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Original article

Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees?

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Abstract – The immune system of bees is influenced by a diversity of factors, some of which have changed in the last 10 years such as the application of pesticides. In addition to pollen, nectar and dust, guttated water of seed-dressed plants might be a new source of contamination to bees. Our experiments demonstrated that guttated water of plants germinated from seeds dressed with neonicotinoids contains neonicotinoids. Maize seeds treated with clothianidin (Poncho® 0.5 mg/seed and Poncho® Pro 1.25 mg/seed) resulted in neonicotinoid concentrations up to 8,000 ng mL^{-1} in the guttated fluid. This concentration decreases rapidly, but remained detectable over several weeks. Seeds treated with Poncho® Pro did not result in higher concentrations in guttated droplets in the first stages of plant development, but the concentration decreased more slowly. Triticale seed treated with imidacloprid contained small quantities of this active agent (up to 13 ng mL^{-1}) in the guttated fluid the following spring after overwintering. During the sampling of guttation fluid, no bees were observed collecting these droplets from triticale or maize. To evaluate the attractiveness of guttation fluid exuded from seed-treated plants under field conditions, more studies are required.

seed coating / neonicotinoids / guttated fluid / *Apis mellifera* / LC-HR-MS

1. INTRODUCTION

The new generation of nicotine-related insecticides, sometimes referred to as neonicotinoids, possess either a nitromethylene, nitroimine or cyanoimine group. Nitromethylenes are derived from the 2-(nitromethyl)pyridine structure, which itself shows weak insecticidal activity, and other similar products such as nithiazine that could not be marketed commercially because of high photo-instability. However, extensive synthesis work in the early 1980s at Nihon Tokushu Noyaku Seizo KK led to the discovery of imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine] with a biological efficacy against the rice green leafhopper *Nephotettix cincticeps* 125-fold greater than nithiazine and a 10,000-fold higher insecticidal activity than (S)-nicotine (Jeschke and Nauen 2008). In addition, imidacloprid and other insecticides from the neonicotyl group such as clothianidin, thiacloprid and thiamethoxam possess enhanced photo-stability and act on the insect central nervous system as potent agonists of the post-synaptic nicotinic acetylcholine receptors (*n*AChRs). Neonicotinoids, such as thiamethoxam, show long-lasting residual activity (Maienfisch et al. 2001). Liu et al. (1993) revealed that the relative toxicity of several neonicotinoids correlate closely with their relative

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affinity for insect *n*AChRs, which suggests that they are likely to represent the principal site of action of these compounds. As a result of the differing mode of action, there is no cross resistance to conventional long-established insecticide classes, and therefore, the neonicotinoids have begun replacing pyrethroids, chlorinated hydrocarbons, organophosphates, carbamates and several other chemical classes of insecticides used to control insect pests on major crops. Currently, the neonicotinoid family accounts for worldwide annual sales of around US $1.56 billion representing nearly 17% of the global insecticide market (Jeschke and Nauen 2008).

In general, systemic insecticides provide the treated plants with prolonged protection from sucking and biting insects like some Heteroptera, Coleoptera and Lepidoptera (Iwasa et al. 2004) as well as soil-dwelling larvae (e.g. larvae of western corn rootworm *Diabrotica virgifera*). The systemic behaviour in plant tissues is based on the high water solubility of the neonicotinoid compounds. When applied, the active ingredient disperses into all plant tissues during the growth and degrades slowly. This xylem mobility is an essential prerequisite for continuous translocation of the active ingredients to the youngest plant tissue. Furthermore, their versatility in application methods leads to high demand in agricultural practice: they can be sprayed on crops in the conventional manner and also can be applied as a seed dressing for many crops. Spraying these insecticides on flowering crops may threaten foraging bees during their flying activity, and the vitality of the colony and the brood might be influenced by consumption of contaminated pollen and nectar (Villa et al. 2000). One major ecological advantage of seed dressing is the drastic reduction in the contaminated area (only few hundred square metres compared to 10,000 m^2 on hectare basis from spray applications). When dressed seeds are applied into soil, the active ingredients go in solution due to soil moisture, and the growing roots take up the systemic protective compounds. In contrast to sprayed application of topical insecticides, the systemic seed dressings are more long-lasting because of the continuous uptake of active ingredients. Therefore, they may have more potential for chronic and sublethal intoxication of bees (Suchail et al. 2001). When registering pesticides for use, a thorough review is conducted to determine if, under good agricultural practice, compounds can reach nontarget organisms, such as honeybees. Although this risk assessment was calculated for the use of neonicotinoids as seed dressing, sowings with neonicotinoid-dressed seed has caused damage on bees. In 2008, over 700 beekeepers with around 12,000 hives in the Rhine Valley, Germany, were affected by contaminated dust during the sowing of maize (Rosenkranz and Wallner 2009).

