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CONTAMINATION OF WILD PLANTS NEAR NEONICOTINOID SEED-TREATED CROPS, AND IMPLICATIONS FOR NON-TARGET INSECTS

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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 LC_{50} 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.

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; Östman et al., 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).

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 LC_{50} 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.

Materials and Methods:

1. SAMPLE COLLECTION METHODS

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).

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 m^2 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 grams of leaves manually gathered from 15-20 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.

1.3. 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 in the field margins and hedges choosing a variety of species representing different plant types (herbaceous or woody) and life history strategies (annuals, biennials and perennials). The plant

species collected in each field boundary varied considerably and depended upon which species were available (Tables S2a-S2e). The average sample distance from the crop edge was 1.5 m (range 1-2 m).

1.4. Potential effects of neonicotinoids on non-target insects

The exposure to toxicity ratio (Hazard Quotient: HQ) was calculated as a quotient of the maximum concentrations (ng/g) measured for each of the neonicotinoids that were detected at quantifiable levels in the foliage samples (*i.e.* thiamethoxam, clothianidin, imidacloprid), divided by oral and/or residual contact LC_{50} values (concentration of a compound that kills 50% of individuals, ng/mL) of short-term exposure (1-7 days) reported in the literature for these compounds in twenty-four species of four insect orders (Table 2). Therefore, realistic worst-case exposure in ng/g (ppb) was divided by lethal concentrations expressed in ng/ml (ppb), assuming equivalence of both units of measurement since the pesticide solutions to test LC_{50}s were prepared with distilled water (ρ = 1 g/ml).

Several studies have shown that for phytophagous and predator insects mortality can result from contact with leaves from plants treated with systemic insecticides, from the consumption of insecticide-contaminated leaf tissue, or both (Prabhaker et al., 2011; Delbeke et al., 1997; Torres and Rubenson, 1994). Oral LC_{50}s were used to calculate HQ values because ingestion of insecticide-contaminated food provides an ecologically meaningful picture of toxic effects. In addition, considering that many parasitoids frequent foliage, where they typically search for hosts, feed, mate, and rest, bioassays evaluating the toxic effects of direct contact with residues on leaf tissue was deemed relevant for our risk assessment. The methods used to obtain LC_{50} values for residual contact in the insects assessed consisted of exposing the individuals to contaminated leaves that were dipped into a neonicotinoid solution (Residual Bioassay, RB) (*e.g.* Hill and Foster, 2000) or where the stem or petiole of the plant was immersed in the neonicotinoid solution to take up the insecticide (Systemic Bioassay, SB) (*e.g.* Prabhaker et al., 2006) (Table 2). When a range of LC_{50}s was given for a single compound in an insect species, the median of the values reported was used to calculate the hazard quotient.

1.5. Residue analysis

- Chemicals and reagents

Certified standards of thiamethoxam, thiamethoxam-d3, clothianidin, clothianidin-d3, imidacloprid, imidacloprid-d4, acetamiprid and thiacloprid, formic acid, ammonium formate, magnesium sulphate, sodium acetate and Supel^{TM}QuE PSA/C18/ENVI-Carb were obtained from Sigma Aldrich UK. All pesticide standards were > 99% compound purity and deuterated standards > 97% isotopic purity. HPLC grade acetonitrile, hexane, methanol and water were obtained from Rathburns UK. Individual standard pesticide (native and deuterated) stock solutions (1 mg/ml) were prepared in acetonitrile (ACN). An additional internal standard mixture of the three deuterated pesticides at 100 ng/ml was also prepared. Calibration points in H_20:ACN (90:10) were prepared weekly from the stock solutions. All stocks were stored at -20^{o}C in the dark.

- Sample preparation for neonicotinoid analyses

Foliage samples

Ten grams of each foliage sample were ground in liquid nitrogen to a fine powder with a pestle and mortar followed by manual homogenisation using a micro-spatula. An aliquot of every sample (1 g ± 0.1 g) was spiked with 1 ng of the deuterated pesticides in ACN and extracted using the QuEChERS method. Organic solvents (3.5 ml of ACN and 1 ml of hexane) were first added to the samples in order to increase the disruption of tissues. Subsequently, 2.5 ml water was added and the samples were extracted by mixing on a multi axis rotator for 10 minutes. Then, 1.25 g of magnesium sulphate: sodium acetate mix (4:1) was added to each tube in turn with immediate shaking to disperse the salt and prevent clumping of the magnesium salt. After centrifugation (13,000 RCF for 5 min), the upper layer of hexane was removed and the supernatant was transferred into a clean Eppendorf tube containing 500 mg of Supel^{TM}QuE PSA/C18/ENVI-Carb and vortexed. The aqueous phase and salt pellet were extracted again using 1 ml ACN and the supernatant combined with the previous ACN extract. The extract was mixed with PSA/C18/ENVI-Carb on a multi axis rotator (10 min) and then centrifuged (10 min). The supernatant was transferred into a glass tube, evaporated to dryness under vacuum, reconstituted with 200 µl ACN:H_2O (10:90) and spin filtered (0.22 µm).

Pollen

The data on neonicotinoid residues detected in oilseed rape pollen from 5 of the 7 fields studied in Botías et al. (2015) were used in the present study in order to establish a comparison with the levels and mixtures of neonicotinoids detected in foliage collected from the same plants.

