: During last years several cases of bee losses have been reported during the period of corn sowing in different European countries. In Italy an institutional system for bee losses survey does not exist and therefore some Italian regions decided to organise an official network to collect data and analyse dead bee samples.

Results: Collected data indicate that the higher number of bee losses events occurred in intensively cultivated flat areas, located in the North of Italy, mainly during or after corn sowing. The chemical analyses of dead bees revealed the presence of three neonicotinoid residues: imidacloprid was found in 25.7% of the sample,

thiamethoxam in 2.8%, clothianidin in 25.7%, both imidacloprid and thiamethoxam in 4.7%. The visual examination and the virological analyses excluded pathological causes.

Conclusion: The spatial and temporal correlation between hive damages and corn sowing and the presence of residues of active ingredients used for seed dressing (imidacloprid, thiamethoxam and clothianidin) in almost half of the samples confirms the connection between spring mortality and the sowing of corn seed dressed with neonicotinoids.

Keywords: honeybee mortality, neonicotinoids, seed dressing, corn sowing, dust dispersion.

Introduction

During last years several outbreaks of honeybee losses have been reported all over Europe and in others countries worldwide. Recently these phenomena became extremely worrying. According to the last researches, the most likely risk factors are bee diseases, agrochemical treatments, poor beekeeping management and climatic changes. These factors can act singularly or simultaneously and can vary depending upon the local circumstances. Among them, the agrochemical treatments performed during spring-summer in intensively cultivated areas seem to have a great impact.

Already in 1999 Italian researchers noted that many reports from beekeepers affected by hive losses coincided with the period of corn sowing and hypothesized that the cause could be dust dispersion from drilling machine during sowing operations of dressed corn seeds. Further investigations demonstrated that a loss of active ingredient (a.i.) through the fan drain of pneumatic seed drills during corn sowing can actually occur [1] and that after the sowing operations flower and grass samples collected near the corn fields are contaminated by the a.i. imidacloprid.[2] The experiments regarded Gaucho® dressed corn seeds.

Bee losses survey is taking place in many European countries including Italy, where it is however not yet well enough organised. At present and waiting for the activation of a national monitoring network, Italian beekeepers can send their reports on hive damages through a specially provided questionnaire, published on the main apicultural magazines and web sites.

The reports in 2008 spring during the period of corn sowing increased exceptionally. In Table 1 the number of reported hive damages in 5 Italian regions is summarized. The total number of affected hives was 6328 and the number of beekeepers 185. These data probably underestimate the total damage, because in Italy beekeepers are not used to report hive damages to the public authorities. For the same period the Italian institution for the surveillance of honey market (Osservatorio Nazionale della Produzione e del Mercato del Miele) estimated a loss of 50,000 hives (http://www.osservatoriomiele.org/2_rapporto2008.htm).

Table 1 Number of reported hive damages during spring 2008 in 5 Italian regions.

Region	n° affected hives	n° affected beekeepers
Lombardy	1513	40
Piedmont	1167	8
Emilia-Romagna	187	7
Veneto and Trentino	1000	20
Friuli Venezia Giulia	2461	110
Total	**6328**	**185**

Aim of the present research is to demonstrate the correlation between colony losses in spring 2008 and the sowing of corn seeds dressed with neonicotinoids.

Experimental methods

In 2008 spring two regions of North Italy (Lombardy and Veneto) decided to organise an institutional network. When beekeepers noted a damage to their bees they had to report it to the local Veterinary Authority and fill in a questionnaire, then the veterinarian should inspect the apiaries and collect samples of dead bees and pollen from surrounding vegetation. Samples were sent to the Istituto Zooprofilattico

Sperimentale of Brescia for the analysis of pathogens and to the CRA-Unità di Ricerca di Apicoltura e Bachicoltura of Bologna for the analysis of neonicotinoid residues (imidacloprid, thiamethoxam, clothianidin).[3,4]

Results

The results of the residue analysis for samples collected in Lombardy and Veneto are summarised in Table 2. A total of 105 dead bee samples were analysed (65 from Lombardy and 40 from Veneto) and 4 samples of pollen from surrounding vegetation in Lombardy. Several samples resulted positive to imidacloprid (25.7%), thiamethoxam (2.8%) and clothianidin (25.7%) and also to both imidacloprid and thiamethoxam (4.7%). Three out of four pollen samples resulted positive to imidacloprid, one also to clothianidin.

