



Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects



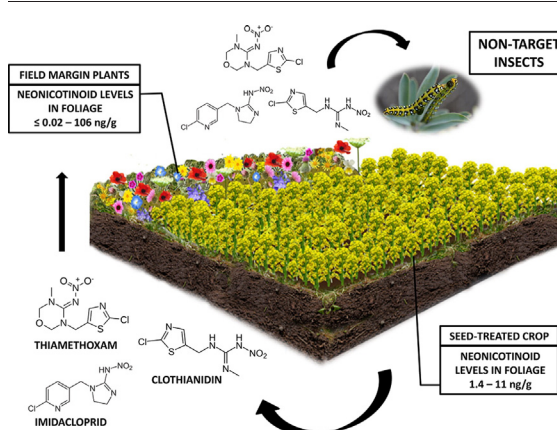
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HIGHLIGHTS

- Seed-coating with neonicotinoids led to contamination of non-target plants, where four different neonicotinoids were detected.
- Neonicotinoids levels in wild plants were very variable, but sometimes overlapped with LC₅₀s reported for some insect species.
- Thiamethoxam and clothianidin differed in pollen and foliage of the same plant species (*Brassica napus* L., oilseed rape).

GRAPHICAL ABSTRACT



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ABSTRACT

Neonicotinoid insecticides are commonly-used as seed treatments on flowering crops such as oilseed rape. Their persistence and solubility in water increase the chances of environmental contamination via surface-runoff or drainage into areas adjacent to the crops. However, their uptake and fate into non-target vegetation remains poorly understood. In this study, we analysed samples of foliage collected from neonicotinoid seed-treated oilseed rape plants and also compared the levels of neonicotinoid residues in foliage (range: 1.4–11 ng/g) with the levels found in pollen collected from the same plants (range: 1.4–22 ng/g). We then analysed residue levels in foliage from non-target plants growing in the crop field margins (range: ≤ 0.02 –106 ng/g). Finally, in order to assess the possible risk posed by the peak levels of neonicotinoids that we detected in foliage for farmland phytophagous and predatory insects, we compared the maximum concentrations found against the LC₅₀ values reported in the literature for a set of relevant insect species. Our results suggest that neonicotinoid seed-dressings lead to widespread contamination of the foliage of field margin plants with mixtures of neonicotinoid residues, where levels are very variable and discontinuous, but sometimes overlap with lethal concentrations reported for some insect species. Understanding the distribution of pesticides in the environment and their potential effects on biological communities is crucial to properly assess current agricultural management and schemes with biodiversity conservation aims in farmland.

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1. Introduction

Agricultural land use affects large parts of the world's terrestrial area, and thus, assessing the impact of farming practices on biodiversity and associated ecosystem services is fundamental to reconcile the conflicting demands for wildlife conservation and increased agricultural production globally (Norris, 2008; Paoletti et al., 1992). Within agricultural landscapes, linear semi-natural habitats of wild plants often define the edges of agricultural fields. These arable field margins support a wide range of associated fauna, some of which may be pest species, while many are beneficial, either as crop pollinators or as pest predators (Dennis and Fry, 1992; Rands and Whitney, 2011). Field margins thus have the potential to support wildlife biodiversity and enhance crop yields (Garibaldi et al., 2016; Ekbom and Bengtsson, 2003; Pywell et al., 2015) and hence they are often the target of agri-environment schemes intended to protect these functions in farmland.

There are growing concerns about the potential contamination of these essential semi-natural habitats with agrochemicals used in the adjacent crops (Bonmatin et al., 2015; David et al., 2016; Goulson, 2013). In particular, the rapid increase in the use of neonicotinoid insecticides worldwide, especially as soil and seed treatments (Jeschke et al., 2011), along with their persistence and water solubility (Bonmatin et al., 2015), may represent an environmental risk in arable land if these compounds transfer to off-crop areas. A very recent study found a strong correlation between the extent of use of these compounds and the rates of decline in farmland butterflies (Gilburn et al., 2015), many of which feed and breed on uncropped edges of arable fields (Feber et al., 1996). The insecticidal activity of these compounds is caused by their affinity to bind to nicotinic acetylcholine receptors (nAChRs), such that even low-dose exposure over extended periods of time has detrimental effects on insects and other invertebrates (Pisa et al., 2014). Their solubility in water and potential for leaching and lateral movement leads to contamination of field margin soils (Sánchez-Bayo et al., 2007; Bonmatin et al., 2015), where there can be residues detected after more than three years after seed-treatment application (Botías et al., 2015; Jones et al., 2014). Being systemic, they are absorbed by plants from the soils and transported throughout their tissues by means of the vascular system, so that boring, sucking, chewing and root-feeding insects (both pests and non-target insects) could consume some amount of these neurotoxic active ingredients when feeding on a contaminated plant (Jeschke et al., 2011; Krischik et al., 2015).

