3.4 Neonicotinoid seed treatment products – Occurrence and relevance of guttation for honeybee colonies

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Abstract

Background: Guttation is a natural botanical phenomenon and describes the active excretion of liquid water (guttation fluid) by some vascular plants in form of droplets on the tips of leaves or on leaf edges. Guttation fluid may contain neonicotinoid residues after plant uptake from seed treatments. To clarify the relevance of the guttation fluid as a water source for honey bee colonies and to assess potential associated risks under conditions of agronomic practice, various studies were performed in key broad acre crops such as maize, sugar beet, potato (in-furrow application), winter barley and oilseed rape by placing honeybee colonies adjacent to freshly emerged fields for several weeks and by following up potential lethal and sub-lethal effects, as well as potential effects on colony performance.

Results: Guttation droplets contained peak residue levels theoretically capable of harming individual honeybees (i.e. several hundred ppm). Residue levels, however, generally decreased with time, as expected based on the physiological process involved. The temporal coincidence of honeybee flight activity and the presence of guttation droplets were generally limited to early morning hours and to a much lesser extent to evening hours. Spatially, honeybees were found to predominately collect water, if any, in the direct vicinity of the hives. Water collection generally ceased within a couple of metres distance to the hives, which renders distance to the crop to be a significant exposure factor, and in turn renders dew and guttation from off-crop vegetation to be more relevant to water collecting honeybees than guttation from the crop. Mortality events, if any, were scarce and generally matched in treatments and in controls. The absolute numbers of dead bees involved in these rare cases were so low that they did not translate into any colony level effects or impacts on bee health or overwintering success, nor on adverse effects on honey production of the involved colonies.

Conclusions**:** Given the overall body of data, the associated intensity of the assessments in each study as well as the worst-case exposure conditions employed, it can be concluded that exposure of honeybee colonies to guttation fluid, excreted from neonicotinoid seed-treated crop plants, did not pose an unacceptable acute or chronic risk to honeybee colony development or survival, and does not adversely interfere with bee keeping practices. Overall, guttation water from seed-treated crop plants was found not to be a significant exposure route for honeybees.

Key Words: Pesticide, honey bee, guttation

Introduction

Honey bees use water to maintain humidity and temperature within the colony as well as for brood care (1). The amount of water required and collected by a colony generally correlates with the outside air temperature, relative humidity, colony strength and the level of brood rearing. Honey bee colonies are typically able to meet their water requirements by collecting nectar and the production of metabolic water during flight. However, when water requirements increase such as during periods of hot temperatures or high brood production, additional water may be required. Water may be collected from a variety of sources including dew, puddles or other surface water bodies or damp earth. Guttation droplets produced by plants under certain environmental conditions may be used as a source of water. Honey bees generally collect water from within the direct vicinity of the colony due to energy required for flight and the fact that water is not an energy source which is however stored inside the honey stomach along with the carbohydrate "fuel".

Guttation is a natural botanical phenomenon that describes the active excretion of liquid water (guttation fluid) by some vascular plants. Droplets are formed either on leaves edges (common in dicotyledonous plants) or only at the leaf tip (common in monocotyledonous plants). In maize, guttation occurs at the end of leaves. Droplets are formed of xylem fluid, which are excreted by root pressure through special structures called hydathodes located at the top and on the edge of leaves. Droplets contain sugars (mono and disaccharides) only in very small amounts, minerals such as potassium (18 to 30 mg/L) and to a lesser degree sodium (0.5 to 1.1 mg/L) and a number of organic acids (2). The phenomenon occurs under certain conditions of soil and atmospheric moisture, which make it difficult to predict. Guttation is more likely when the soil is waterlogged and air is moist enough for evaporation from the leaves to occur and is strongly influenced by plant growth stage (3). The volumes of fluid involved are in the range of µL per leaf.