Another threat to honeybees may be through the guttated water of plants treated with neonicotinoids. Guttation is an active process occurring in plants, when leaves are not able to transpire because the surrounding air reached the dew point (Harries 1999). To keep up the internal water flow, usually driven by transpiration, it is essential for plants to exude water at the leaf tips as guttation. This phenomenon occurs in many monocotyledons and dicotyledons. Even fungi exude water in this manner. In experiments with a xylem-mobile fungicide, significant quantities of the fungicide were excreted with the guttated fluid of winter wheat seedlings (Harries 1999). It is unclear whether honeybees use the collected water, and if they do, for what purpose, e.g. for climate regulation inside the beehive or feeding the brood. Nevertheless, the exposure of bees to systemic insecticides via guttation must be considered.

Honeybees live in colonies in an interdependent relationship, which means they depend on each other for survival. They play an important part in pollination, but today's landscape is agrarian-oriented and that is why honeybees are increasingly influenced by contamination with pesticides. Employing environmental risk assessments, ingredients could be categorised in relation to their environmental risk. The relation between the predicted no-effect concentration (PNEC), calculated by the no-observed effect level concentration (NOEC), and the predicted environmental concentration (PEC) indicates

whether there might be a risk to the environment. If PEC is higher than PNEC, it can be assumed that undesirable side effects could occur.

It is worthy to note that pesticide effects on bees may be associated with colony collapse disorder (CCD). This is a described phenomenon in the USA of dead and dying bee colonies, which is characterised by specific symptoms, including rapid loss of adult worker bees and the lack of dead worker bees within and surrounding the affected hives (vanEngelsdorp et al. 2009). Numerous causes of CCD have been proposed, but supporting data are often missing (Oldroyd 2007). Several parameters may be responsible, such as honeybee mites (*Varroa destructor*), pathogens such as apian viruses, and pesticide residues. Other factors such as poor nutrition or stress related to environmental change and migratory beekeeping are also under discussion (vanEngelsdorp et al. 2009). The sub-lethal exposure to pesticides can weaken the bees' immune system hampering the ability of honeybees to fight off miscellaneous infections (Desneux et al. 2007). Up to now, no data are available concerning the long-term effects of low concentrations of neonicotinoids in guttated water collected by honeybees.

This paper deals with monitoring the concentration of neonicotinoid insecticides in the guttated droplets of selected monocotyledonous crops beginning in spring, when colony growth starts and colony water requirement increase due to the amount of uncapped brood. Imidacloprid is known to affect bees' cognitive behaviour such as the proboscis extension reflex (Decourtye et al. 2005). In addition to imidacloprid, clothianidin is another active ingredient with high efficacy against insects and is also used for seed coating in various crops. Cutler and Scott-Dupree (2007) report that clothianidin-treated canola had no long-term effect on honeybees. However, in view of the contact LD_{50} of 44 ng/bee and the oral LD_{50} of 3.8 ng/bee for clothianidin (Schmuck and Keppler 2003), it may have an acute toxic impact if bees become contaminated. It is still unclear if there is a risk to the colony if the concentration of neonicotinoids in guttated water is high.

2. MATERIALS AND METHODS

2.1. Experimental site

The guttation experiments were carried out in the experimental area “Heidfeldhof” of the Institute of Plant Breeding (University of Hohenheim) located in Stuttgart, Germany. The site is situated at 400 m above sea level, with a mean total annual precipitation of 685 mm and mean annual temperature of 8.5°C. The prevalent type of soil is luvisol (WRB) with partial silt.

