Translocation of imidacloprid from coated rape (*Brassica napa*) seeds to nectar and pollen

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Implications

Bees and other pollinating insects contribute significantly to the world's production of edible crops. Bees and bumblebees are threatened by modern agricultural practices like monocropping, destruction of natural habitats and pesticide use. Organic farming practice avoids the pesticides, but should also actively support the living conditions for pollinating insects, e.g. by maintaining flowering strips and trees. A recent ban on imidacloprid as rapeseed coating in Norway may encourage research in alternative ways of protecting rapeseed against ground flees (*Phyllotreta* spp.). Such studies may be beneficial also for organic rape growers.

Background and objectives

Already in the middle of 1990's, French beekeepers began to report alarming losses of bees, caused by honeybees not returning to their hives (Maxim and van der Sluijs, 2013). Later, significant loss of honeybees, also named Colony Collapse Disorder (CCD), has been reported in many countries and different parts of the world. This serious threat against our valuable pollinators has called for a careful consideration of the reasons for disappearing honeybees. The picture is complex, and there might be several factors involved. One theory is stress related to long distance transportation, especially in regions such as the United States where bees are commonly utilised for pollination more than for honey production. Another important factor is the lack of diversity in modern agriculture, where large areas of monocropping reduce the food offer for a range of insects including bees and bumblebees. Cropping of less protein rich plants result in less glucose oxidase in the nectar (Eishen and Graham, 2008; Aluax et al, 2010), which has a protecting role in the bee community to reduce attacks from virus/fungus/mite as well as the stress linked to exposure to insecticides, out of which several may be toxic to bees. Neonicotinoid pesticides, especially thiamethoxam, clothianidine and imidacloprid, are extremely toxic for bees. Early 2013, The European Food Safety Authority (EFSA) published a report where risks connected to use of imidacloprid, especially for crops that are attractive for bees such as rape, were described (EFSA 2013). Pesticide use is regulated by national law in Norway and of the three neonicotinoid pesticides, only imidacloprid has been authorized by the Norwegian Food Safety Authority (NFSA). NFSA decided in March 2013 to restrict the use of this pesticide to indoor use (greenhouses, decoration plants) and as seed coating on potatoes. From 2014, rapeseed coated with imidacloprid is no longer permitted in Norway.

In 2012, NFSA engaged Bioforsk to study if imidacloprid coated on seeds of rape (*Brassica napa* cv. Mosaikk) used in Norwegian agriculture could be translocated to nectar and pollen. Imidacloprid-coated rape seeds were cultivated on ground in a 50 m^2 plot in greenhouse. Nectar and pollen were sampled during full flowering. Nectar was sampled by capillary tubes and pollen was sampled by careful shaking of the flowers. Nectar and pollen from flowers of non-coated seeds, grown in the same soil as the coated seeds but in another greenhouse, were used as control.

In total, six nectar and four pollen samples from flowers from imidacloprid-coated seeds were analysed. Imidacloprid-D4 was added to the samples as internal standard before sample preparation. Use of internal standard is necessary for the analysis of small samples with low concentrations. The samples were analysed by LC-MS/MS, which has good sensitivity and specificity. The concentrations of imidacloprid were determined by

quantification against standards with known concentrations, and adjusted for the internal standard.

The study was designed as an "early warning" study, to reveal if translocation of imidacloprid to nectar and pollen could be found from coated rape seed used in Norwegian agriculture, and thus potentially expose bees and other pollinators.

Key results and discussion

Imidacloprid was detected above limit of detection (LOD=1.5 ng g^{-1}) in five out of six nectar samples and in all pollen samples. The concentrations in nectar and pollen ranged from below LOD to 3.1 ng g^{-1} and 1.8—2.8 ng g^{-1}, respectively. Control samples showed no traces of imidacloprid. Our lowest standard was 0.0002 µg ml^{-1}, which corresponds to 0.8 ng g^{-1} in a 50 mg sample.

In the EFSA report (EFSA 2013) the data were transformed to *residual unit dose* (RUD). This is an expression which is used in order to compare residual concentrations of pesticides in nectar and pollen between different studies independent of the amount of pesticide applied. The RUD value of imidacloprid for pollen and nectar is referring to an application rate of 1 kg ha^{-1} or 1 mg $seed^{-1}$; meaning that the analyzed residue level will be extrapolated to a pesticide application rate of 1 kg ha^{-1} or 1 mg $seed^{-1}$ if necessary. In our study, the maximal estimated RUD values of imidacloprid were 0.155 mg kg^{-1} in nectar and 0.110 mg kg^{-1} in pollen. These values fall within the range of RUD values reported by the EFSA (2013), which were 0.017-0.159 mg imidacloprid kg^{-1} in nectar, and 0.069-0.156 in pollen.

According to EFSA LD_{50} for acute effects on honey bees after contact and oral exposure is 0.081 and 0.0037 µg bee^{-1}, respectively (EFSA 2008). Chronic lethal effects and behavioral impact including bee hive development of imidacloprid via dietary exposure is given as 24 ng g^{-1} and 20 ng g^{-1} (EFSA 2008). Since field bees only feed on nectar it is relevant to compare these effect values with our measured nectar residue - up to 3.1 ng g^{-1}. Compared with a field situation, rape flowers cultivated in a greenhouse with controlled watering regime are exposed to higher humidity, higher temperatures and no wind exposure. Via their influence on plant transpiration (higher humidity and temperature – higher transpiration, no wind – lower transpiration), these conditions may impact the translocation and concentrations of imidacloprid in various plant organs. However, the results show that imidacloprid might be found in concentrations up to 2-3 ng g^{-1} in nectar and pollen in flowers from coated rape seed used in Norwegian agriculture. A follow-up study will investigate whether important bee-attractive plants commonly found around greenhouses or along potato fields, such as wild raspberries, may contain imidacloprid.

Acknowledgement

We thank The Norwegian Food Safety Authority, for funding this project via "The action plan on reducing risk from the use of pesticides (2010-2014)" (Pesticide National Action Plan). A special thanks goes to Marit Randall and Siri Nesbakken, both in NFSA, for valuable contribution of knowledge and information.

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