In 2009 a group of scientists in Italy published evidence showing that guttation fluid produced by plants grown from seeds treated with systemic insecticides, could contain residues of these insecticides and when sugar was added as a phagostimulant to the guttation droplet and fed to honey bees death shortly followed (4). This raised the concern that exposure to neonicotinoid insecticides via guttation water could be a significant route of exposure for honey bees. However, there is evidence to conclude that this is in fact a minor source of exposure (5, 6) due to guttation fluid being of limited interest as a source of nutrition or water to honey bees which was occurring on plants of limited attractiveness. Also the frequency of honey bees returning to the colony with water is rather low (less than 5%) compared to those returning with nectar (7).

Consequently in order to clarify the relevance of guttation fluid as a water source for honey bee colonies and to assess potential associated risks for honeybees under conditions of agronomic practice, various studies were performed by Bayer CropScience in key broad acre crops such as maize, sugar beet, potato, winter barley and oilseed rape. The findings from a range of studies which were performed in comparison between “control hives” and “treatment hive” with appropriate replication are summarised in this paper.

Experimental methods

Preparation and sowing of treated seeds

Field studies to determine the occurrence and effect of exposure to guttation water from neonicotinoid seed treatment products were conducted over a number of years in Germany and France. Studies focused on the five agronomically most relevant seed-treated or soil treated broad acre crops in Europe: winter cereals, winter oil seed rape, sugar beet, maize and potato. The investigated seed loadings reflected authorized rates in the European Union at the time of study conduct. In our experiments, cereal seeds were seed-treated with a combination of imidacloprid (IMD) + clothianidin (CTD) at a rate of 55 g total neonicotinoid a.s./100 kg seeds. Winter oil seed rape seeds were treated with CTD at a rate of 7 g a.s./kg. Sugar beet seeds were prepared as pills with a combination of IMD + CTD corresponding to a rate of 0.9 mg total neonicotinoid/pill. For maize, the seeds were seed-treated with CTD at a rate of 0.5 mg a.s./seed.

Fields were sown so that there was about 110 g total neonicotinoid/ha via seed-treated winter cereals, about 30 g CTD/ha via seed-treated winter oil seed rape, about 120 g total neonicotinoid/ha via treated sugar beet pills and about 50 g CTD/ha via seed-treated maize. For potato, IMD was applied at the rate corresponding to about 180 g a.s./ha via an in-furrow treatment at planting. At control sites seeds of the same crop variety as at the treated sites were sown, but were not treated with neonicotinoid seed- or soil treatment products. In the studies with winter barley, winter oil seed rape and maize, honeybee colonies were present directly adjacent at the edge of fields at the time of sowing and were as such also exposed to seed-treatment dust, generated during the sowing operation.

Replication, location of trials and honey bee colonies

The majority of studies (all except maize) were conducted in Germany at a range of geographical locations and over a period of years to ensure a wide range of natural and typical agricultural conditions. The winter cereal study was replicated five times with five honey bee colonies (in total, 25 colonies in treatment and control, respectively). Studies in sugar beet and potatoes consisted of two neonicotinoid treated and untreated plots, each with eight honey bee colonies per site, so conclusions are based on in total 16 colonies in treatment and control for each crop, respectively. Winter oil seed rape trials were set up so that there were three replicated study plots each for neonicotinoid treated and untreated plots. Five honey bee colonies were placed at each winter oil seed rape location, so conclusions are based on in total 15 colonies in treatment and control. Maize studies were placed at four different regions in France (Alsace, Champagne, Languedoc-Roussillon and Aquitaine) each containing a single neonicotinoid-treated and untreated field with six honey bee colonies each, so conclusions are based on in total 24 colonies in treatment and control.

Average field sizes were 6.4, 5.0, 2.7, 1.7 and 2.2 ha for winter cereals, winter oil seed rape, sugar beet, potato and maize respectively. The smallest field was 1.6 ha (potato) and the largest 11 ha (oil seed rape) reflecting the commercial scales of cultivation. However, giving the rather low water-foraging range of honeybees, field size as such is not a driving factor of exposure (see below).

Study set up and methodology

As there are no internationally recognized methods for the evaluation of the acute and long-term risk to colony survival and development from potential guttation exposure, methods were developed and based upon the most up to date guidance for honey bee field trials OEPP/EPPO Guideline No. 170(4) (2010).