UHPLC-MS/MS analyses

The UHPLC-MS/MS method described in Botías et al. (2015) was used for the analysis of samples. UHPLC-MS/MS analyses were carried out using a Waters Acquity UHPLC system coupled to a Quattro Premier triple quadrupole mass spectrometer from Micromass (Waters, Manchester, UK). Samples were separated using a reverse phase Acquity UHPLC BEH C18 column (1.7 µm, 2.1 mm × 100 mm, Waters, Manchester, UK) fitted with a ACQUITY UHPLC BEH C18 VanGuard pre-column (130 Å, 1.7 µm, 2.1 mm X 5 mm, Waters, Manchester, UK) maintained at 22 °C. Injection volume was 20 µl and mobile phase solvents were 95% water, 5% ACN, 5 mM ammonium formate, 0.1% formic acid (A) and 95% ACN, 5% water, 5 mM ammonium formate, 0.1% formic acid (B). Initial ratio (A:B) was 90:10 and separation was achieved using a flow rate of 0.2 ml/min with the following gradient: 90:10 to 70:30 in 10 min; then from 70:30 to 0:100 in two minutes and held for 7 min, and return to initial condition and equilibration for 7 min.

MS/MS was performed in Multiple Reaction Mode (MRM) using ESI in the positive mode and two characteristic fragmentations of the protonated molecular ion $[M+H]^+$ were monitored; the most abundant one for quantitation and the second one used as a qualifier as reported in Botías et al. (2015). Mass calibration of the spectrometer was performed with sodium iodide. Samples were analysed in a random order and QC samples (*i.e.* standards) were injected during runs every 10 samples to check the sensitivity of the machine. Data were acquired using MassLynx 4.1 and the quantification was carried out by calculating the response factor of neonicotinoid compounds to their respective internal standards. Concentrations were determined using a least-square linear regression analysis of the peak area ratio versus the concentration ratio (native to deuterated). At least five point calibration curves (R^2> 0.99) were used to cover the range of concentrations observed in the different matrices for all compounds, within the linear range of the instrument. Method detection and quantification limits (MDL and MQL,

210 respectively) were determined from spiked samples which had been extracted using the
211 QuEChERS method. Non-spiked samples were also prepared. MDLs were determined as the
212 minimum amount of analyte detected with a signal-to-noise ratio of 3 and MQLs as the minimum
213 amount of analyte detected with a signal-to-noise ratio of 10, after accounting for any levels of
214 analyte present in non-spiked samples (Table 1).

215 *Quality control*

216 One blank workup sample (*i.e*. solvent without matrix) per batch of eleven samples was included
217 and injected on the UHPLC-MS/MS to ensure that no contamination occurred during the sample
218 preparation. Solvent samples were also injected between sample batches to ensure that there
219 was no carryover in the UHPLC system that might affect adjacent results in analytical runs.
220 Identities of detected neonicotinoids were confirmed by comparing ratio of MRM transitions in
221 samples and pure standards. Recovery experiments performed on spiked foliage samples (1 ng/g
222 dw, n=4 and 5 ng/g dw, n=4) gave absolute recovery values ranging from 72 ± 15 to 115 ± 6% for
223 the five pesticides (Table S3). The concentration of any pesticides detected in unspiked samples
224 was also determined and subtracted from the spiked concentration to estimate the true
225 recovery of the test chemical.

226 1.5. Statistical analysis

227 All statistical analyses were carried out using SPSS 21 software. Non-parametric Mann-Whitney
228 U-tests were used to compare the concentrations of neonicotinoids present in foliage vs. pollen
229 collected from OSR flowers, foliage from OSR plants vs. foliage from wild plants, foliage from
230 wild herbaceous vs. woody plants, and finally wild annual vs. non-annuals plants (perennials and
231 biennials). When comparisons were performed in the latter group, biennials and perennials
232 were considered as one single group since both plant types overwinter at least once and were
233 thus potentially exposed to multiple neonicotinoid treatments applied in the same fields. To
234 perform the statistical analyses, all concentrations that were over the limits of detection (≥MDL)
235 but below the limits of quantification (<MQL) were assigned the value considered as the MDL in
236 each case (Table 1). Concentrations below the MDL were considered to be zero.

237 Spearman's rank correlation was used to assess the relationship among levels of neonicotinoids
238 in pollen and foliage collected from the same sites in the OSR fields.

239

240 **2. Results and Discussion**

241 2.1. Neonicotinoid residues in oilseed rape plants

242 All foliage samples collected from oilseed rape plants (N = 15) contained thiamethoxam (TMX,
243 the seed dressing applied), at an average concentration of 1.04 ± 0.88 ng/g (mean ± SD; median
244 = 1.04). Clothianidin (CLO), the major metabolite of thiamethoxam, and used in the seed
245 dressing in the previous year in all the five studied fields, was also present in all the foliage
246 samples, being at higher mean concentrations than thiamethoxam (2.92 ± 2.08 ng/g; median =
247 2.09; U (28) = 36, Z = -3.18, *P* = 0.001). Maximal concentrations in OSR foliage were 2.3 ng/g for
248 thiamethoxam and 8.7 ng/g for clothianidin. Furthermore, imidacloprid, which had not been
249 applied in these fields in at least the previous three years, was also detected in 20% of the
250 samples, albeit at low concentrations (0.23 ± 0.79 ng/g), and with only one sample showing

concentrations as high as 3.1 ng/g. Although the conversion of thiamethoxam to toxicologically relevant concentrations of clothianidin and the additional presence of imidacloprid would extend the duration of crop protection, the simultaneous presence of more than one neonicotinoid in the plants may put additional selection pressure on crop-infesting pest insects, increasing the chances of cross-resistance to these compounds (Nauen et al., 2002; Prabhaker et al., 2005). Thiacloprid and acetamiprid, which were not applied to these fields in the previous three years but are licensed for use in the UK, were not detected in any of the oilseed rape foliage samples.