Four colonies of bees (*Apis mellifera carnica* P.), from stock of the Apicultural State Institute (University of Hohenheim) that were successfully wintered and comparable in development, were placed at the “Heidfeldhof” field site. Today's landscape is marked by intensive agriculture, and it is more or less impossible to position hives with no contact to agriculture. Apart from canola (*Brassica napus* L.), various types of grains, e.g. triticale (x *Triticosecale* M.) and maize (*Zea mays* subsp. *mays* L.) are major crops in German agriculture. Experimental plots of wheat and maize were within flying distance of the apiary so that field observations of water foraging bees during the sampling of guttation fluid were possible.

2.2. Plant material

2.2.1. Triticale experiments

Seeds of triticale were treated with a combination of the insecticide Gasur® (per seed—1.75 mg imidacloprid, Bayer CropScience AG, Leverkusen, Germany) and the fungicide Efa® (per seed—0.06 mg triazoxide, 0.0225 mg tebuconazole, 0.225 mg fluoxastrobin, 0.15 mg prothioconazole; Bayer CropScience AG, Leverkusen, Germany).

The surrounding triticale was treated only with the fungicide Landor® (per seed—0.001875 mg fludioxonil, 0.0015 mg difenoconazol, 0.000375 mg tebuconazol). Seeds were not treated with neonicotinoids and can therefore be regarded as a control. Sampling of the surrounding area was carried out randomly. Sowing took place on 07 October 2008 into a field prepared with broad bean *Vicia faba* L.

2.2.2. Maize experiments (four different seed treatments)

This experiment included four different seed treatments:

1. Maxim XL® (per seed—0.00625 mg fludioxonil, 0.0025 mg metalaxyl-m; Syngenta International AG, Basel, Switzerland), Thiram® (per seed—0.6 mg thiram; Bayer CropScience, Leverkusen, Germany), Mesurol® (per seed—1.5 mg methiocarb; Bayer CropScience, Leverkusen, Germany)
2. Poncho® (per seed—0.5 mg clothianidin; Bayer CropScience AG, Leverkusen, Germany), Maxim XL® (per seed—0.00625 mg fludioxonil, 0.0025 mg metalaxyl-m; Syngenta International AG, Basel, Switzerland), Thiram® (per seed—0.6 mg thiram; Bayer CropScience, Leverkusen, Germany), Mesurol® (per seed—0.75 mg methiocarb; Bayer CropScience, Leverkusen, Germany)
3. Poncho® Pro (per seed—1.25 mg clothianidin; Bayer CropScience AG, Leverkusen, Germany)
4. Poncho® Pro (per seed—1.25 mg clothianidin; Bayer CropScience AG, Leverkusen, Germany), Maxim XL® (per seed—0.00625 mg fludioxonil, 0.0025 mg metalaxyl-m; Syngenta International AG, Basel, Switzerland), Flowsan FS® (per seed—0.64 g thiram; TAMINCO N.V., Gent, Belgium), Mesurol® (per seed—0.75 mg methiocarb; Bayer CropScience, Leverkusen, Germany)

In each lot, 80 grains of *Zea mays* subsp. *mays* L. were seeded in four rows. No untreated maize was between the lots.

The surrounding maize seeds were treated only with Mesurol® flüssig (methiocarb; Bayer CropScience, Leverkusen, Germany) and TMTD 98% SATEC® (thriam; SATEC international GmbH, Rheinbreitbach, Germany) and were regarded as a neonicotinoid-free control.

Sowing was on 25 April 2009 into a field prepared with clover and pasture.

2.3. Sampling

Guttation fluid was investigated from April to July 2009. The exact sampling was a function of the state of plant growth. Guttated fluid was sampled using pasteur pipettes (VWR International, Germany), equipped with rubber bulbs (Roth, Germany). Single drops were transferred into small glass vials (1.5 mL, Trott, Germany) and were sealed with screw caps after sampling. The guttated fluid was sampled from various plants within the single experimental lots. The maximum fluid amount was 3 mL. The sampling interval was 2 days when the weather conditions were suitable. Glassware was used only once and disposable gloves were used when sampling in order to avoid carry-over effects. The samples were stored in the dark at 0–8°C.