Studies were conducted under standard agricultural conditions with honey bee colonies sited at the edge of either fields sown with insecticide treated or untreated seed. The studies were set up to provide appropriate conditions so that there were no major flowering crops present within 3 km of the test locations and that there were no open water bodies close to the test location or within 300 m to the field, to ensure that the colonies collected any water necessary for their needs from the immediate area as either guttation fluid, dew or rainfall. Due to the high energetic cost of flying, bees will collect water from their immediate vicinity (8).

The studies investigated the following parameters:

- Occurrence and proportion of guttation on the crop and off-crop
- Observation of honey bee visiting the crop and off-crop areas
- Behaviour of the bees in the crop and around the hive
- Honey bee mortality (as mean number of dead bees per colony per day)
- Condition of the colonies (e.g. colony strength, brood, food storage) and health status (e.g. presence and levels of *Varroa*, viruses and other pathogens)
- Overwintering performance of exposed colonies (all except maize)
- Levels of neonicotinoid insecticide residues in guttation fluid (winter barley, winter oil seed rape, sugar beet and potato).

As winter crops are sown in autumn there are potentially two guttation periods to which honey bees could be exposed in a year time; one in autumn shortly after crop emergence and before overwintering and again in the spring after winter hibernation. In the cereal and oil seed rape studies, the same colonies were exposed to both guttation periods. Sugar beets, maize and potato are drilled in the spring and hence have one guttation period during that time. After exposure to guttation the colonies were relocated and monitored at non-agricultural sites.

Results and discussion

At all test locations and for each of the five crops guttation was observed. In winter cereals and winter oil seed rape, guttation was a common occurrence in both the autumn and spring exposure periods. Bees were similarly likely to be active on days where guttation occurred in winter cereals in autumn as they were in spring (Table 1). However, far fewer bees (as a proportion of those observed at the study sites) were observed to be collecting guttation water in the autumn compared to the spring. This can be explained by the fact that in autumn the colonies are declining in size and preparing to overwinter and in the spring colonies are active and increasing in size as egg laying recommences after the overwintering period. Thus, the autumn colonies have a lower demand for resources compared to those in spring. During the autumn guttation occurred frequently in the morning but was generally observed to have declined or decreased on average by midday (winter barley, Hesse). In spring, guttation was a very rare during the evening with only 0.5 – 1.1% incidence.

In contrast, guttation was far less common in sugar beet, potato and maize than observed for winter cereals. Bees were active on days when guttation occurred but were not observed to visit the fields sown with either treated or untreated seed or tubers for sugar beet, potato or maize and bees were not observed collecting guttation water at any time during these experiments from crop plants at either treated or untreated locations. Water from dew and guttation from the off-crop area close to the colonies was observed to be collected in some studies.

Table 1 Exposure of honey bees to guttation fluid

Crop	% of days where guttation was observed	Guttation coincides with bee flight	% of total bees observed that were seen collecting guttation fluid in crop
Cereals (winter wheat and barley)	90% (autumn) 86% (spring)	64% (autumn) 63% (spring)	1.2% (autumn) 14% (spring)
Winter oil seed rape	80% (autumn) 76% (spring)	76% (autumn) 54% (spring)	0.5% (autumn) 5.0% (spring)
Sugar beet	25% (spring only)	Yes	0%
Potato	50% (spring only)	Yes	0%
Maize	68% (spring only)	Yes	0%