Consistent with the findings above, and as reported in a previous study (Botías et al. 2015), oilseed rape pollen samples, collected from the same plants as the foliage samples, also all contained thiamethoxam (Table S1), with the concentrations in both matrices showing a positive correlation (Spearman rank's correlation, r_S (13) = 0.61, P = 0.016) (Figure 1), *i.e* plants with more thiamethoxam in their leaves tended to have more in their pollen. However, the levels of thiamethoxam detected in pollen (mean ± SD: 3.5 ± 2.5 ng/g) were three fold higher than in foliage (U(28) = 31, Z = -3.4, P = 0.001) (Figure 2). Clothianidin was also present in all pollen samples, but in this case, levels (1.9 ± 2.4 ng/g) were significantly lower than in foliage (U(28) = 57, Z = -2.3, P = 0.021), and no correlation was found between concentrations detected in both matrices for this compound (r_S (13) = 0.27, P = 0.33). To our knowledge, this is the first study comparing levels of thiamethoxam and clothianidin in foliage and pollen from the same plants. A previous study also found differences in the average concentrations for imidacloprid in different tissues of maize seed-treated plants, with higher average levels detected in foliage (6.6 ng/g) than in pollen (2.1 ng/g) (Bonmatin et al., 2005). The discrepancy in the relative levels of thiamethoxam and clothianidin in foliage and pollen may reflect differences in the translocation rates from the plant xylem to the pollen grains for these two active ingredients, or perhaps differences in their rates of degradation according to tissue type. This possible difference in the uptake rates for these two compounds in plants is also suggested by our previous findings (Botías et al., 2015), where levels of thiamethoxam detected in soil were positively correlated with the levels in pollen of the oilseed rape plants growing in that soil, while the same correlation was not found for clothianidin. Clothianidin is known to be highly persistent in foliage (Kim et al., 2012) and earlier studies have shown that high levels of thiamethoxam are not always associated with detectable levels of its main metabolite (clothianidin) in pollen, flowers and bees (Botías et al., 2015; Hladik et al., 2016; Stewart et al., 2014). The frequency and factors involved on the simultaneous presence of both active compounds in the pollen of treated and non-treated plants should be further studied, since the combined exposure to thiamethoxam and clothianidin has been shown to have detrimental effects on bees (Fauser-Misslin et al., 2014; Sandrock et al., 2014). In general, the effects of simultaneous exposure of insects to multiple pesticides are very poorly understood.

Imidacloprid and thiacloprid also showed different patterns for foliage and pollen. While imidacloprid was present in 20% of the foliage samples and not detected in any of the pollen samples, thiacloprid, absent in foliage, was detected in 80 % of the pollen samples (1.9 ± 2.1 ng/g), with 7.3 ng/g as the highest concentration. Our results suggest that the persistence of these compounds in different matrices may depend on the specific chemical structure of each pesticide, the metabolic enzymes involved in their degradation (which have not yet been examined in plants, Simon-Delso et al., 2015), and on the route of contamination in each case

(*i.e.* root uptake from the residues in soil and soil water, spray drift or contaminated dust emissions during coated-seeds sowing). Thiacloprid is less toxic to insects than the other neonicotinoids detected (Iwasa et al., 2004), but nonetheless its presence in pollen is of serious concern since we are unable to identify the source of this environmental contamination. This active substance is widely used as spray in gardens and also in orchards and crops in the UK (PAN-UK, 2016; Garthwaite et al., 2013), so drifting from neighboring farms and/or gardens to the studied fields (Langhof et al., 2005) may explain the residues detected in our pollen samples.

