APPLICATION RATES OF NEONICOTINOIDS IN SEED COATING AS SOURCES OF ENVIRONMENTAL CONTAMINATION

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Abstract
To assess technical variability in actual dosages, the application rates of neonicotinoid insecticide active ingredients in seed coatings were determined and compared for commercial seeds of different maize varieties. Theeffect oflong storage and coating by unique equipment were assessed. Application rates in different pesticide treatment modes (seed coating, spray or soil granule applications) were also compared. Results indicate that the three technologies utilize similar amounts of the active ingredients per hectare.

Introduction
The use of seed coatings is rapidly increasing throughout the world, as pesticides applied directly to the surface of the seed provide long term protection to crops: the seed coating technology offers an effective method for protecting the seeds during storage or in the soil from pathogens, insects and other pests, and contributes to the uniform stand establishment of a variety of crops produced. Neonicotinoids are nowadays the most widely used insecticides in the world. However, the EU Commission withdrew authorization of three neonicotinoid ingredients (imidacloprid, thiamethoxam and clothianidin) as seed coatings, and restricted their use in 2013 [1]. Based on the environmental risk assessment by the European Food Safety Authority (EFSA) [2], a high risk for bees cannot be excluded unless further restrictions are imposed. According to EU Decision 2015/495 [3] these compounds are now on the watch list and their concentrations in the aquatic environment should be monitored. Reassessment of the above mentioned three neonicotinoids started in 2015 by EFSA with a first publications [4-6], and the risk assessment process is scheduled to be completed by January, 2017. Among neonicotinoids, currently only thiacloprid is authorized in EU for seed coating of maize.
Among the benefits of seed treatment, increased precision and effectiveness are emphasized by placing the crop protection product on the seed to protect it during germination. Estimations claim that the precise application of a crop protection product via seed treatment reduces soil surface exposure by up to 90 percent compared to in-furrow applications and up to 99 percent compared to a surface application [7]. As an environmental impact, lower off-target exposure has been claimed to be expected, yet movement of the neonicotinoid active ingredient in the seed coating in the soil [8], as well as uptake by plants and dispersal in their guttation fluid [9-10] have been evidenced.
Polymers are also applied in seed coatings to bind crop protection products directly to the seed, largely eliminating dust during sowing. It lowers exposure to people who handle and plant the seed, as well as to non-target organisms. Due to its precise application directly to the seed, which is then planted below the soil surface, seed treatment reduces potential off-target exposure to plants and animals. Recommended doses for coating of maize seeds are, however, alarmingly high, 1 mg/seed from thiacloprid (TCL), 1.25 mg/seed clothianidin (CLO) and 0.63 or 1.25 mg/seed from thiamethoxam (TMX). In the current study, to assess true environmental load of neonicotinoids in seed coating, actual levels of neonicotinoid active ingredients TLX, CLO and TMX were determined in coated seeds of various maize varieties.

Experimental

Analyses of samples were carried out 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, determined with standard solutions lied at 10 ng/ml for TMX and below 10 ng/ml for CLO.

Samples were obtained from commercially available maize seeds or from those that were coated uniquely on a farm. DECALB 449 (CLO), OCCITAN 380(TMX), and LG 30490 (TCL), were prepared by a relatively new technology, while PR36K67 (CLO) and two MSG seeds were coated earlier. Ingredient contents of coated seeds have been checked by extraction of target compounds from individual seeds with 10 ml of water using ultrasound agitation for 10 minutes, carried out in 10 or 15 replications. Solutions were analyzed after ten-fold or hundred-fold dilution, and filtration through a 0.45 µm hydrophilic polytetrafluoroethylene syringe filter (Labex Ltd, Hungary). Non-coated seeds were used as blank control.

Results and discussion

Results for commercially available new seeds are summarized in Table 1. Analytical determinations indicated that average values are in accordance with current rate of application of CLO or TMX to maize crops (1.25 mg/seed or 0.63 mg/seed), but higher insecticide content was determined for TCL coated seeds, where the recommended dose are 1 mg/seed. High standard deviation (54.3%) observed for Decalb seeds indicates uneven ingredient content. There were more than four-fold difference between lowest and highest value that can also effect the ingredient uptake and individual levels in guttation liquid of maize plants. According to our earlier results average levels in guttation liquid measured in the first period after plantation depend on the amount of ingredient in seed coating material as well. Values determined for plants emerged from commercial seeds (Table 1, line 3) were 50 to 150-fold higher than that of emerged from seeds containing only the 5.4 % of recommended dose (Table 1, line 3)

Table 1. The concentration of three neonicotinoid active ingredients in seed coating on commercial seeds of various maize varieties

Maize variety	**Active ingredient**	**Concentration (mg/seed)**	**RSD (%)**	**Number of seeds**	**Year**
DECALB 449	clothianidin	1.217	54.34	15	2013
OCCITAN 380	thiamethoxam	0.605	12.30	10	2013
LG	thiacloprid	1.182	11.15	10	2015

Earlier coated and stored seeds involved in our investigation (see Table 2) contained less insecticides in their coating material compared to the recommended doses. Although deviations were somewhat higher compared to the best values about 10%, they still fall in an acceptable range. There occurred about two-fold differences between the lowest and highest values measured for seeds of maize variety PR36K67 that could have only slight effect to the ingredient uptake and individual levels in the guttation liquid of maize plants.