Residue analysis of neonicotinoid insecticides (and their metabolites) in guttation fluid produced by winter sown crops (winter barley and winter oil-seed rape) consistently shows that residue levels during springtime are far lower than those observed in autumn, with peak residues at or shortly after emergence. This can be explained by the fact that the older the plants, the more biomass the plants have built up and the more biological dilution occurs; concurrently, the bioavailability of the substances for plant uptake decreases over time and is highest directly after emergence. This becomes particularly apparent in spring, when the plants are older, larger and in a phase of rapid growth, in contrast to the plants in the autumn, when they are about to enter winter. Consequently, while residues are higher in autumn, bees are far less likely to collect guttation water compared to the spring when residues are lower. A systematic approach to residue measurement was taken in winter barley with regular samples being taken in autumn and spring for analysis where sufficient guttation fluid was produced (Figures 1 and 2). A peak residue of 8.5 mg/L of clothianidin and of 6.7 mg/L of imidacloprid was recorded in autumn 2001 which declined to levels often close to the limit of quantification in the following spring, with maximum values of 0.15 and 0.07 mg/L of clothianidin and imidacloprid, respectively. In contrast, e.g. the residue levels in guttation fluid produced by sugar beet plants in spring (i.e. shortly after emergence) were at least an order of magnitude lower than the residues found in guttation fluid produced by winter cereals and oil seed rape in the autumn.

Table 2 Range of concentrations of neonicotinoid insecticides and metabolites in guttation fluid

Crop	Residues in guttation fluid of treated crops (mg/L)	
	Imidacloprid treated (min –max)	Clothianidin treated seed (min-max)
Winter barley	IMD autumn <LOQ – 6.7	CTD autumn <LOQ – 8.5
	IMD spring LOD – 0.068	CTD spring LOD – 0.15
Winter OSR	IMD not tested	CTD autumn <LOQ – 0.41
		CTD spring <LOQ 0.02
		TZNG: <LOD – <LOQ
		TZMU: <LOD – <LOQ
Sugar beet	IMD: 0.018 – 0.061	CTD: 0.15 – 0.33
	IMD 5-hydroxy: 0.007 – 0.016	TZNG: 0.035 – 0.057
	IMD olefin: 0.002 – 0.004	TZMU: 0.036 – 0.053
Sugar beet	IMD: 0.003 – 0.01	CTD: 0.064 – 0.017
	IMD 5-hydroxy: 0.001 – 0.004	TZNG: 0.029 – 0.012
	IMD olefin: <LOQ – 0.001	TZMU: 0.031 – 0.11

Note: The Limit of Quantitation (LOQ) of each analyte in guttation fluid was 0.01 mg/L and the Limit of Detection (LOD) of each analyte was 0.001 mg/L, respectively. IMD = imidacloprid; CTD = clothianidin; TZNG = N-(2-chlorothiazol-5- ylmethyl)-N-nitroguanidine; TZMU = N-(2-chlorothiazol- 5-ylmethyl)-N-methylurea.

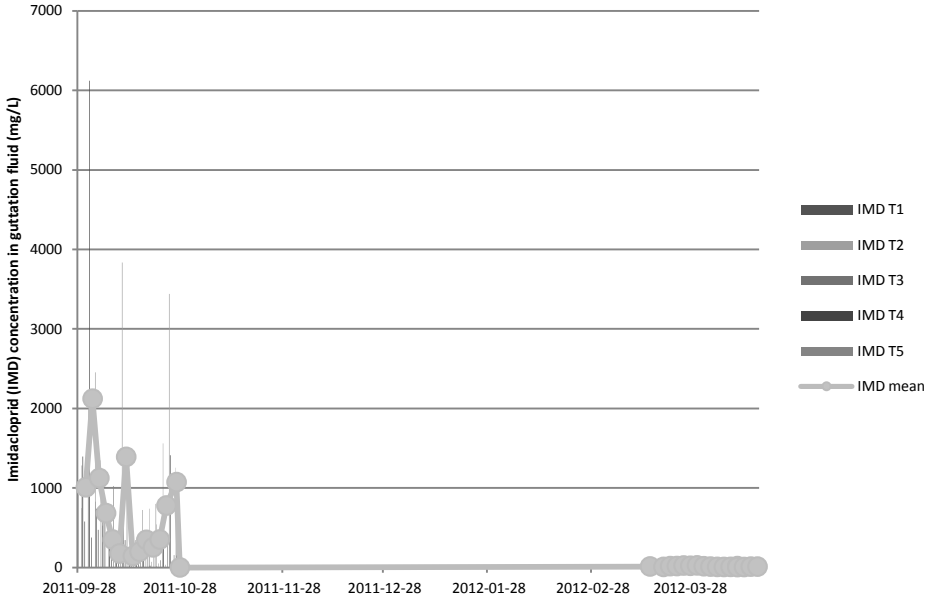

Figure 1 Range of concentrations of imidacloprid in guttation fluid collected in autumn and spring from treated winter cereals (2011/2012). T1 – 5 indicate individual fields, IMD mean is the average concentration of imidacloprid in guttation fluid per day across all 5 fields.