Previous research found neonicotinoid contamination in wild plants growing in field margins or surrounding areas of seed-treated crops, but these studies analysed residues in just one plant species (Krupke et al., 2012), or pooled several species by site for testing (Botías et al., 2015; Greatti et al., 2006; Rundlöf et al., 2015; Stewart et al., 2014), meaning that differential propensity of individual species, genera, or types of plant to accumulation of pesticide residues could not be determined.

Identifying which wild plant species tend to accumulate higher levels, and understanding the factors involved in this process, may improve our ability to predict which non-target organisms would be most likely to be at risk of neonicotinoid exposure through contaminated field margin plants. Furthermore, studying the variable persistence and behaviour of these active compounds in the different plant matrices (e.g. pollen and foliage) may help us understand which organisms are most at risk and to what concentrations and mixtures of neonicotinoids they would be more likely exposed depending on what part of the plant they feed on. The majority of attention on neonicotinoid toxicity in recent years has been focused on the risks to bees, which are exposed through nectar and pollen collected from plants, with very little information available about the toxicity of neonicotinoids and levels of exposure for most non-target groups that live in farmland such as butterflies (Pisa et al., 2014).

In this study, we compared levels of neonicotinoid residues in pollen and foliage of a seed-treated plant, oilseed rape, to further understand the relation between concentrations and mixtures of neonicotinoid residues present in different matrices of an individual plant species. We

also analysed concentrations of neonicotinoids in foliage from a number of plant species growing in the oilseed rape field margins, representing different types (herbaceous or woody) and life history strategies (annuals, biennials and perennials), in order to detect possible differential propensities to absorb and accumulate these compounds by different groups of plants. Finally, the maximum concentrations detected in the foliage samples, which represent the worst-case scenario, were compared against the LC₅₀ values (concentrations of a compound that kills 50% of individuals) reported in the literature for ingestion of the active substance and residual contact with treated leaves in a set of relevant insect species with the aim of setting the maximal concentrations detected in our study into an ecological effects context.

Determining the quantity, distribution and prevalence of neonicotinoid residues present in non-target vegetation is highly relevant for agricultural management and biodiversity conservation, since the persistence of these neurotoxic insecticides in field margin plants may turn these habitats, which are regarded as refuges and sources of food for much farmland wildlife, into reservoirs of neonicotinoid residues, leading to chronic exposure of a broad range of non-target invertebrates.

2. Materials and methods

2.1. Sample collection methods

2.1.1. Sampling locations

Five oilseed rape fields (sown at the end of August 2012) were selected at random from three conventional farms located in East Sussex, South-East England, UK. The selected fields had varying cropping history following normal farming practices in the region (the predominant crops being winter wheat, spring barley and oilseed rape). Previous crops in these fields had been treated with a range of pesticides, including use of clothianidin for at least the two previous years (wheat and barley crops in 2010 and 2011 in the studied fields were all seed-treated with Redigo Deter®, active substances: 50 g/l prothioconazole and 250 g/l clothianidin; application rate for clothianidin: ~100 g a.s./ha). The seeds from the oilseed rape fields were all treated with Cruiser® seed dressing in 2012 (active substances: 280 g/l thiamethoxam, 8 g/l fludioxonil and 32.2 g/l metalaxyl-M; application rate for thiamethoxam: ~33.6 g a.s./ha).

2.1.2. Sample collection in oilseed rape crops

Foliage and pollen samples were collected in the 5 oilseed rape fields approximately ten months after sowing (May–June 2013), when rape plants were in bloom. Three sites of 50 m² within each oilseed rape field were sampled for foliage and pollen, and sites were at least 100 m apart (Table S1). Whereas foliage samples were specifically collected and analysed for the present study, oilseed rape pollen samples were analysed as part of a previous study where 7 oilseed fields were sampled (see Botías et al., 2015). Thus, in this study we used the data obtained from the 5 oilseed rape fields where foliage samples were also collected in order to compare levels and mixtures of neonicotinoids present in different tissues (foliage and pollen) of a single plant species (*Brassica napus* L., oilseed rape).

Foliage samples consisted of 10 g of leaves manually gathered from 15 to 20 oilseed rape plants. Pollen samples were obtained directly from the oilseed rape flowers using methods described previously (Botías et al., 2015). All samples were stored on ice in coolers in the field and then frozen immediately in the laboratory and kept at –80 °C prior to pesticide extraction and analysis.

Samples collected from wild plants in the oilseed rape field boundaries.

Field boundaries sampled in the 5 oilseed rape fields consisted of a hedge of woody plants separated from the crop by a 0–2 m strip of herbaceous vegetation. Ten grams of foliage were collected from 45 plant species (mean ± SD: 14.2 ± 7.6 species per field) that were present

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