Guttation and the risk for honey bee colonies (*Apis mellifera* L.): a worst case semi-field scenario in maize with special consideration of impact on bee brood and brood development

Malte Frommberger[1], Jens Pistorius[1], Ina Joachimsmeier[1], Detlef Schenke[2]

[1]Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11-12, 38104 Braunschweig, Germany, Email: jens.pistorius@jki.bund.de

[2]Julius Kühn-Institut, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Strasse 19, 14195 Berlin, Germany

DOI: 10.5073/jka.2012.437.015

Abstract

Background: The possible risk of guttation for bees was investigated in two semi-field studies with maize treated with clothianidin. In a worst-case scenario set-up the effects on adult and brood mortality of bees with special consideration of the brood development of the bees were assessed.

Results: Due to the weather conditions in the first experiment guttation occurred only once, which caused a high mortality and a brood-termination rate of up to 100 % in the worst-case scenario without additional water supply but no clear increase of mortality or brood termination was observed when water was supplied. In the second experiment guttation in maize occurred on 5 of 10 days. The mortality in treated variants with water supply and control variants with untreated seeds was on a similar level and within normal range. The brood-termination rate was in the control below 16 %, in the treatment from 16 to 43 %.

Conclusion: In the first experiment in the variant with treated maize and no additional water supply, an artificial and extreme situation a high impact on mortality and also on the brood development was observed, indicating the sensitivity of the test system but representing an unrealistic worst case scenario.

In variants with treated maize and additional water supply no clear effects on adult mortality and brood were observed in the first and the second experiment.

Keywords: honey bees, guttation, pesticides, clothianidin, seed treatments, brood development

Introduction

Like other organisms, a bee colony needs water to maintain its vital functions. Except availability of water in the beehive and excessive water from collected fresh nectar, the remaining water demand must be collected outside the beehive. Frequent water sources are dew, rain, streams, lakes and also guttation drops.

The use of guttation drops as water source may be a potential route of exposure for bees to systemic active substances used for seed treatment of different crops (1). Residues of clothianidin in guttation drops of small maize plants (treated with Poncho Pro®) may reach up to about 100 mg/l directly after emergence (2). The residue levels detected in guttation drops of maize may result in high mortality if consumed by bees (3). Exposure in the laboratory by adding sugar water to guttation water does not represent realistic exposure for water foraging bees and hive bees, to which these liquid may be passed on. Therefore in a semi-field study it was investigated whether guttation drops may be used as a water source and may pose a potential relevant route of exposure. In two experiments with clothanidin treated maize the effects on adult bees and bee brood with or without access to alternative, uncontaminated water sources were investigated.

Experimental methods

Two succeeding experiments were performed under semi-field conditions in Lucklum (Braunschweig, Germany), in seed treated (a.s. clothianidin, Poncho Pro®, 0.5 mg/kernel) and untreated maize crops. Four tents (96 m^2, 16 x 6 m) were set up on the treated, 2 tents on the control plot, covered with a gauze permeable for wind and rain but impenetrable for bees. The study was repeated twice, in the first experiment (BBCH 13-15) with two treatment variants, one with and one without artificial water source containing uncontaminated tap water and one control for each variant. In the second experiment (BBCH 15-19) at the same field location, all 4 colonies in the treatment and 2 in control had an additional uncontaminated water source.

Bee colonies used were of similar size with approximately 10.000 bees, (one-room, 'Zander'), had an oviparous one year old queen bee and contained sufficient honey and pollen stores in the hives. The bees were allowed to forage on additional sugar feeding paste and pollen sources provided *ad libitum* in the tents.

The mortality of bees was assessed daily in dead bee traps and on linen sheets in the crop. The flight activity and behaviour of bees at the hive entrance and in three flight squares were determined once daily. The observation period in the tents was 11 (1st run) and 10 (2nd run) days. An observation of brood development of 100 individual brood cells per hive was conducted for about four weeks following the protocol of Schur et al., 2003 (4), using a digital brood assessment system. The evaluation of the cells was done with a test version of digital image processing software (RIFCON GmbH). Over the whole study the occurrence of guttation was documented and guttation droplets were sampled daily for residue analyses. After the exposure period the bees were relocated for observation of further brood development and placed at the Julius Kühn-Institut near the city of Braunschweig with very few agriculture in the surrounding.

Results

In the first experiment the study was performed in six flight tents in maize (BBCH 13-15) with one bee hive per tent. Two treatment tents with and two without artificial water source containing uncontaminated tap water, one control for each variant in the untreated field side.

Due to the weather conditions in the first experiment guttation of maize occurred only once, after small amount of precipitation (<1mm) occurred in the night from the 27th to very early morning of the 28th of May. Guttation droplets were immediately used by the bees which had no access to any water outside the hive for 3 days. The use of guttation droplets as water source caused a high adult bee mortality of more than 1000 bees/hive and day (colony 1: 1900, colony 2: 160) in the waterless variant (Fig.1). The mortality was clearly increased for several days. In colonies set up in tents in treated maize, which had access to guttation droplets containing residues and to an uncontaminated, alternative water source no increase in mortality compared to the control was observed.

