3.2 Neonicotinoids and bees: A large scale field study investigating residues and effects on honeybees, bumblebees and solitary bees in oilseed rape grown from clothianidin-treated seed

Nadine Kunz [1], Malte Frommberger [1], Anke C. Dietzsch [1], Ina P. Wirtz [1], Matthias Stähler [2], Eva Frey [3], Ingrid Illies [4], Winfried Dyrba [5], Abdulrahim Alkassab [6], Jens Pistorius [1]

[1] Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland
[2] Julius Kühn-Institut, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection
[3] Apicultural State Institute, University Hohenheim
[4] Bavarian State Institute for Viniculture and Horticulture, Department of Honey Bee Research & Beekeeping
[5] Beekeeping State Association Mecklenburg-Vorpommern, Beekeeping Centre Bantin
[6] Ruhr-Universität Bochum, Department for Biology and Biotechnology, Bochum
[*]Corresponding author: Nadine Kunz, Julius Kühn-Institut, Inst. A., Messeweg 11-12, D-38104 Braunschweig. nadine.kunz@jki.bund.de

Abstract

In 2013, the European Food Safety Authority (EFSA) has highlighted several data gaps regarding the exposure and risk of pesticides to honeybees, bumblebees and solitary bees, including the risks from exposure to contaminated nectar and pollen. This study aims to contribute data, results and conclusions to obtain more information on exposure and risks of flowering oilseed rape seed treated with the neonicotinoid clothianidin, to pollinators. Semi-field and field trials were conducted at five different locations across Germany, using the Western honeybee (*Apis mellifera* L.), the buff-tailed bumblebee (*Bombus terrestris* L.) and the red mason bee (*Osmia bicornis* L.) as study organisms.

Highest amounts of clothianidin residues were measured in single samples of mud cell walls (7.2 µg kg $^{-1}$) and pollen (5.9 µg kg $^{-1}$) from solitary bee nests. Residues in nectar from honey sacs, honeybee combs and bumblebee nests (2.2, 2.9, and 3.0 µg kg $^{-1}$ respectively) showed no clear differences in the amount of residues, neither did residues in pollen (1.5, 1.8, and 1.3 µg kg $^{-1}$ respectively). These results suggest differences in the risk profiles of those three bee species.

Keywords: clothianidin, residues, honeybees, bumblebees, solitary bees, field, semi-field

Background

Honeybees, bumblebees and solitary bees are important crop pollinators. To date, potential side effects of oilseed rape seeds treated with neonicotinoids on the behavior, mortality, colony development and reproduction have been mainly investigated for honeybees. [1] Hardly any higher tier studies in semi-field or field conditions are available for solitary bees and bumblebees, and official test guidelines as well as validated methods to evaluate potential risks of pesticides are still lacking. This study aims to evaluate exposure to translocated residues of the systemic neonicotinoid clothianidin in nectar and pollen as well as their potential effects for honeybees and other commercially used pollinators. Residues of clothianidin were measured in a semi-field and field study by investigating nectar and pollen of honeybees, bumblebees and the red mason bee as highly relevant routes of exposure for bees. Residues in nesting material (mud cell partitions of *O. bicornis*) were also analysed.

Experimental methods

Field trials and semi-field trials in flowering oilseed rape (OSR, *Brassica napus* L. variety SHERPA® or AVATAR®) cultivated from treated and untreated seed (control) were conducted in five federal states of Germany in spring 2014. Control OSR seed were coated with a fungicidal seed coating (TMTD 98% Satec, DMM) and cultivated in at least 2.5 km distance from treatment fields. Treatment seed was additionally coated with clothianidin ('ELADO FS 480®', Bayer CropScience AG, Germany). The coating contained clothianidin (10 g kg $^{-1}$ seeds) and (beta-) cyfluthrin (2 g kg $^{-1}$

seeds, a non-systemic pyrethroid insecticide). Seeds were sown at seed rates of 500,000 up to 800,000 seeds ha $^{-1}$. No other plant protection products containing clothianidin were used.