2.4. Residue analysis

Samples of 50 μL of guttated water were mixed with 10 μL of internal reference standard imidacloprid-d4 (10 μL of 1 μg mL^{-1} in methanol).

2.4.1. Chromatographic and mass spectrometric measurement

Compounds were identified and quantified by LC-HR-MS (LTQ-Orbitrap spectrometer, Thermo Fisher Scientific, Bremen, Germany). Retention times and exact masses were consistent with the reference standards (Sigma-Aldrich, Steinheim, Germany). Separation was performed on a Surveyor-LC HPLC system (Thermo Fisher Scientific, Bremen, Germany) with a quaternary, low-pressure mixing pump with vacuum degassing, an autosampler with temperature-controlled tray (T=8°C) and a column oven (25°C). Injection volume was 10 μl. For mass spectrometric detection, nitrogen was used as sheath gas (six arbitrary units). The separations were performed by using a Nucleodur Gravity column (1.8 μm, 3× 50 mm) (Macherey-Nagel, Düren, Germany) with a H_2O (+10 mM ammonia acetate and 0.1% HCOOH) (A)/methanol (+0.1% HCOOH) (B) gradient (flow rate 0.4 ml min^{-1}). Samples were analysed by using a gradient programme as follows: 100% A isocratic for 1 min, linear gradient to 100% B within 9 min. After 100% B isocratic for 11 min, the system was returned to its initial condition (100% A) over 1 min and then equilibrated for 6 min. The spectrometer was operated in positive mode (one spectrum per second; mass range, 80–400) with mass resolving power of 60,000 at m/z 400 with a scan rate of 1 Hz. Automatic gain

control was applied to provide high-accuracy mass measurements within 2 ppm deviation using one internal lock mass; m/z 391.284290; bis-(2-ethylhexyl) phthalate. Compounds were monitored at their exact masses, imidacloprid (m/z of 256.059–256.060; Rt 8.43 min), imidacloprid-d4 (m/z of 260.084–260.085; Rt 8.43 min), thiacloprid (m/z of 253.0305–253.0315; Rt 9.25 min), clothianidin (m/z of 250.0150–250.0170; Rt 8.55 min) and thiamethoxam (m/z of 292.026–292.028; Rt 7.84 min). Various concentration levels of the analytes (standard and spiked matrix solutions) ranged from 1 to 100 ng mL^{-1} were run to measure the detection limits of the compounds. The limit of quantitation of the target analytes was defined as corresponding to a signal-to-noise ratio of 10:1 (imidacloprid 5 ng mL^{-1}, thiacloprid 5 ng mL^{-1}, clothianidin 10 ng mL^{-1} and thiamethoxam 30 ng mL^{-1}). External calibration alongside the internal standard was performed at the concentration levels of 0.5, 2, 10, 50, 200, 1,000 and 5,000 ng mL^{-1} in water/methanol (80:20, *v*/*v*).

3. RESULTS

During the sampling of guttated fluids at the field site "Heidfeldhof", water-collecting bees were observed collecting the exuded fluids from *Potentilla* plants along the edge of the plot (Figure 1). These observations illustrate that guttated fluid is attractive to honeybees as a source of water for the colony, although no bees were observed collecting guttated fluid from triticale or maize.

3.1. Residues in guttation fluid

3.1.1. Triticale experiments

A total of 28 samples of guttated fluid were collected from April 1 to June 17, 2009. The samples were collected after germination until grains were still green with lactic content. The guttation fluid exuded by triticale, germinated from imidacloprid-treated seed, showed concentrations in the range up to 13 ng mL^{-1} imidacloprid (Figure 2). Translocation of neonicotinoids in a wintered triticale field was monitored, but no trend was observed. This may be because the first guttation fluids after germination were not sampled. Weather conditions like rain influenced the measured concentrations in guttated fluid so that concentrations were not constantly linked with each other.

In guttated fluid of the surrounding triticale, treated only with the fungicide Landor®, no residues of neonicotinoids were detectable (Figure 2). Thus, the soil was not contaminated.

Figure 1. Bee collecting water from the guttated fluid of *Potentilla reptans* L. (picture by Wallner 2009).