Uniquely coated maize seeds contained the recommended dose or intentionally less pesticide active ingredient than applied in the usual seed treatment technology. In spite of our expectations, seed coating performed on a farm resulted in similar or somewhat lower values for standard deviations of insecticide content as seeds obtained had previously been in long storage. There occurred less than two-fold difference between the lowest and the highest ingredient content, likely causing no significant differences in levels in maize guttation liquid.

Table 2. The concentration of three neonicotinoid active ingredients in seed coating on commercial seeds after long storage

Maize variety	Active ingredient	Concentration (mg/seed)	RSD (%)	Number of seeds	Year
PR36K67	clothianidin	0.997	24.24	15	2009
MSG-I	thiamethoxam	0.295	18.60	10	2007
MSG-II	thiamethoxam	0.259	15.30	10	2007

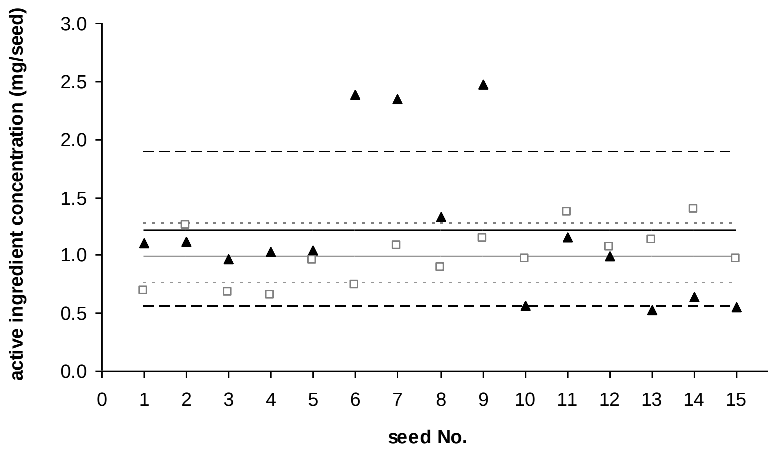

Fig 1. Concentration of active ingredient clothianidin (mg/seed) on individual seeds of maize varieties Decalb 449 (*black triangles*) and PR36K67 (*grey open squares*). Average concentration values (*solid lines*) and standard deviations (*dashed/dotted lines*) determined for 15 seeds are also depicted.

Table 3. The concentration of three neonicotinoid active ingredients in seed coating on uniquely coated maize seeds

Maize variety	Active ingredient	Concentration (mg/seed)	RSD (%)	Number of seeds	Year
1	clothianidin	0.610	16.81	10	2014
2	thiamethoxam	0.145	20.45	10	2014
3	thiacloprid	0.054	9.65	10	2015

Seed coating is considered to be a targeted pesticide delivery method [7] due to being applied directly at the site of the intended protection measure against early insect pests. Application of the active ingredient is secured at the time of sowing, and insecticide efficacy appearing far less dependent on weather conditions than in the case of spay applications. Applied dosages (g of a.i./ha) are also considered to be reduced in seed coating compared to spray or soil granule applications [11], yet this comparison seems to be misleading as it compares different insecticide active ingredients to each other (neonicotinoids in seed coating to carbamate/phenyl-pyrazole compounds (carbofuran, fipronil) in soil disinfectant granules and a chlorinated hydrocarbon (lindane) in spray). Moreover, some of these active ingredients (carbofuran, lindane) are banned in most countries. An approximate comparison of the practically applied dosages of neonicotinoids in seed coating and spray or soil granule formats reveals that seed coating corresponds to 30-85 g active ingredient/ha (0.6-1.22 mg a.i./seed at 50-70 thousand (maize) plants/ha), while typical dosages in spray and in soil granule applications are 20-70 g active ingredient/ha (20-70 mg a.i./l at 1000 l/ha) and 110 g active ingredient/ha (10 g a.i./kg at 11 kg/ha), respectively. This indicates that the three technologies utilize similar amounts of the active ingredients per hectare, and therefore, seed coating is more favorable in terms of pesticide consumption only if spray applications are needed to be used several times during the vegetation period (the number of registered applications is limited in two sprayings per season). Worthy of note that application of seed coating as well as granules contain insecticide active ingredient in a concentrated form. Thus, their uptake by plants occurs rapidly and high concentrations can be observed in guttation liquid (peak values are often above 100 µg/ml). Due to their good water solubility (e.g. 0.34 g/L and 4.1 g/L for CLO and TMX, respectively), neonicotinoids readily leach into ground and surface water, and consequently are widely detected as pollutants in water resources. Very high contamination rates (up to 100%) were detected in US and Canada in spring. Our environmental monitoring surveys in Hungary showed lower rates: Their occurrence was at 0.017-0.040 ng/ml and 0.040-0.030 ng/ml as diffuse contaminant for CLO and TMX, respectively, and their point source type occurrence at 10-41 ng/ml measured from temporary shallow water bodies [12]. Neonicotinoid uptake processes from different applications and spread of ingredients in the environment are strongly influenced by soil type, and probably soil moisture also affects the transport pattern. According to our previous results of soil column experiments, binding capacity of soils plays an important role in the movement of ingredients [8]. Therefore, contamination of surface waters is more pronounced near to sandy soils, whereas soils of high clay and/or organic matter content retain the components resulting long release of compounds.

Conclusion

Developments in seed coating technology as well as in sowing machines led to decrease of pesticide drift and dust during the sowing of coated seeds. In this way off-target exposure can be eliminated for many species. However, reproducibility of seed coating requires exact technologies and further efforts to ensure controlled doses and environmental effects in the future.

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