The Western honeybee (*Apis mellifera* L.), the buff-tailed bumblebee (*Bombus terrestris* L., purchased from Biobest, Belgium) and the red mason bee (*Osmia bicornis* L., purchased from WAB-Mauerbienenzucht, Germany) were exposed to flowering OSR for 23 to 26 days (mean 25 days). Four commercial honeybee hives and four bumblebee colonies, as well as three artificial trap nests with solitary bee cocoons (33 male and 33 female cocoons each) were placed right next to flowering OSR on each of ten field plots. In addition, a total of 40 tents were set up before flowering of OSR. Each tent (40 m^2) covered flowering OSR, held one small honeybee colony, two small bumblebee colonies and three trap nests with solitary bees, resulting in 20 small colonies of *A. mellifera*, 40 colonies of *B. terrestris* and 60 trap nests with *O. bicornis* in each of the control and the treatment semi-field setup. Samples of honey sacs and pollen sacs from foragers, honey and pollen from hives or nests, as well as mud from solitary bee nests were continuously collected and analysed for residues of clothianidin. Chemical analysis was done using HPLC-MS/MS (Dionex UltiMate 3000 – AB SCIEX QTRAP 5500), with acetamiprid as a surrogate. Limit of quantification (LOQ) was 0.6 µg kg $^{-1}$, limit of detection (LOD) was 0.2 µg kg $^{-1}$, with a weight per sample of 1.0 g.

Results

Residues of clothianidin in nectar and pollen taken from honeybee individuals during exposure were detected up to a maximum concentration of 2.9/1.0 and 1.8/3.2 µg kg $^{-1}$ respectively (field/semi-field, Table 1, Figure 1). Clothianidin residues in nectar and pollen taken from bumblebee colonies did not exceed 3.0 in the field; in the semi-field the highest concentration was below LOQ (Table 1, Figure 1). In solitary bee pollen a maximum of 1.4/5.9 µg kg $^{-1}$ clothianidin was measured (field/semi-field, respectively). In mud cell walls of *O. bicornis* a maximum of 7.2 µg kg $^{-1}$ clothianidin was measured in the field (Table 1).

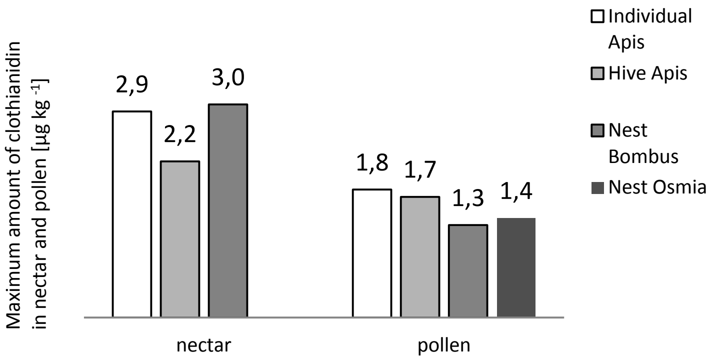

Fig. 1 Maximum amounts of residues of clothianidin in nectar and pollen [µg kg $^{-1}$] from treated OSR in the field trial.

Tab. 1 Residues of clothianidin in different matrices foraged during exposure at OSR clothianidin treatment (T) or control (C) sites. NA=sample not available, <LOQ=clothianidin concentration smaller than limit of quantification, <LOD=clothianidin concentration not detectable.