2.2. Neonicotinoid residues in wild plants from the field margins

Drilling equipment has been identified as a source of dispersion of the abraded seed coating during seed sowing that can contaminate air, vegetation, surface soil and water surrounding the fields (Tapparo et al., 2012; Nuyttens et al., 2013), and it is highlighted as an area of concern and relevant contamination route for off-crop areas (EFSA, 2013). Additionally, neonicotinoids are water-soluble and mobile in soil, so that plants adjacent to crops whose seeds are treated with neonicotinoids can unintentionally take up excess residues if there is significant lateral movement of the pesticide (Goulson, 2013). Indeed, we detected neonicotinoid residues in 52% of the foliage samples collected from wild plants growing in OSR field margins (N = 100) (Table 1), with an average total concentration of 10 ± 22 ng/g. The maximum levels for thiamethoxam were 106 ng/g in a sample of *Cirsium vulgare*, 11 ng/g for clothianidin in *Rubus fruticosus* (field 2, margin 1) (Table S2c) and 26 ng/g for imidacloprid in *Cirsium vulgare* (field 4, margin 1) (Table S2d). These concentrations of total neonicotinoid residues in wild plants were significantly higher than in the OSR foliage (4.2 ± 3.1 ng/g) (M-W test: U(113) = 470, Z = - 2.42, P = 0.016). However, the median values of total neonicotinoids were higher in OSR foliage (3.30 ng/g) than in wild plants (0.10 ng/g) due to highly variable quantities of residues in the 45 wild plant species evaluated, ranging between non-detectable levels to more than 106 ng/g (Tables S2a-S2e). According to conclusions by the European Food Safety Authority (EFSA, 2013), the predicted percentage of thiamethoxam deposition in off-field vegetation would be 2.7 % of the rate applied to the seed-treated oilseed rape crop (0.91 g a.s./ha in our studied fields, *i.e.* 2.7 % of 33.6 g a.s./ha). However, as reported above, some off-field plants showed concentrations that would exceed the predicted contamination due to deposition, as they were in some cases higher than the levels detected in the seed-treated plants, suggesting an additional route of contamination apart from dust drift (*e.g.* run-off from the crop to the field margin soil).

Thiamethoxam was the most frequently detected residue (35% of the samples) in field margin plants, and was detected at higher average concentrations in long-lived plants (perennials-biennials: 9.5 ± 24 ng/g) than in annuals (7 ± 13 ng/g), although statistical comparisons failed to show statistical significance for this difference (M-W test: U(98) = 901.5, Z = -1.619, *P* = 0.106). Clothianidin was detected in 22% of the wild plant samples and at significantly higher concentrations in annual plants (0.58 ± 1.4 ng/g) than in perennials-biennials (0.48 ± 1.8 ng/g) (M-W test: U(98) = 856, Z = -2.4, *P* = 0.018). Conversely imidacloprid, not applied for at least 3 years but present in 29% of the wild plants, showed significantly higher concentrations in perennials-biennials (1.21 ± 4.73 ng/g) than in annuals (1.15 ± 3.19 ng/g)(M-W test: U(98) = 824, Z = -2.44, *P* = 0.015). This slightly higher presence of imidacloprid in long-lived plants (biennials and perennials) may reflect a longer persistence and bioaccumulation of imidacloprid (Castle et al., 2005), with levels increasing in field margin plants over time for this compound, whereas

clothianidin may be metabolised relatively faster in perennials, and be more persistent in annuals according to our results. However, although statistical comparisons showed significant differences between plant types for these two compounds, the differences in mean levels were minimal, and the number of samples analysed for each group was not even (68 perennial and biennial plants vs. 32 annual plants) (Tables S2a-2e). A bigger sample size and an experimental design where plants with different life history strategies are exposed to these compounds in the same environmental conditions would be needed to better understand this issue. Annual plants have shorter longevity and higher relative growth rate than perennials, which leads to faster metabolic rates (Garnier, 1992). They also have smaller rooting depths and lateral root spreads than perennials (Jochenk Schenk and Jackson, 2002). These differences in the physiological and morphological traits of annuals and long-lived plants (perennials and biennials) might affect the uptake capacities and the metabolic pathways of xenobiotics in these two groups of plants, which may in part explain our findings.

Neonicotinoid residues detected in foliage of herbaceous and woody plants were also compared, and we found imidacloprid to be at significantly higher concentrations in herbaceous plants (1.5 ± 4.7 ng/g) than in woody plants (M-W test: $U(98) = 494$, $Z = -3.03$, $P = 0.002$), where this compound was below the method detection limits (≤ 0.02) in all samples. In addition, total neonicotinoid residues were in general detected at higher average concentrations in foliage of herbaceous plants (11.22 ± 22.20 ng/g) than in woody plants (6.95 ± 18.93 ng/g), probably due to residual neonicotinoid concentrations decreasing in relation to the plant biomass (Balfour et al., 2016; Krischik et al., 2007), which is generally higher in woody plants. However, since this last trend was not statistically significant (M-W test: $U(98) = 509.5$, $Z = -1.67$, $P = 0.095$) and the number of samples analysed from each group was very different (81 herbaceous plants vs. 19 woody plants tested) (Tables S2a-2e), further exploration to confirm this observation is warranted.

Acetamiprid, which had not been used before in the studied farms, was present in 1% of the foliage samples (Table 1). As with thiacloprid, the origin of these residues requires investigation.

