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Neonicotinoid Insecticide Uptake by Maize and Appearance in Guttation Liquid

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Abstract

Movement of *clothianidin* (CLO) and *thiamethoxam* (TMX) applied as maize seed dressing or spray application has been investigated in different soil types (sandy, clay or loam), and subsequent appearance of these compounds in the guttation liquid of maize are presented. Elution profiles for different soil types were determined in order to explore differences in binding capacity. Soil characterized by high organic matter content retained the ingredients, whereas high clay content resulted in delayed release of the compounds.
Neonicotinoid uptake by non-coated maize plants was also determined *via* guttation liquid measurements after the neonicotinoid ingredients had been applied in spray format. The highest peak concentrations of TMX and CLO (0.546 and 1.83 µg/ml, respectively) were measured from plants planted in sandy soil, but these levels were still substantially lower than levels in samples taken from plants emerged from neonicotinoid-coated seeds (above 100 µg/ml). Moreover, the time of ingredient appearance in guttation liquid was also strongly influenced by soil type.
Cross-contamination was established by measuring neonicotinoid concentrations in guttation liquid in plants emerged from differently coated (CLO and TMX) seeds potted near to each other and the effect of soil type has also been explored. Results for coated (CLO or TMX) and non-coated plants also confirmed that cross-contamination may occur by uptake through soil from neighboring seeds. Differences between non-coated and coated seeds gradually disappeared. This is the first record of neonicotinoid levels in guttation liquid of plants emerged from non-coated maize seeds.

Introduction

After the introduction of the first commercially available insecticide with a neonicotinoid active ingredient (*imidacloprid*) into the pesticide market in 1991, neonicotinoids rapidly became the most important class of insecticides. Their share in the total global market of insecticides was 28.5% in 2011 [1]. Except for organic farming all of maize seeds planted in North America are coated with neonicotinoids, mainly with *clothianidin* (CLO) or *thiamethoxam* (TMX). They are also routinely applied in developing countries and non-coated seeds are often unavailable for purchase. The current use of systemic pesticides is not sustainable globally [2]. Over 80% of ingredients in seed dressings are not uptaken by target crops [3], and off-target drifting similarly reduces the efficacy of spray applications and causes environmental contamination. Neonicotinoids are persistent in soils under appropriate conditions. Their reported half-lives vary by compound and environmental conditions, but range 150-6900 days for CLO and 35-3000 days for TMX [3]. Due to their good water solubility (e.g. 0.34 g/l and 4.1 g/l for CLO and TMX, respectively) they leach into ground and surface water, and consequently are widely detected in water resources.
Neonicotinoids act as neurotoxic agents to insects and may affect the orientation of bees. Concerns have been raised that their extensive use may contribute to pollinator decline, affecting ecosystem services. Although these compounds were initially regarded less toxic to non-target species compared to other insecticides, they still may pose long-term negative

effects on a wide range of different organisms. Neonicotinoid-related toxic effects on the neuronal connections in the molluscan nervous system have been reported recently [4], but many other examples exist in the literature reviewed in 2015 [5].
Cases of severe bee poisoning and updated risk assessment by EFSA in January 2013 led EU Commission to the conclusion [6] that a high risk for bees cannot be excluded except by imposing further restrictions involving withdrawal of authorization of neonicotinoids and ban of coated seeds for different crops. The restriction applies to the use of CLO, *imidacloprid* and TMX for seed treatment, soil application (granules) and foliar treatment on crops attractive to bees, including certain cereals. Reassessment of these neonicotinoids was scheduled by the EU Commission to be executed by the end on 2015.
Due to their systemic action neonicotinoids are translocated in the entire plant, conferring a long-lasting control of insects. Novel ways of intoxication for bees have been explored *via* the guttation liquid [7], which is excreted in the form of water droplets on the tips of leaves of certain vascular plants. Maize showed high guttation capacity [8], therefore, we selected it as a model species in the present work.
The aim of this work was to study the spread of ingredients in different soil types and to explore the effect of soil quality to CLO and TMX content in guttation liquid. We have also studied the uptake of these active ingredients applied as sprayed format, and their appearance in the guttation liquid of maize plants that emerged from non-coated seeds. Cross-contamination occurring between seeds coated by CLO and TMX has also been studied in different soils. In addition, cross-contamination between coated (CLO or TMX) and non-coated seeds has also been investigated.

Experimental

Analyses of samples were done on Younglin YL9100 HPLC system equipped with a YL9150 autosampler. A C18 column (150 mm × 4.6 mm i.d., 5μm) was used for the separation at 40ºC. Eluent flow rate was 1.0 ml/min with isocratic elution for 5 minutes (70:30 = A:B eluents, A = 90% water : 10% MeOH, B= MeOH). UV detector signals were recorded at λ=269 nm for CLO and λ=252 nm for TMX. Limits of detections (LODs), determined with standard solutions lied at 10 ng/ml for TMX and below 10 ng/ml for CLO.
Maize seedlings were grown in laboratory in pots and they were obtained from commercially available seeds (DECALB 449 and OCCITAN 380), coated with CLO or TMX. Ingredient contents of coated seeds have been checked.
For investigation of the absorption of neonicotinoids in soils and for spray applications a stock solution containing 2.06 mM of CLO and TMX was prepared and diluted to 8.24 μM in soil adsorption experiments or to 16.5 μM for pre-emergence spray applications.
Three different soil types (sandy, clay, loam) and pumice have been used as a media for growing the maize plants. For the extraction of target compounds from soils a modified QuEChERS method [9] had been chosen as a sample preparation procedure.
The movement of TMX and CLO through a soil column was studied by determination of the elution profiles for clay, loam or sandy soils. Equal molar quantities ($8.24 \cdot 10^{-7}$ mol) of CLO and TMX were loaded onto the columns, which were then washed with successive 10 ml volumes of water. The neonicotinoid content of the leachates obtained by elution of the active ingredients was determined, as well as the residues in soil were quantified by HPLC.
Vertical spreading of neonicotinoids followed pre-emergence spray application to soil was investigated in pots filled with different soils (loam, clay, sandy). CLO and TMX uptake by plants emerged from non coated maize seeds were studied by analyzing their guttation liquids collected at leaf edges.
Cross-contamination and neonicotinoid uptake by plants from neighboring coated seeds were investigated either with differently coated maize seeds (CLO and TMX) or with non-coated

and coated (CLO or TMX) seeds. Two maize seeds were placed into the same hole and guttation liquid produced by plants were collected and analyzed separately.