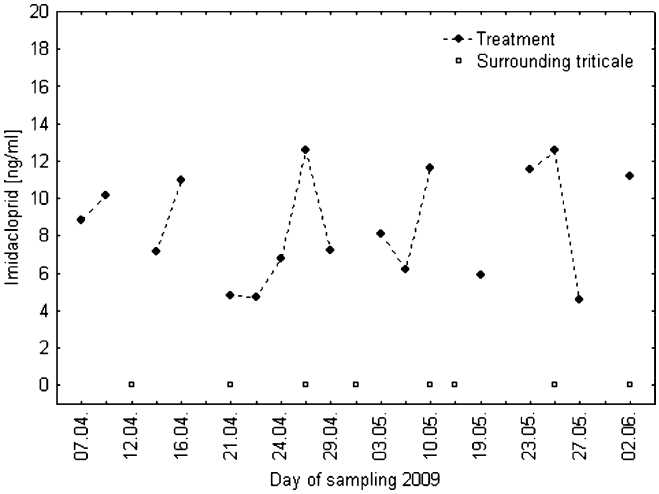

Figure 2. Translocated neonicotinoid concentrations in water guttated by triticale from seed treated with imidacloprid under field conditions in comparison to the surrounding fungicide-dressed triticale.

3.1.2. Maize experiments

From May 12 to July 3, 2009, 23 samples of guttated fluid were collected from each seed treatment experiment. The samples were collected after germination when the first leaves unfolded until growth occurred and the third stem nodes were noticeable.

The guttated fluid concentrations of the plants from seeds treated with clothianidin of experiments 2, 3 and 4 are shown in Figures 3, 4 and 5. Concentrations up to 8,000 ng ml^{-1} clothiandin were measured. There was a clear trend from the highest concentration on small maize plants at the beginning of guttated droplet collection to a lower concentration from older plants. The variation in the concentration of clothianidin (for example elevated concentration on 31 May 2009) may be attributable to the weather conditions such as strong rain, the days before

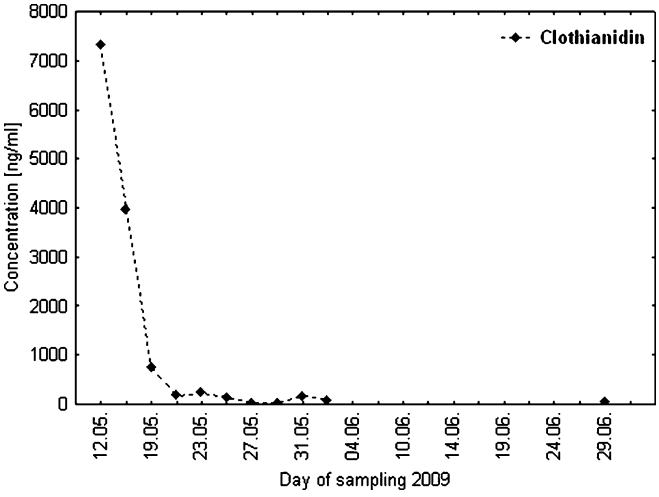

Figure 3. Translocated neonicotinoid concentration in water guttated by maize (experiment 2) from seed treated with clothianidin (Poncho®) under field condition.

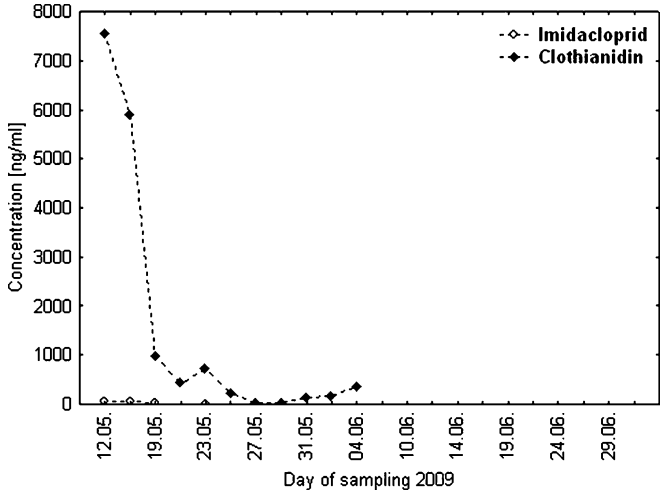

Figure 4. Translocated neonicotinoid concentration in water guttated by maize (experiment 3) from seed treated with clothianidin (Poncho® Pro) under field conditions.