2.3. Potential effects of neonicotinoids on non-target insects

The hazard quotient (HQ) approach was used to put the maximal concentrations detected in the wild plants from field margins, which represent the worst-case scenario, into an ecological effects context (Candolfi et al., 2001; Bonmatin et al., 2015). Overall, the results demonstrate considerable variation in the predicted impact of neonicotinoids on different species within each insect order, with the highest levels of neonicotinoid residues found in foliage being lower than most of the reported lethal levels for acute exposure in the insects evaluated. Considering the EU guidance document on risk assessment procedures for plant protection products with non-target arthropods and the guidelines on terrestrial ecotoxicology (Candolfi et al., 2001; European Commission, 2002), if the risk indicator (Hazard Quotient: HQ) based on the active substance is greater than or equal to 2, a potential hazard is concluded and a higher tier test must be carried out, and only if it is well below this HQ trigger (e.g. 100-fold), studies with the formulation could be considered dispensable due to no unacceptable impact on the studied organisms. This threshold value of 2 is expected to be conservative as it is indicated for laboratory tests performed with two non-target arthropod sensitive species (Candolfi et al., 1999), of which the

exposure is maximized on a glass plate. Moreover, the HQ for non-target arthropods in the EU risk assessment regulation is defined as the ratio of the predicted exposure concentration (PEC, g/mL a.s. per ha) divided by the lethal rate that kills 50% of the test organisms (LR_{50}, g/mL a.s. per ha). However, in our study we calculated HQs as the ratio of realistic worst-case exposure (ng/g or ppb) divided by lethal concentration that kills 50% of the test organisms (LC_{50}, ng/ml or ppb). Therefore, it is important to note that we used the threshold values described in ESCORT II guidance document (Candolfi et al., 2001) to put the residue levels detected into a context of risk assessment and to understand the possible impact that the detected concentrations may cause in the field, but they are not deemed as decision making criteria and they should be interpreted with caution.

Our results show that from the twenty-four species assessed, only three presented a HQ ≥ 2, with HQ = 6.27 for thiamethoxam in *Aphis glycines* (Hemiptera: Aphididae), HQ = 2.02 for imidacloprid in *Homalodisca coagulata* (Hemiptera: Cicadellidae) and 1.77-2.12 for thiamethoxam in *Podisus nigrispinus* (Hemiptera: Pentatomidae) (Table 2), meaning that the highest concentrations found for these compounds in our foliage samples would be potentially lethal for them in the short term. Four more hemipterans (*Aphis pomi* (Aphididae), *Myzus persicae* (Aphididae), *Orius laevigatus* (Anthocoridae), and *Hyaloides vitripennis* (Miridae), and one lepidopteran (*Danaus plexippus* (Nymphalidae)), were only 10-fold below the trigger value 2 used for non-target arthropods in the EU risk assessment guidelines, indicating potential environmental risk for these organisms at the peak exposure levels detected in our study. Four out of the remaining sixteen insect species (*i.e. Anaphes iole* (Hymenoptera: Mymaridae), *Aphelinus mali* (Hymenoptera: Encyrtidae), *Bombyx mori* (Lepidoptera: Bombycidae) and *Anoplophora glabripennis* (Coleoptera: Cerambycidae)) presented HQs ranging from 10 to 100-fold below the HQ trigger of 2 (from HQ = 0.06 for thiamethoxam in *Anaphes iole* to HQ = 0.16 in *Aphelinus mali* for imidacloprid), with the other twelve species having HQs all below 100-fold this threshold value. It should be noted that some of the species evaluated are considered as pests for some crops, and some are not present in the studied area (South-East England), as for instance the above mentioned hemipterans *Aphis glycines* and *Homalodisca coagulata* (Magalhaes et al., 2008; Prabhaker et al., 2006) (Table 2). It is also worth mentioning that the use of the maximal concentrations detected to calculate HQ values reflect a worst-case scenario, and predicting the ecological consequences of this non-intended contamination of field margin plants is challenging due to the high variability in the residue concentrations detected, and also in the susceptibility to the exposure for the different insect species. Nonetheless, the fact that 17 out of 35 wild plant foliage samples with detectable levels of thiamethoxam (49%) showed concentrations over the lethal concentration for *Aphis glycines* (LC_{50} = 16.9 ng/mL) calls for further consideration of the possible impact of exposure for non-target insects that could be potentially more susceptible to the highest levels of residues present in foliage. Furthermore, the exposure-toxicity ratio analysis (HQ) suggests that some non-target organisms which play an important role as biocontrol agents for some pests, such as the hemipteran *Orius laevigatus* or the hymenopteran *Aphelinus mali*, present in the UK, might be potentially affected by the acute exposure to the highest concentrations of neonicotinoid residues detected in this study (*O. laevigatus*: HQ range residual contact = 0.09-0.65, HQ range oral ingestion = 0.01-0.02; *A. mali*: HQ residual contact = 0.16). Predatory invertebrates may become exposed to neonicotinoids by ingestion of contaminated plant tissue, through residual contact by moving on contaminated

leaves, or by consuming pests that fed on contaminated plants (Armer et al., 1998; Lundgren, 2009; Naranjo and Gibson, 1996), and these systemic insecticides can persist in the environment for long periods (Bonmatin et al., 2015; Goulson, 2013; Jones et al., 2014).