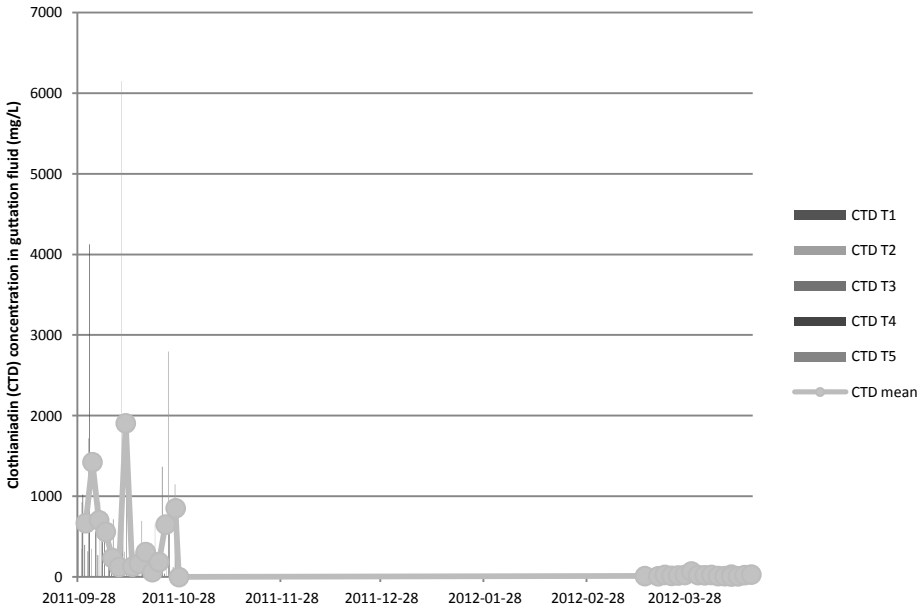

Figure 2 Range of concentrations of clothianidin in guttation fluid collected in autumn and spring from treated winter cereals (2011/2012). T1 – 5 indicate individual fields, CTD mean is the average concentration of clothianidin in guttation fluid per day across all 5 fields.

In the wheat, barley and oil seed rape occasional daily peaks of mortality were observed (in both, treatment and control) and where possible samples of dead bees were analyzed for the presence of neonicotinoid insecticides and metabolites. Very low levels were found or the sample did not contain detectable residues. Overall, no pattern between bee death or residue levels could be established. In addition, average daily mortalities were monitored for an extended period of time (see Table 3), corresponding to the period where guttation was observed in the crop and bees were generally active (i.e. no mortality counts were made during winter, but observations were resumed in detail during early springtime, and potential effects on colony and overwintering performance of the colonies exposed to the autumn-sown crops was assessed). The levels of mean daily mortality were similar at both treated and untreated sites and there was generally more variation between sites than between treatments, indicating that exposure to neonicotinoid insecticide seed treated crops was in the vast majority of all cases not a source of increased mortality over the exposure period or thereafter (when assessed). In all studies, no differences in behaviour were noted between the colonies exposed to treated crops compared to untreated crops and colony strength and health status (e.g. presence of *Varroa*, viruses and other pathogens) were unaffected (data not shown). The rate of overwintering success was also similar between colonies which had been sited at the guttating neonicotinoid insecticide seed treated crops compared to those sited at untreated locations (Table 4). These observations are consistent with those from other published studies where honey bee colonies were exposed to guttation fluid from plants grown from neonicotinoid treated seed under both semi-field and field conditions (9, 10).

