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Investigations on neonicotinoids in guttation fluid of seed treated sugar beet: Frequency, residue levels and discussion of the potential risk to honey bees

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ABSTRACT

Little is known about the likelihood of occurrence of guttation fluids in sugar beet, and the extent to which its occurrence is related to weather conditions. There is also uncertainty regarding the extent to which the concentration of neonicotinoid residues in guttation fluid of seed treated sugar beet represents an environmentally relevant route of exposure for honey bees. In our study in 2009 and 2010, guttation monitoring, sampling and analysis of neonicotinoid residues in guttation fluids were conducted on 31 field sites consisting of differing landscapes with frequent cultivation of sugar beet. High concentrations of neonicotinoids in the guttation fluid up to 9 mg clothianidin L^{-1} were recorded, levels that would result in lethal effects to honey bees consuming such droplets. However, we demonstrated that the occurrence of guttation in sugar beet was very rare especially compared to the occurrence of guttation in the vegetation adjacent to sugar beet fields or the occurrence of dew. Therefore, we concluded that guttation fluids from seed treated sugar beet might not be a preferred water source for water foraging bees. Thus, an exposure of honey bee colonies to neonicotinoids dissolved in guttation fluids of seed treated sugar beet is very unlikely.

1. Introduction

In sugar beet (*B. vulgaris* L.) cultivation, systemic substances from the group of neonicotinoids are used as insecticides in seed coatings to protect the crop against many pests and associated diseases (Märländer et al., 2003). Currently, three neonicotinoids (imidacloprid, thiamethoxam, clothianidin) are used at different dosages in seed coatings of almost all sugar beet seeds in Germany (Buhre et al., 2014). These neonicotinoids are classified as highly toxic for bees (Iwasa et al., 2004; EFSA, 2013a, b, c), but used in seed coatings they have long been considered to pose an acceptable risk for bees because negligible or no exposure of beneficial organisms to these active substances was assumed. In 2013, the European Commission suspended the use of imidacloprid, clothianidin and thiamethoxam in seed treatments of several crops because the European Food Safety Authority (EFSA) identified "in particular high acute risks for bees from exposure via dust as regards several crops, from consumption of residues in contaminated pollen and nectar as regards some crops and from exposure via guttation fluid as regards maize" (EU regulation No 485/2013). Sugar beet was not affected by this ban because dust emissions during drilling are minimal and they do not flower if grown for sugar production. However, so far little is known about guttation of sugar beet.

Guttation is defined as the excretion of xylem fluid (hereinafter referred as guttation fluid) in form of droplets along the edges or tips of plant leaves (Bresinsk et al., 2001). This process naturally occurs in many plant species, especially in monocotyledons, when leaves are not able to transpire because the dew point of the surrounding air is reached. A wide range of crops produce guttation fluid, e.g. maize, barley, rye, wheat, oilseed rape, sunflower and rice (Lippmann, 1925; Biles and Abeles, 1991; Goatley and Lewis, 1966; Magwa et al., 1993).

The composition of guttation fluid from untreated plants depends on its way of excretion (passive and/or active hydathodes) and the growth stage of the plant or leaf (Stein-Dönecke, 1993; Ehrhardt, 1978). Goatley and Lewis (1966) found that guttation fluid from untreated wheat or barley predominantly contains inorganic substances (nutrients) such as carbonate, several xylem mobile sugars such as xylose and arabinose (total content of sugar: 27.1 mg L^{-1} (wheat), 59.8 mg L^{-1} (barley)). Klepper and Kaufmann (1966) calculated that

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these concentrations were much lower than those circulating in the vascular tissues of the plants. Considering the threshold values for sugar response of honey bees determined by von Frisch (1927, 1928, 1930) none of these components can be considered as a source of sugar for honey bees. However, honey bees do not only forage nectar and pollen, but also collect water (Lindauer, 1954). Nicolson (2009) for example concluded that the importance of water foraging on the colony level is particular for two main reasons: for cooling of the brood area (Winston, 1987) and diluting the stored honey from late winter to early spring. Water is also required for the preparation of larval food and the supply of minerals (Piscitelli, 1959). Contrary to nectar and pollen, the collected water is not stored in the bee hive and therefore, has to be collected when needed (Seeley, 1997).

Depending on location of hives, various water sources can be available. In most cases the colony's water need is met by collection of fresh nectar or water condensed within the bee hive (Kuehnholz and Seeley, 1997). However, during periods with low amounts of nectar entering the bee hive further water sources may be used more frequently. These sources can be permanent ones such as lakes, ponds and ditches or be of a spontaneous nature for example drew-, guttation- and raindrops. Whether bees show a certain preference for a specific water source has so far not been investigated. However, several experiments in maize conducted in Germany and Italy demonstrated that systemic substances in seed treated plants, can be excreted via guttation fluid in concentrations relevant for honey bees (Wallner, 2009; Girolami et al., 2009; Joachimsmeier et al., 2010; Schenke et al., 2010, 2011; Reetz et al., 2011; Tapparo et al., 2011) whether or not guttation fluids of seed treated sugar beet contain harmful amounts of systemic neonicotinoids and whether or not bees are attracted to collect water from the guttation fluid of sugar beet is not known. Moreover, the frequency of guttation events in seed treated sugar beet is also unknown. This factor is particularly important to define the probability of bees being exposed to guttation fluid. Therefore, in 2009 and 2010, a monitoring of the occurrence of guttation in sugar beet fields as well as in the adjacent vegetation was performed, initiated by the Julius Kühn-Institute together with the Institute of Sugar Beet Research and the German National Meteorological Service (DWD).