(Figure 6). Experiment 2 involved only half the clothianidin concentration on the seed compared to experiments 3 and 4. Low concentrations up to 64 ng mL^{-1} imidacloprid in experiment 3 (Figure 4) were measured. Figure 7 shows the result of maize experiment 1 (seed not treated and sown into a field prepared with clover and pasture). Low concentrations of clothianidin were detected starting from 04 June 2009. No neonicotinoid could be detected in the guttated water gathered from the surrounding maize.

4. DISCUSSION

When registering pesticides, a thorough review is conducted into whether or not they can reach non-target organisms such as honey-

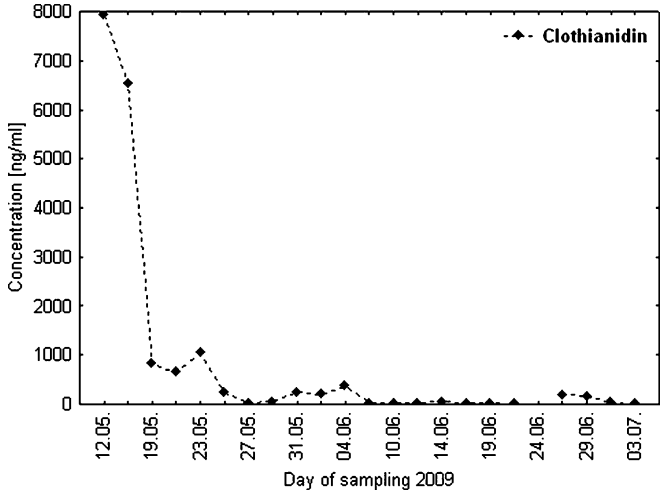

Figure 5. Translocated neonicotinoid concentration in water guttated by maize (experiment 4) from seed treated with clothianidin (Poncho® Pro) under field conditions.

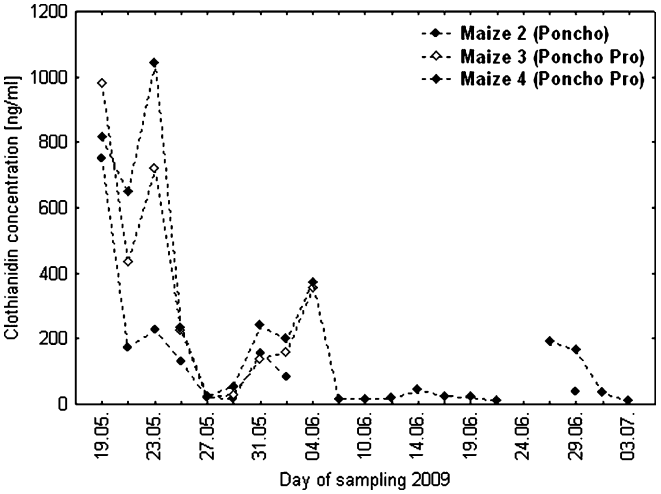

Figure 6. Detailed graph of the translocated concentrations in water guttated by maize (experiments 2, 3 and 4) from seed treated with clothianidin under field conditions.

bees under good agricultural practices. Seed coating with neonicotinoids is an application method that has been regarded as more ecologically friendly than spraying because the insecticides are put into soil, even though neonicotinoids act as antagonists to the central nervous system of insects and therefore have a high potential for toxicity to insects. However, recent knowledge about the translocation of neonicotinoids from coated seed into guttated fluid necessitates a review. The uptake of the latter by honeybees may have a direct influence on the vitality of foraging bees, and furthermore, contaminated water being transported into the hive may constitute a risk for the colony. But to evaluate the ensuing risk for non-target