Our data clearly show that non-target insects living in field margins are likely to be chronically exposed to highly variable concentrations of neonicotinoids, often in mixtures. These concentrations are typically below the lethal concentrations of these pesticides, but there remains cause for concern. The toxicity studies upon which these calculations are based are short-term exposure (1 to 7 days), yet these insects are likely exposed throughout their lives. This is of particular concern as it has been reported that neonicotinoids, like many other toxicants, increase their toxicity when exposure is extended in time, so that much lower concentrations eventually result in death (Rondeau et al., 2014; Sánchez-Bayo and Goka, 2014; Suchail et al., 2001). Apart from lethal effects, a number of studies have found sub-lethal impacts on larval development, reproductive rate and susceptibility to disease after exposure to field-realistic doses of neonicotinoids on insects (Di Prisco et al., 2013; Kullik et al., 2011; Lashkari et al., 2007; Magalhaes et al., 2008; Pecenka and Lundgren, 2015), highlighting the need of long-term chronic tests for pesticide exposure where other side effects apart from mortality are recorded. The effect of the combined exposure to mixtures of neonicotinoids should also be considered in risk assessment test. Our HQ calculations are based on studies in which insects were exposed to a single pesticide, yet we found that up to three neonicotinoids (*i.e.* thiamethoxam, clothianidin and imidacloprid) can be detected in foliage from a single plant (46.3 % of the foliage samples with residues had detectable levels of two or more neonicotinoids).

In summary, our results show that a proportion of the seed-applied neonicotinoid does not come into contact with the target pests, but instead is dispersed into the surrounding area. Concentrations in plant tissues and sap between 5 and 10 ppb are generally regarded as sufficient to provide protection against pest insects (Goulson, 2013), and as shown by our results, the levels detected in foliage of field margin plants are very variable but can often exceed this threshold, at times overlapping with LC_{50} values reported for some non-target insects. The widespread presence of these compounds in field margin wild plants raises concerns over the potential effects of exposure for non-target wildlife living in these habitats, which are often managed for biodiversity through agri-environmental schemes (Pywell et al., 2006; Wood et al., 2015). Our data are consistent with the hypothesis that declines of farmland butterflies could be driven by exposure to neonicotinoids in field margin vegetation (Gilburn et al. 2015). Hedgerows and field margins contribute to enhance crop yields by providing nest sites, forage resources for pollinators and acting as reservoirs for natural enemies of crop pests (Hannon and Sisk, 2009; Pywell et al., 2015), as well as increasing the nature conservation value of agricultural landscapes (Dennis and Fry, 1992; Paoletti et al., 1992). If these functions are being impaired by contamination with persistent, systemic insecticides, then this may be a matter with significant ecological and economic implications.

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691 Figure 1. Concentrations of thiamethoxam and clothianidin (ng/g) in pollen of oilseed rape
692 flowers as a function of their levels present in the foliage of the same plants.

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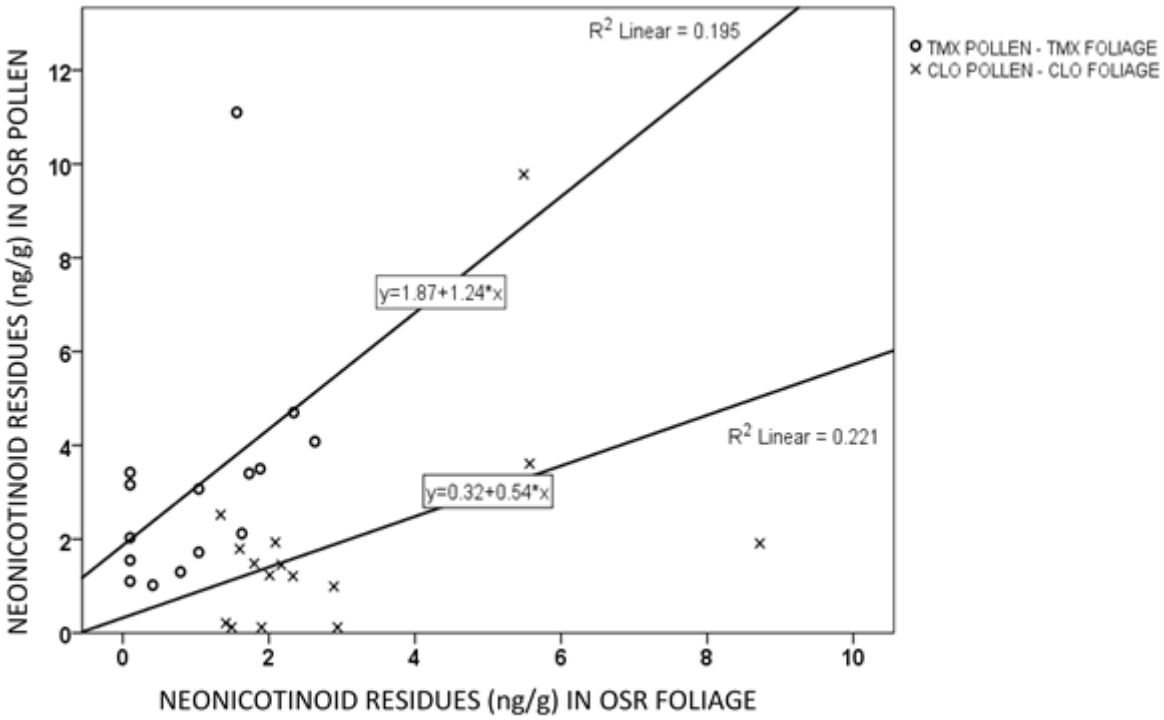

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Figure 2. Concentrations of thiamethoxam and clothianidin (ng/g) detected in foliage and pollen from OSR plants. (Black horizontal bars inside boxplots are median values. The upper and lower whiskers represent scores outside the inter-quartile range; open circles represent mild outliers and asterisks are extreme outliers).