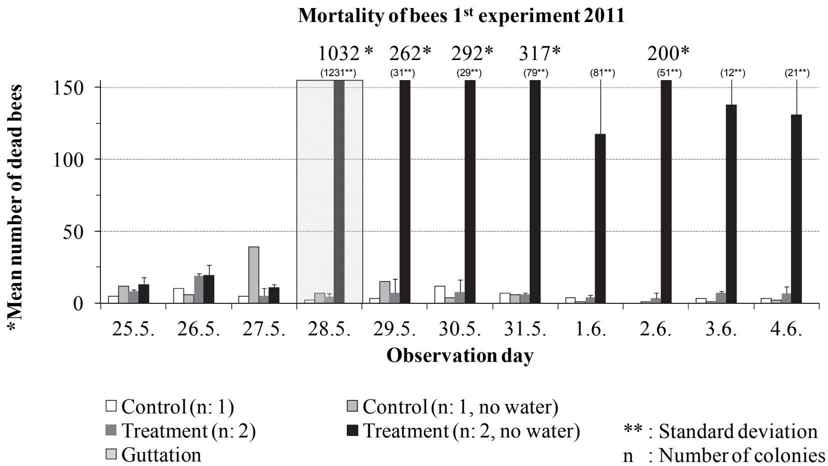

Fig. 1 Effects on adult mortality in the different variants of the 1st experiment. One treatment and control without an additional water source. Guttation took place only on the 28th.

It seems unlikely that all 1900 bees of colony 1 were actively collecting guttation droplets directly, thus it can be assumed that water was passed on rapidly also to other hive bees. The residues in sampled guttation droplets were between 0.010 and 0.018 mg/l clothianidin in the control and 7.953 to 46.55 mg/l clothianidin in treated variants. The residues detected in dead bees were between 0.021 to 0.079 mg/kg in variants without water source and below limit of detection in the control. Colony 2 had a lower but still clearly treatment related highly increased mortality. The difference between the two colonies may be assigned due to individual water use and water need of the colonies. In dead bees, high residues even higher than the oral LD_{50} were detected (Table 1).

Tab. 1 Residues detected in dead bees in the first experiment in control and treated variants without artificial water source

Date	Variation	Residues of clothianidin (mg/kg)	ng/bee
28.05.2011	Treatment hive 1, no water	0.037	3.79
	Treatment hive 2, no water	0.079	7.80
29.05.2011	Treatment hive 1, no water	0.022	2.37
	Treatment hive 2, no water	0.021	2.02
29/30.05.2011	Control hive 1+2	Not detected	Not detected

The condition of the colonies and the amount of adult bees, eggs, young larvae, capped brood, nectar and pollen was assessed one day before set up in the tents and a second time after the exposure period, 16 days after first colony assessment. At the start of the study the colonies showed an equal distribution of the different brood stages and the total covered area was also on a similar level in the six colonies. At second colony assessment in the first experiment the area of capped brood cells of the waterless variant (treatment) was decreased compared to the other variants.

As the brood termination rate ranged up to 100 % in the waterless variant and also in both control variants of the first experiment, the results are of limited value. The confined semi-field conditions may have caused stress for the colonies, which might have been the reason for a high or total termination rate. Nevertheless, it can neither be concluded nor be fully excluded that some treatment related brood mortality occurred in this trial.

The second experiment of the study was performed at later BBCH (15-19) development of maize with four treatment and two control tents (in the untreated field side), all with an additional artificial water source containing uncontaminated tap water. Bee colonies were of similar size (adult bees and bee brood) like in the first run.

In the second experiment guttation of the maize occurred on 5 of 10 days. The mortality in treated and control variants was on 3 days with guttation at a similar level and within normal range and on 2 days slightly increased in the treatment (Fig. 2). The residues of guttation droplets sampled in tents were between 0.006 to 0.008 mg/l clothianidin in the control and 0.205 to 1.710 mg/l clothianidin in treatment.

In second experiment the condition of the colonies and the amount of adult bees, eggs, young larvae, capped brood, nectar and pollen was assessed two days before set up in the tents, and a second time after the exposure period, 11 days after first colony assessment. The colony strength in the control and treated variants were at a similar level and in a normal range.

In all variants of the second run, most marked eggs reached the expected stages up to the last BFD and hatched successfully (Fig.3). A termination rate under 30% is considered to be in the normal range in confined semi-field conditions. In one of the 4 treated variants a moderate increase of the termination rate was observed between BFD +5 and BFD +11 (29th of June).