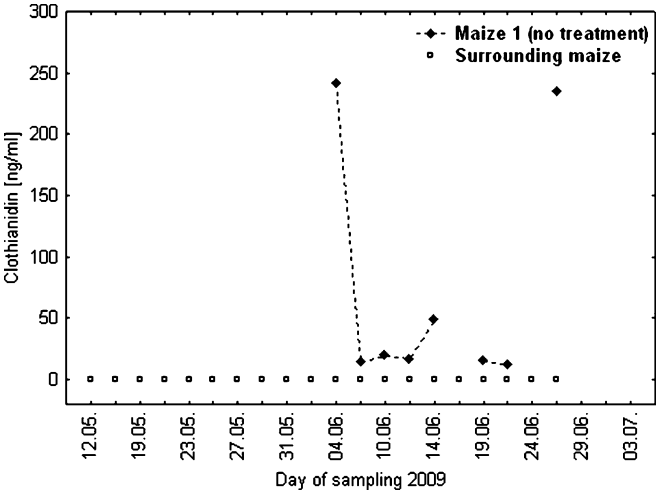

Figure 7. Translocated neonicotinoid concentration in water guttated by maize (experiment 1) from seed not treated with neonicotinoids under field conditions in comparison to the surrounding maize, dressed only with Mesurol®. Uptake of ingredients due to root growth in adjacent parcels treated with neonicotinoids.

insects, more data are needed concerning the amount of contaminated water transported and its use inside the hive. During the sampling of guttated fluid on one of the field sites, water foragers could be observed collecting guttated fluid on a weedy plant, *Potentilla*, located on the edge of the plot that was not seed-treated (Figure 1). It is still unclear whether contaminated guttated fluid from treated plants may threaten honeybees since it is not known to what extent water-collecting bees take up the active ingredients and metabolise them.

Our results represent the first step in a risk evaluation, with respect to the translocation of neonicotinoids in various agricultural crops under field conditions, beginning in spring until summer. The next stage to consider is the conceivable toxicity to foraging bees and, in a wider sense, to the colony that would be expected according to our present knowledge. We made an assumption to arrive at a relationship between the concentrations of neonicotinoids measured in guttated water and the potential effects on honeybees. Wallner (2006) demonstrated that foraging bees transport an average 36 μl and up to a maximum of 82 μl nectar in the honey sac. Current literature provides information about the honey sac volumes of nectar foraging bees, but there are no data on water foragers, their metabolism during foraging, the use within the hive and a variety of other factors. Thus, there are various possibilities for handling the results given above, e.g. they could be compared with LD_{50} oral or NOEC. The following scenario is based on the assumption that foraging bees collect 45 μL contaminated guttated fluid and digest it undiluted.

The maximum concentration was 13 ng mL^{-1} imidacloprid in guttated fluid from triticale, so under this assumption, a maximum of 0.585 ng imidacloprid would be taken up by a bee. Taking into account the LD_{50} oral (3.7 ng/bee, Schmuck et al. 2001), the imidacloprid concentration in guttated droplets would appear not to have acute toxic effects. However, sub-lethal and chronic effects occur in the range of picogram per bee (Halm et al. 2006), and in view of chronic NOEC of 10 ppb (Schmuck 2004), effects cannot be excluded. The biotransformation of imidacloprid inside the honeybee is reported to occur within hours, and resulting metabolites may be toxic to bees (Suchail et al. 2004).

The low concentration, up to 64 ng mL^{-1} of imidacloprid in guttated fluid of maize in experiment 3 (Figure 4), was the result of seed contamination during the coating process. Soil contamination prior to the experiment can be excluded, since other experiments were carried out on the same soil near the plot of experiment 3, and the samples of the surrounding maize revealed no residues. Taking into account the maximum amount of 64 ng ml^{-1} imidacloprid and the oral LD_{50} of 3.7 ng/bee (Schmuck 1999), there might be a higher probability of toxicity. However, possible sub-lethal effects must be considered in relation to the NOEC of 10 ppb (Schmuck 2004).

Concentrations of clothianidin up to 250 ng mL^{-1} were detected in guttated fluids in experiment 1 (seed not treated and sown into a field prepared with clover and pasture), starting from 04 June 2009 (Figure 7). This was a consequence of the close proximity of the experimental plots. Lot 1 was next to lots 2 and 4. Thus, the roots of the untreated maize from lot 1 spread into the soil horizon of the treated lots. Residues of neonicotinoids in the soil can be excluded, because none could be measured in the water guttated from the surrounding maize. Taking this concentration into account the LD_{50} oral (3.68 ng/bee, Schmuck and Keppler 2003), acute toxic effects could be expected.