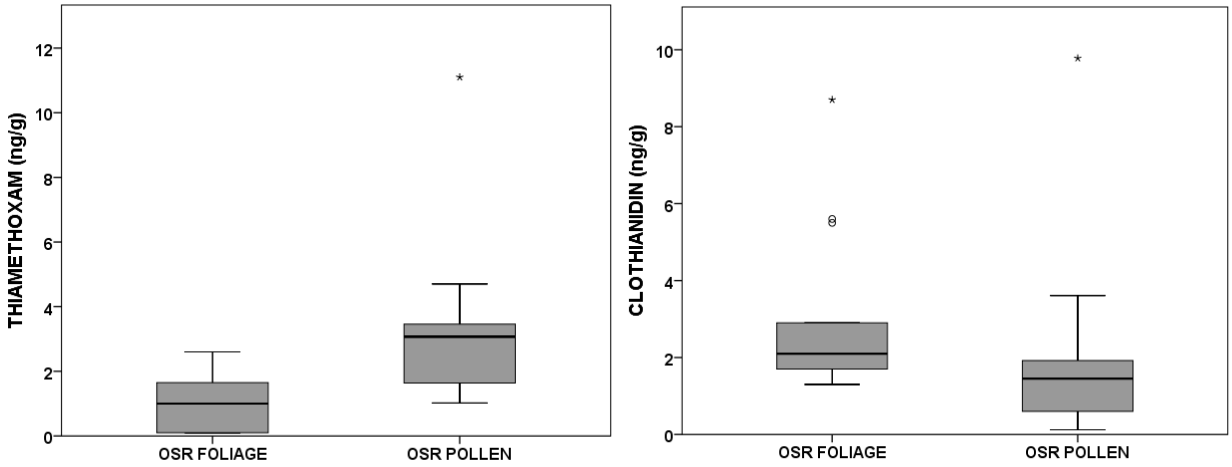

Figure 2. Concentrations of total neonicotinoid residues in foliage collected from oilseed rape plants and wild plants from oilseed rape field margins. (Black horizontal bars inside boxplots are median values. The upper and lower whiskers represent scores outside the inter-quartile range; open circles represent mild outliers and asterisks are extreme outliers).

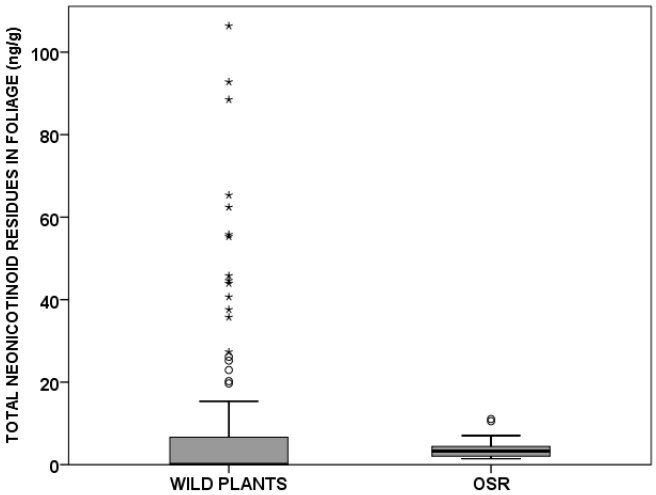

Table 1. Number of samples analysed, percentage with detectable levels of neonicotinoid insecticides, mean and range of levels found (Mean ± Standard Deviation) in pollen and foliage samples collected from oilseed rape (OSR) plants and foliage from wild plants collected from the margins of the OSR fields (TMX: thiamethoxam, CLO: clothianidin, IMC: imidacloprid, THC: thiacloprid, ACT: acetamiprid).

			TMX	CLO	IMC	THC	ACT
POLLEN	**N**	*Method detection limit* (MDL)(ppb)	0.12	0.12	0.16	0.04	0.04
		Method quantification limit (MQL)(ppb)	0.36	0.36	0.48	0.12	0.12
OSR FLOWERS	15	FREQUENCY OF DETECTIONS (%)	100%	100%	0%	80%	0%
		RANGE (ng/g)	1.02 - 11.10	≤ 0.36 - 9.78	≤ 0.16	≤ 0.04 - 7.25	≤ 0.04
		MEAN ± S.D. (ng/g)	3.15 ± 2.48	1.90 ± 2.39		1.87 ± 2.14	
		MEDIAN (ng/g)	3.07	1.45		1.27	
FOLIAGE	**N**	*Method detection limit* (MDL)(ppb)	0.10	0.20	0.20	0.02	0.02
		Method quantification limit (MQL)(ppb)	0.30	0.60	0.60	0.06	0.06
OSR PLANTS	15	FREQUENCY OF DETECTIONS (%)	100%	100%	2%	0%	0%
		RANGE (ng/g)	≤ 0.10 - 2.60	1.30 - 8.70	≤ 0.20 - 3.10	≤ 0.02	≤ 0.02
		MEAN ± S.D. (ng/g)	1.04 ± 0.88	2.91 ± 2.08	0.23 ± 0.80		
		MEDIAN (ng/g)	1.04	2.09	≤ 0.20		
FIELD MARGIN WILD PLANTS	100	FREQUENCY OF DETECTIONS (%)	35%	22%	29%	0%	1%
		RANGE (ng/g)	≤ 0.10 - 106.2	≤ 0.20 - 11.45	≤ 0.20 - 26.06	≤ 0.02	≤ 0.02 - ≤ 0.06
		MEAN ± S.D. (ng/g)	8.71 ± 21.13	0.51 ± 1.67	1.19 ± 4.28		≤ 0.02
		MEDIAN (ng/g)	≤ 0.10	≤ 0.20	≤ 0.20		≤ 0.02