Table 2 Results of the analysis of samples collected in 2008 spring in Lombardy and Veneto.

	Lombardy		**Veneto**		**Total**	
	Number	**%**	**Number**	**%**	**Number**	**%**
Analysed samples	69		40		**109**	
Dead bee samples	65		40		**105**	
Positive dead bee samples	30	46.1	22	55.0	**52**	**49.5**
Dead bee samples positive to imidacloprid	19	29.2	8	20.0	**27**	**25.7**
Dead bee samples positive to thiamethoxam	2	3.0	1	2.5	**3**	**2.8**
Dead bee samples positive to clothianidin	13	20.0	14	35.0	**27**	**25.7**
Dead bee samples positive to fipronil	0	0	0	0	**0**	**0**
Dead bee samples positive to both imidacloprid and clothianidin	4	6.1	1	2.5	**5**	**4.7**
Pollen samples	4				**4**	
Positive pollen samples	3	75.0			**3**	**75.0**
Pollen samples positive to imidacloprid	3	75.0			**3**	**75.0**
Pollen samples positive to thiamethoxam	0	0			**0**	**0**
Pollen samples positive to clothianidin	1	25.0			**1**	**25.0**
Pollen samples positive to both imidacloprid and clothianidin	1	25.0			**1**	**25.0**

The concentrations of a.i. found in dead bee samples (52 positive samples) ranged from 1.01 to 240.6 ng/g for imidacloprid, from 3.67 to 39.2 ng/g for clothianidin and from 24.8 to 138 ng/g for thiamethoxam. The concentration of a.i. found in pollen samples (3 positive samples) ranged from 7.3 of clothianidin to 311.45 ng/g of imidacloprid.

The inspection carried out by the Veterinary Services and the results of virological analysis excluded any pathological cause.

In Table 3, we report the data of the questionnaire completed by beekeepers and veterinarians, related to the 65 apiaries affected in Lombardy, corresponding to 1513 hives. All the reports came from cultivated areas, located mostly in the plain area; the main surrounding crop was corn and in 96.2% of cases the damage occurred during or after corn sowing. Affected hives had rich brood combs and abundant stores and the foraging activity was intense. In 91% of the affected hives an anomalous behaviour of workers was observed, consistent with those reported after intoxication with neonicotinoids.[5,6]

Table 3 – Results of the questionnaire filled in by beekeepers in 2008 spring in Lombardy region.

Number of questionnaires	**65**
Number of affected hive in each apiary	from a minimum of **3** to a maximum of **170**
Hives types	**93%** sedentary; **7%**migratory
Range of dead bees for each hive	from few hundreds to many thousands (**up to 15,000-20,000**)
Areas	**69%** plain; **20%** hills; **11%** mixed areas
Main surrounding crop	**96%** corn; **55%** wheat; **33%** meadows
Period	**96.2%** of cases during or after corn sowing
Stores	Presence of rich brood combs and abundant honey and pollen stores
Foraging activity	Intense at the time of sowing (presence of foragers with pollen loads in the **95.8%** of cases)
Worker behaviour	Anomalous in **91%** of cases: rolling **71.4%;** disorientation **57.4%;** aggressiveness **23.8%;** incapability to enter the hive **52.3%.**

Discussion and conclusions

The results of the study allow some relevant conclusions:

- there is a spatial and temporal correlation between hive damages and corn sowing;
- the presence of residues of a.i. used for seed dressing (imidacloprid, thiamethoxam and clothianidin) in almost half of the samples confirms the relationship between spring mortality and the sowing of corn seed dressed with neonicotinoids.

The fact that half of the analysed samples did not contain residues is not enough to exclude the responsibility of neonicotinoids in hive damages. Many factors can influence the presence of residues and their level: the way of exposure of bees to the a.i., that can be direct during corn sowing or indirect via pollen and nectar of surrounding flora; dead bee samples could have been collected with some delay after intoxication or could have not been properly stored with a consequent degradation of the a.i.

Following these evidences, on 17th of September 2008 the Italian Government decided the precautionary suspension of use of all the four a.i. registered for seed dressing - imidacloprid, thiamethoxam, clothianidin and fipronil - although the latter was never found in dead bee samples.