Table 3 Mortality of honey bee colonies exposed to guttation fluid from neonicotinoid insecticide seed treated crops

Crop	Location	Duration of exposure (days)	Mean number of dead bees/colony/day	
			Treated	Control
Winter cereals	Germany/Hesse	45 (autumn)	28.4 ± 13.8[a]	36.0 ± 22.7
		54 (spring)	17.9 ± 9.0[a]	17.1 ± 8.2
Sugar beet	Germany/Baden-Württemberg	42	16.6 ± 5.4 [b]	12.9 ± 4.7
Sugar beet	Germany/Baden-Württemberg	40	14.1 ± 3.0 [b]	13.1 ± 2.9
Potato	Germany/Baden-Württemberg	58	13.8 ± 4.9 [c]	16.0 ± 2.8
Potato	Germany/Baden-Württemberg	57	15.8 ± 3.8 [c]	18.5 ± 10.1
Maize	France/Aquitaine	48	12.7 [d]	10.0
Maize	France/Alsace	43	46.3 [d]	29.8
Maize	France/Champagne	36	9.5 [d]	11.4
Maize	France/ Languedoc-Roussillon	32	38.4 [d]	42.6

Notes: aImidacloprid+clothianidin 50 + 87.5 g a.s./100 kg seed; bClothianidin+Imidacloprid 0.6+0.3mg/pills; cImidacloprid in furrow application at 180 g a.s./ha; dClothianidin at 0.5 mg a.s./seed.

Table 4 Overwintering success of honey bee colonies exposed to guttation fluid from neonicotinoid insecticide seed treated crops

Crop	Location	No. colonies overwintering successfully	
		Treated	Untreated
Winter cereals	Germany/Hesse	25/25	25/25
Sugar beet	Germany/Baden-Württemberg	16/16	16/16
Winter oil seed rape	Germany/Baden-Württemberg	15/15	15/15
Potatoes	Germany/Baden-Württemberg	Ongoing	Ongoing

Conclusions

All summarized studies consisted of replicated "treatment colonies" (hives placed adjacent to fields with neonicotinoid seed treatment) and "control colonies" (hives placed adjacent to fields without neonicotinoid seed treatment) within the same landscape to distinguish potential effects from guttation water uptake from other factors affecting colony performance. The studies were set up to provide appropriate conditions so that there were no major flowering crops present within 3 km of the test locations and that there were no open water bodies close to the study site or within 300 m to the field. Taking into account the long exposure period and the generally low bee-attractiveness of early growth-stages, study conditions thus certainly represent worst-case conditions.

Guttation droplets contained peak residue levels theoretically capable of harming individual honeybees (i.e. several hundred ppm) at very early growth stages. Residue levels, however, generally decreased with time, as expected based on the physiological process involved. The temporal coincidence of honeybee flight activity and the presence of guttation droplets was generally limited to early morning hours and to a much lesser extent to evening hours. Spatially, honeybees were found to predominantely collect water, if any, in the direct vicinity of the hives. Water collection generally ceased within a couple of metres distance to the hives, which renders distance to the crop to be a significant exposure factor, and in turn renders dew and guttation from off-crop vegetation to be more relevant to water collecting honeybees than guttation from

the crop. Considering off-crop grassland as likely surrounding for honeybee colonies, this vegetation will always provide more droplets / m^2 than the sown crops at early stage. Mortality events, if any, were scarce and generally matched in treatments and in controls, and the absolute numbers of dead bees involved in these rare cases were so low that they did not translate into any colony level effects or impacts on bee health or overwintering success, nor on adverse effects on honey production of the involved colonies. Given the overall body of data, the associated intensity of the assessments in each study as well as the realistic worst-case exposure conditions employed, it can be concluded that exposure of honeybee colonies to guttation fluid, excreted from neonicotinoid seed-treated crop plants, did not pose an unacceptable acute or chronic risk to honeybee colony development or survival, and does not adversely interfere with bee keeping practices. Overall, guttation water from seed-treated crop plants was found not to be a significant exposure route for honeybees.

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