Sample type	Setup	Treatment	Number of samples [N]	Number of samples >LOD [N]	Max. concentration clothianidin [µg kg $^{-1}$]
Nectar from hive (*A. mellifera*)	Semi-field	C	5	0	<LOD
		T	5	0	<LOD
	Field	C	11	1	<LOQ
		T	12	7	2.2
Nectar from bee (*A. mellifera*)	Semi-field	C	2	0	<LOD
		T	4	0	<LOD
	Field	C	15	1	<LOQ
		T	14	10	2.9
Nectar from nest (*B. terrestris*)	Semi-field	C	4	0	<LOD
		T	3	0	<LOQ
	Field	C	22	2	<LOQ
		T	24	17	3.0
Bee bread from hive (*A. mellifera*)	Semi-field	C	5	1	1.0
		T	6	1	3.2
	Field	C	11	0	<LOD
		T	12	6	1.7
Pollen from bee (*A. mellifera*)	Semi-field	C	3	0	<LOD
		T	5	2	1.6
	Field	C	8	1	<LOQ
		T	10	4	1.8
Pollen from nest (*B. terrestris*)	Semi-field	C	12	0	<LOD
		T	8	0	<LOD
	Field	C	18	0	<LOD
		T	20	7	1.3
Pollen from nest (*O. bicornis*)	Semi-field	C	1	1	<LOQ
		T	1	1	5.9
	Field	C	8	0	<LOD
		T	8	8	1.4
Cell wall from nest (*O. bicornis*)	Semi-field	C	0	NA	NA
		T	1	1	<LOD
	Field	C	7	2	<LOQ
		T	6	4	7.2

Discussion and Conclusion

In this study of the ongoing ABO-project (‘*Apis-Bombus-Osmia*’), numerous data on residues were obtained from five different field sites in Germany. Further data on effects of clothianidin on overwintering of honeybees are currently evaluated and prepared for publication.

In the field trials, residues were detected in some samples of all of the five control study sites, confirming that it was extremely difficult to find adequate control sites without any other accessible (neonicotinoid treated) oilseed rape within bee flight distance. Residues found in samples from control field sites in 2014 may have originated from treatment fields in the further surrounding of the investigated control oilseed rape areas. However, residues in pollen from honeybees and bumblebees were detected in only one of 37 samples in the control field sites, and in four of 48 nectar samples. In solitary bee pollen in the control field sites, no residues were found (n=8). Honeybees and bumblebees have a larger foraging distance than the red mason bee [2] and may have been attracted to pollen and nectar over longer distance. *Osmia bicornis* only covers shorter distances and is likely to rely on OSR pollen collected at fields in the closer proximity. Since oilseed rape is only grown from seed without any neonicotinoid treatment at present, residue data obtained in field trials in 2015 are expected to differ from data obtained in 2014.

In the semi-field trials, no residues of clothianidin were detected in any of the 11 control nectar samples but in two samples out of 21 pollen/bee bread control samples. This result is surprising since bees did not have access to treatment OSR in the semi-field trial. In both semi-field and field trials residues and maximum values measured at different locations were in the range of previously reported values for honeybees, where concentrations ranged between 1-8.6 µg kg $^{-1}$in nectar and between 1-4 µg kg $^{-1}$ in pollen collected by honey bees. [1] Nevertheless, a slightly higher value was detected in one of the semi-field treatment pollen samples collected by *O. bicornis*. In contrast, residues in pollen of bumblebees and honeybees in the same tunnel were low. These results suggest differences in the risk profiles of those three bee species; they differ in their biology and foraging behavior and may also be exposed to different quantities of residues. [3] There is no clear explanation for the different results obtained for *O. bicornis*. Further trials in 2015 and additional data on residue in *O. bicornis* pollen from other locations will help to further clarify the exposure of solitary bees to clothianidin.

Acknowledgements

This project was funded by the German Federal Ministry of Food and Agriculture (BMEL) with additional support from the Federal Office of Consumer Protection and Food Safety as part of the 'ABO 2014' project, coordinated by the Julius Kühn-Institut, Braunschweig, Germany.

References

1. EFSA (European Food Safety Authority), Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees). *EFSA Journal* **10** (5): 2668 (2012).
2. Greenleaf SS, Williams NM, Winfree R and Kremen C, Bee foraging ranges and their relationship to body size. *Oecologia* **153** (3): 589-596 (2007).
3. Van der Valk H, Koomen I, Nocelli RC, Ribeiro MdF, Freitas BM, Carvalho S, et al. Aspects determining the risk of pesticides to wild bees: risk profiles for focal crops on three continents. *Hazards of pesticides to bees – 11th International Symposium of the ICP-BR Bee Protection Group, Julius-Kühn-Archiv* **437** :142-158 (2012).