Taking the maximum concentration into account of 7,930 ng mL^{-1} clothianidin in fluid guttated from maize and the oral LD_{50} (3.68 ng/bee, Schmuck and Keppler 2003), a maximum quantity of 357 ng clothianidin could be carried by the bee. Even 1 μL of this solution exceeds the oral LD_{50}, so it can be assumed that this contaminated water could have an acute toxic effect if it enters the bee's metabolism. These translocated concentrations persist over a period of some weeks. If we assume that these

concentrations of insecticides enter the beehive and are diluted and mixed with royal jelly and feed to brood and queen, there may be additional sub-lethal effects. Clothianidin residues could be detected in bee products like pollen, nectar and honey when colonies were exposed to treated canola fields, but the amounts were below those reported to evoke toxic effects in honeybees (Cutler and Scott-Dupree 2007; Wallner 2009). But canola crops have become more prevalent in various countries over the past several years, and as canola is a major source of honey, it is likely that bees came into contact with active compounds. Although Cutler and Scott-Dupree (2007) concluded that the entry of pesticides decreases after the blooming period and there is no long-term impact of exposure to clothianidin-treated canola on honeybees, there is little evidence concerning the impact of contaminated water inside the beehive and on bee development. It should be noted that the impact of systemic insecticides on honeybees is not limited to the parent compound alone. There might also be an impact from exposure to pesticide mixtures or their metabolites (Halm et al. 2006).

To minimise the risk of contamination with pesticides, it might be possible to focus bees on specific watering places away from agricultural fields. However, focusing bees on special watering places is difficult to control. Special watering places would need regular sanitation and management to prevent the distribution of diseases and parasites, in particular, nosemosis. Increasing the distance between beehive and treated fields is another possible solution, because water-collecting bees normally work within short distance around the hive in order to react as quickly as possible to the colony's water demand. If there are no nearby water sources, bees do forage farther from the hive to collect water. Visscher et al. (1996) reported on water-collecting bees, which normally forage up to 500 m from the hive, but in the absence of water they will extend their radius up to 2 km. Kühnholz and Seeley (1997) positioned troughs for observations on water foragers in a distance of 250 m away from the hive. From this knowledge, it can be assumed that water foragers in the temperate zone choose between a diversity of sources and can find sufficient sources within short distances from the hive. Like nectar-collecting bees, water-collecting bees can differentiate between sources on the basis of their composition (Butler 1940), and apart from that, they might avoid contaminated sources. There may also be differences in water usage within the beehive depending on the diversity of water sources and their quality.

The results reveal that active ingredients from the group of neonicotinoids can reach the plant surface via the guttated drops and their presence could constitute a risk to bees when collecting water for the colony. The concentration of neonicotinoids in guttated fluid depends on the crops and the stages of plant development. In general, the concentrations detected in the drops guttated from maize were higher than those in wheat that was seeded the previous year. The final evaluation of our findings and its relevance for bees is still under discussion. Crucial information is not known concerning the amount of water required by a colony, daily water foraging activity and dispersion of foragers among water sources and the uses and the proportion of the water used inside the beehive. Our evaluation, based on the concentration of the active ingredients in the water reservoir available to bees and the reported LD_{50} values, is a worst case scenario.

It is important to clarify in laboratory and field studies to what extent bees are attracted to the guttated drops and whether they are able to change their behaviour and the intensity of their flights when water sources are contaminated. Hence, it is essential to develop and to design specific methods to obtain further information on water-collecting bees for subsequent trials. It will be possible to obtain reliable answers to the questions raised above by using highly sensitive analytical methods.

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Présence d'insecticides néonicotinoïdes dans les gouttelettes exsudées par guttation sur les plants de maïs et de blé issus de graines traitées par enrobage: une menace pour les abeilles?

graines traitées par enrobage / néonicotinoïdes / guttation / *Apis mellifera* / LC-HR-MS

Freisetzung neonicotinoider Insektizide aus der Saatgutbeizung in Guttationstropfen bei Mais und Getreide: Eine Gefahr für Honigbienen?

Saatgutbeizung / Neonicotinoide / Guttationswasser / *Apis mellifera* / LC-HR-MS

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