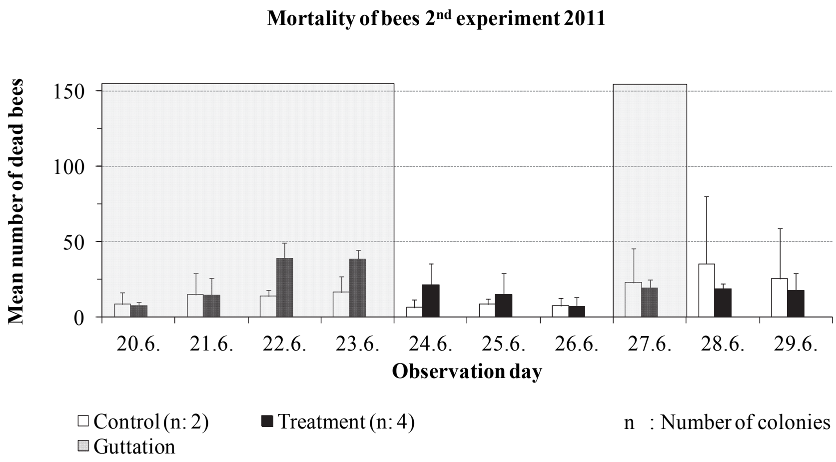

Fig. 2 Adult mortality in the treatment and control variants of the 2nd experiment. All variants with an additional uncontaminated water source. Guttation was present from 20th to 23rd and on the 27th of June.

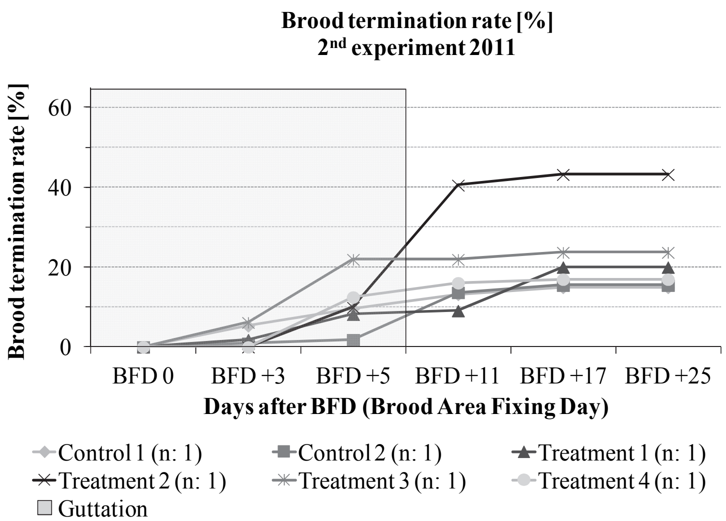

Fig. 3 The brood assessment of the 2nd experiment reflected the termination rate between BFD 0 at the beginning and BFD +25 at the end of the trial. Guttation was present from 20th to 23rd (BFD+5) and on the 27th of June.

Conclusions

In the first experiment, bees suffered lack of water in the artificial extreme situation without any additional water supplies for three days before first occurrence of guttation. As guttation droplets, which contained high residues of the active substance used as seed treatment, first occurred they were immediately used by bees as a water source. A high impact on adult mortality was observed especially in one of the two colonies of this treatment group, which can be clearly linked to the uptake of guttation water from maize plants. It can also be concluded that some water foraging bees collected guttation drops and managed to pass these on to other hive bees and potentially also to bee brood. A very high termination rate was observed in the two waterless variants by the assessment of 100 individual brood cells per hive. But the reason for this keeps unclear, because of a high termination rate also in the control variants. In the colony assessment of the first experiment the area of capped brood cells of the waterless variant (treatment) was decreased compared to the other variants.

The first experiment demonstrated the sensitivity of the test system but represents a unrealistic worst case scenario. In variants with treated maize and additional water supply, no effects on adult mortality and brood mortality and brood development were observed. In the second experiment, no such high increase of mortality was observed as all variants had an additional water source. A moderate increase of brood termination rate was observed in one out of four colonies. The potential exposure to clothianidin (residue in guttation) was clearly lower in the second experiment, though still being present at toxic levels which would result in lethal effects on adults after consumption of only a few microlitres of guttation fluid per bee.

To assess the potential risk for bee colonies as used by beekeepers, further field studies should be conducted to investigate if an increase of mortality and effects on bee brood development may occur in realistic field conditions caused by systemic and bee toxic substances in guttation.

Acknowledgements

RIFCON GmbH for supplying a test version of digital brood evaluation software.

References

1. Wallner K, Guttation: Tropfen, die es in sich haben. Deutsches Bienen-Journal 4: 18-19 (2009).
2. Schenke, D; Joachimsmeier, I.P; Pistorius, J; Heimbach, U: Verlagerung von Pflanzenschutzmittelwirkstoffen aus behandeltem Saatgut in Guttationstropfen – Erste Ergebnisse, Julius-Kühn-Archiv 428, 131 (2010).
3. Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, Greatti M, Giorio C and Tapparo A, Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: A novel way of intoxication for bees. J. Econ, Entomol. 102 (5): 1808-1815 (2009).
4. Schur A, Tornier I, Brasse D, Mühlen W, Von der Ohe W, Wallner K, Wehling M: Honey bee brood ring-test in 2002: method for the assessment of side effects of plant protection products on the honey bee brood under semi-field conditions; Bulletin of Insectology, ISSN 17218861, 56 (1), 91-96 (2003).