Results & discussion

Water, acetone and acetonitrile were tested as extraction solvents of soil samples, but finally a QuEChERS based sample preparation procedure proved to be suitable method for extraction of TMX and CLO from soils used in this study. Remarkable differences in recoveries concerning water extractions indicate that rate of the leaching can be variable even for these water soluble ingredients.

The effect of soil types on the movement of CLO and TMX in a soil column experiment was significant and elution profiles varied by soil types studied. Poor binding potential of sandy soil probably accounted for high levels of neonicotinoids detected in the first 5^{th}-6^{th} eluates. High clay content soil showed a greater capacity to bind both TMX and CLO, reflected in their retarded downward movement, continuous leaching and long dissipation. Loam displayed an even stronger retention of both compounds, possibly due to its high organic matter content compared to the two other soil types. According to water solubility data, concentrations measured for TMX were generally higher than that of for CLO. The overall molar ratios of the eluted TMX related to CLO lied at 1.11, 1.46 and 1.33 and 1.26 for sand, clay and loam, indicating the higher mobility of TMX than CLO in all cases. Results are in accordance with binding capacity of soils and with higher water solubility of TMX.

Vertical movement of CLO and TMX and neonicotinoid uptake by non-coated maize plants followed pre-emergence spray application of insecticides to soil show characteristic differences by soil types. The compounds were leached into soil down to the level of seeds with uneven rates, so their uptake and excretion *via* guttation occurred differently. CLO and TMX could be detected immediately in plants emerged in sandy soil. Loam soil retained the substances for a longer period of time, while in the case of clay both compounds appeared shortly after emergence. Peak concentrations determined in guttation liquids of these plants were significantly lower (below 2.0 µg/ml) than the highest levels (over 100 µg/ml) measured for plants emerged from coated seeds. Results reflect the different retention characteristics of investigated soils. Compounds were more retained by higher clay or organic matter content. **P**lants grown in clay have taken up only 0.021% of applied CLO and 0.042% of TMX, whereas higher rates (0.076 % of CLO, 0.094% of TMX) were detected for plants emerged in sand. Amounts measured for plants grown in loam are negligible.

Leaching of neonicotinoids from one coated seed to another in close proximity and their uptake by plants was evidenced. In case of differently coated seeds (CLO and TMX), cross-contaminations have been observed. Binding capacity of soils plays an important role in the movement of ingredients, therefore, cross-contamination is more pronounced in sandy soil than in soils of high clay and/or organic matter content. When coated and non-coated seeds are potted in close proximity to each other, the neonicotinoids from coated seeds appeared after emergence also in the guttation liquid of plants that emerged from non-coated seeds. In the first period, guttation liquid of maize plants emerged from CLO-coated seeds contained substantially higher amounts of the active ingredient than that of neighboring plants from non-coated seeds (Figure 1) and the concentration rapidly decreased from very high levels. However, significant levels were detected in the guttation liquid of neighboring plants from non-coated seeds as well, whereas there were practically no differences between the two concentrations later from the 24^{th} day on. Ratio between the amounts of CLO measured in guttation liquids and coating material was 0.57%. Amounts of CLO taken up by non-coated plants and detected in guttation liquids was 45.7% compared to that of coated seeds.

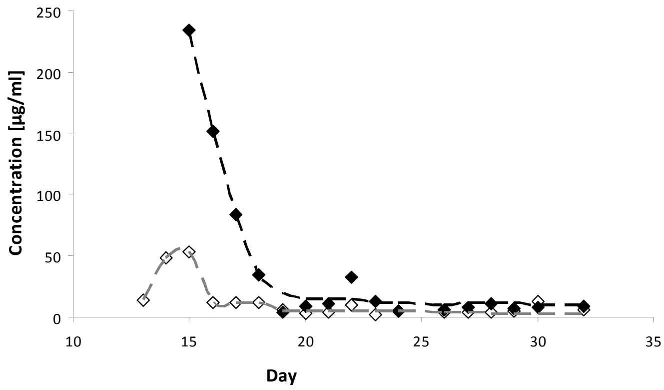

Figure 1. CLO levels in the guttation liquid of plants from CLO coated seeds (♦) and neighboring non-coated seeds (◊) grown in sandy soil

Conclusion

Actual concentrations of neonicotinoids in leachates or in guttation drops are influenced not only by the type of seedling material, but effect of soil type and the application mode (spray or seed coating) also influences the levels of occurrence of these compounds. Our results confirm that high levels of neonicotinoids occur in the guttation liquid of maize. Despite of the fact that neonicotinoids applied as seed coating are targeted to the seed zone, they do not remain associated with the sub-surface soil solution. Our findings provide further evidence of the mobility of these water soluble ingredients, where soil characteristics are of high importance. Insecticides from the field surface move to root zone, and they are also uptaken by non-treated plants. Thus, their systemic effect is not restricted to the plants emerged from coated seeds and could result in exposure of non-target animal species.

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