The aim of this study was to (i) determine guttation frequency in sugar beet in contrast to adjacent vegetation (mainly grasses), (ii) quantify the concentration of active substances (imidacloprid, clothianidin, thiamethoxam) in guttation droplets at different times during the vegetation period and (iii) find causal relationships between weather data and occurrence of guttation in sugar beet or adjacent vegetation to forecast occurrence of guttation in sugar beet. Furthermore the probability of honey bees being exposed to guttation fluid for seed treated sugar beet was considered in order to assess the potential risk posed to the bees by this fluid.

2. Material and methods

2.1. Study sites and duration

In 2009, 2010, observations of guttation on sugar beet (*B. vulgaris*) and their adjacent vegetation were carried out on 29 field sites in Germany and on one site in the Netherlands and one in Sweden. All sites were situated in typical sugar beet cultivation areas (soil values: 25–90). The sugar beet was conventionally cultivated without any irrigation and treated with insecticide seed coatings containing neonicotinoids (Table 1).

Field observations were carried out from plant emergence (20th April the earliest) to canopy closure of the plants (24th July the latest). Observations of guttation on sugar beet were conducted twice a week (2009) or daily (2010) once a day at early morning (around 6.00 am). This survey period was pre-set since previous greenhouse tests had shown that guttation occurs in all crops mainly early in the morning and at young growth stages (Joachimsmeier et al., 2012). The growth stages of the sugar beet were estimated during every observation using the BBCH Monograph (Meier et al., 1993).

2.2. Experimental set-up

The occurrence of guttation and dew was monitored within four 1 m^2 blocks (approx. 8–10 plants per block) in sugar beet fields and within one 1 m^2 block in the adjacent vegetation (mainly grasses). Within sugar beet fields, two blocks were established in the area of the headland (compact soil), the other two blocks outside of the headland within the field. In case guttation of sugar beet was detected, guttation fluid was picked up for subsequent residue analysis from the edges of the leaves using a Pasteur pipette. If possible, a total volume of 50 µl guttation fluid was sampled form several plants and stored in Eppendorf vessels at −18 °C until residue analysis.

2.3. Climatic conditions

In 2009, weather data from local weather stations for the German meteorological service (DWD) were provided. In 2010, each field was equipped with an individual weather station (DALOS 535-M, F&C GmbH) placed directly in the experimental sugar beet field. In both years, the parameters air/soil temperature and relative humidity were considered. On rainy days, monitoring of guttation was usually not possible. Thus, the number of observation days at different sites varied. In total, guttation in sugar beet was investigated in 758 observations, guttation in adjacent vegetation in 660 observations.

2.4. Residue analysis

The thawed guttation fluid was mixed with the same volume of internal standard solution (imidacloprid-d_4 in acetonitrile) and thiamethoxam, clothianidin and imidacloprid were analysed by liquid chromatography coupled with tandem mass spectrometry - LC-ESI/MS/MS (PerkinElmer Series 200 - API 2000 Applied Biosystems in 2009 and Dionex Ultimate 3000 QTrap 5500 Applied Biosystems in 2010). The limit of detection (LOD) was 0.005 mg L^{-1} for all three substances in 2009. In 2010 the LOD for imidacloprid was 0.0005 mg L^{-1} and 0.0001 mg L^{-1} for clothianidin and thiamethoxam, with a signal-noise ratio > 3:1 in both years.

Since the speed of metabolism of imidacloprid and thiamethoxam is rather high (Nauen et al., 2003) their metabolites were also analysed and later added to the values analysed for their parent substances considering their molecular mass. However, only degradation products, which are potentially relevant for bees, were analysed in 2010 (parent substance: imidacloprid with the metabolites imidacloprid-olefin (LOD: 0.001 mg L^{-1}) and imidacloprid-5-hydroxy (LOD: 0.0005 mg L^{-1}); parent substance: thiamethoxam with the metabolite clothianidin). Overall 44 samples of guttation fluid were analysed (Table 1).

2.5. Statistical analysis

All statistical evaluations were carried out with the R software version 3.0.2 (R Core Team, 2015). Generalized linear mixed effects models (*glmer*) using the family class 'binomial' were employed using the package lme4 (Bates et al., 2015). Model selection and validation to estimate effects on the presence of guttation in sugar beet and adjacent vegetation were conducted following Zuur et al. (2009) in separate models (model 1: guttation in sugar beet, model 2: guttation in adjacent vegetation). The global binomial model 1 was performed with block location (headland, within the field), presence of dew, BBCH, air and soil temperature and relative humidity as fixed explanatory variables as well as environment nested within sampling day as random factors. The variables block location, presence of dew, BBCH and soil temperature were excluded from the initial model 1 for guttation in sugar beet either due to its collinearity with other explaining variables or due to its non-