The future implementation of an Italian national bee monitoring network (APENET), which hopefully will be implemented by 2009, will certainly contribute to the knowledge of the extent and causes of this phenomenon. [7]

References

1. Greatti M, Sabatini AG, Barbattini R, Rossi S, Stravisi A, Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. Bulletin of Insectology **56**: 69-72 (2003).
2. Greatti M, Barbattini R, Stravisi A, Sabatini AG, Rossi S, Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds. Bulletin of Insectology **59**: 99-103 (2006).
3. Rancan M, Sabatini AG, Achilli G, Galletti GC, Determination of Imidacloprid and metabolites by liquid chromatography with an electrochemical detector and post column photochemical reactor. Analytica Chimica Acta **555**: 20-24 (2006).
4. Rancan M, Rossi S, Sabatini AG, Determination of Thiamethoxam residues in honeybees by high performance liquid chromatography with an electrochemical detector and post-column photochemical reactor. Journal of Chromatography A **1123**: 60-65 (2006).
5. Bortolotti L, Montanari R, Marcelino J, Medrzycki P, Maini S, Porrini C, Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bulletin of Insectology **56**: 63-67 (2003).

6. Medrzycki P, Montanari R, Bortolotti L, Sabatini AG, Maini S, Porrini C, Effects of imidacloprid administered in sub-lethal doses on honey bee behaviour. Laboratory tests. Bulletin of Insectology **56**: 59-62 (2003).
7. Porrini C, Sgolastra F, Sabatini AG, Rete per il monitoraggio dei fenomeni di spopolamento e mortalità degli alveari in Italia (APENET). Apoidea **5**: 83-87 (2008).

Sprayed and seed dressed pesticides in pollen, nectar and honey of oilseed rape

Klaus Wallner

University Hohenheim, Apiculture Institute, D-70593 Stuttgart
E-mail: Klaus.Wallner@uni-hohenheim.de

Introduction

Oilseed rape is almost exclusively produced as an intense cultivation. Seeds are treated before sowing with systemic insecticides, nowadays primarily with neonicotinoids. In the blooming period, sprays against fungi (*Sclerotinia sclero)* or pests (e.g. *Ceutorhynchus assimilis*) with different non hazardous pesticides are common. These substances are known to reach pollen and nectar. Contaminants in food sources are actually discussed as sublethal factors influencing colony health. Residues are adverse for the image of honey.

Experimental

In a study to quantitative study the presence of residues in nectar, pollen and honey conditions, two fungicides were sprayed into an 8 ha blooming cultivation in accordance with normal agricultural practice in Germany. Over a 7 day period, residues of the seed dressed insecticide and the sprayed fungicides were measured in the pollen and nectar loads of returning foragers. Unripe honey from combs and extracted honeys were analyzed.

16th April 2007: Application of the fungicides Cantus® (boscalid, 500g a.i./kg), 0,5 kg/ha) and Proline® (prothioconazol, 250g a.i./kg, 0,7 kg/ha) in an 8 ha oilseed rape field (variety *Smart)*, seed dressed with the insecticide Elado Premiumbeize® (clothianidin a.o.). Both fungicides act systemically and can be combined with non hazardous pyrethroids or neonicotinoids insecticides. The fungicides were sprayed in combined application with 250 l water per ha.

Two apiaries (2 respectivally 7 colonies) at 200 m distance to the sprayed oilseed rape field were used for the experiments.

Returning foragers were caught at the hive entrance with a special vacuum cleaner. The bees were immediately shock frozen with carbon dioxide snow and stored at -20°C until preparation. Thus, starting from the day before application, at least three series with around 100-150 bees were collected per day over a 7 day period

In the lab, the pollen loads and the collected nectar of the honey sacs were prepared separately for each trapped group of forager bees. The pollen loads were sorted by color and the origin was checked under the microscope. Only oilseed rape pollen was used for further analysis. In total 22 pooled pollen and 22 pooled nectar samples were prepared with an adapted QuEChERS-multi method and analyzed with tandem LC-MS/MS. The quantitation limits for the different substances in the analysis were as follows:

- Boscalid in pollen, nectar and honey: 0,001 mg/kg
- Prothioconazol in nectar und honey: 0,001 mg/kg
- Prothioconazol in pollen: 0,010 mg/kg
- Clothianidin in pollen, nectar und honey: 0,001 mg/kg