Table 2. Lethal concentrations (LC_{50}) reported for twenty-four insect species from four different orders, maximal concentrations detected in the foliage samples collected from wild plants in OSR field margins, and exposure-toxicity-ratio (HQ) for each species defined as the pesticide concentrations divided by the LC_{50} (a HQ of 1 = LC_{50}). The exposure routes used to obtain the LC_{50} values (ng/mL) were oral ingestion (O) or contact with neonicotinoid-treated leaves following systemic bioassay (SB) or residual bioassay (RB). HQs equal or above 0.01 (≥ 1% of the LC_{50}) are highlighted in bold numbers.

* median value calculated from all the LC_{50}s reported for *Homalodisca coagulata* after 48 h exposure to imidacloprid (range LC_{50}: 0.087 – 53.09 ng/ml (ppb), range HQ: 0.49 – 298.85).

** median value calculated from all the LC_{50}s reported for *Homalodisca coagulata* after 48 h exposure to thiamethoxam (range LC_{50}: 644.26 – 704.45 ng/ml (ppb), range HQ: 0.15-0.16).

† introduced species

†† domesticated species

INSECT ORDER	SPECIES	DEVELOPMENTAL STAGE	COMPOUND	MAXIMUM LEVELS ng/g (ppb)	LC_{50} (time exposure; route of exposure) ng/mL (ppb)	HQ	ROLE	DISTRIBUTION	REFERENCE
Hymenoptera	*Diadegma insulare*	Adults	Imidacloprid	26	2,000 (24 h; RB)	**0.01**	Biocontrol of pests	North America	Hill and Foster, 2000
	Anaphes iole	Adults	Thiamethoxam	106	1,700 (48 h; RB)	**0.06**	Biocontrol of pests	North America	Williams and Price, 2003
	Aphelinus mali	Adults	Imidacloprid	26	160 (24 h; RB)	**0.16**	Biocontrol of pests	North America, Cosmopolitan†	Cohen et al., 1996
	Eretmocerus eremicus	Adults	Thiamethoxam	106	1,010,000 (48 h; SB)	1.05E-04	Biocontrol of pests	USA	Prabhaker et al., 2011
			Imidacloprid	26	1,930,000 (24 h; SB)	1.35E-05		Southern Europe†	
	Encarsia formosa	Adults	Thiamethoxam	106	397,000 (48 h; SB)	2.67E-04	Biocontrol of pests	Cosmopolitan	
			Imidacloprid	26	980,000 (24 h; SB)	2.65E-05			
	Gonatocerus ashmeadi	Adults	Thiamethoxam	106	1,440,000 (48 h; SB)	7.36E-05	Biocontrol of pests	North America	
			Imidacloprid	26	2,630,000 (24 h; SB)	9.89E-06			
	Aphytis melinus	Adults	Thiamethoxam	106	105,000 (24 h; SB)	1.01E-03	Biocontrol of pests	USA	
			Imidacloprid	26	246,000 (24 h; SB)	1.06E-04		Southern Europe†	
Lepidoptera	*Bombyx mori*	2nd instar larvae	Imidacloprid	26	1,270 (96 h; O)	**0.02**	Economically important	Cosmopolitan††	Yu et al., 2015
			Thiamethoxam	106	2,380 (96 h; O)	**0.04**			
	Danaus plexippus	Neonate larvae	Clothianidin	11	15,63 (36 h; O)	**0.70**	Pollinator/high cultural value	North America; Southern Europe; Oceania	Pecenka & Lundgren, 2015
	Cydia pomponella	Neonate larvae	Clothianidin	11	2,400 (24 h; O)	4.58E-03	Agricultural pest	Cosmopolitan	Brunner et al., 2005
	Pandemis pyrusana	Neonate larvae	Clothianidin	11	186,000 (24 h; O)	5.91E-05	Agricultural pest	North America	
	Choristoneura rosaceana	Neonate larvae	Clothianidin	11	75,000 (24 h; O)	1.47E-04	Agricultural pest	North America	
Hemiptera	*Aphis glycines*	Adults	Imidacloprid	26	31.29 (7 days; SB)	**0.83**	Agricultural pest	Asia	Magalhaes et al., 2008
			Thiamethoxam	106	16.91 (7 days; SB)	**6.27**		North America†	
	Aphis pomi	1st instar nymphs	Imidacloprid	26	64 (72 h; O)	**0.41**	Agricultural pest	Europe	Lowery and Smirle, 2003
		2nd instar nymphs			54 (72 h; O)	**0.48**		Western Asia	
		3rd instar nymphs			67 (72 h; O)	**0.39**		North Africa	
		Adults			165 (72 h; O)	**0.16**		North America	
	Homalodisca coagulata (= *H. vitripennis*)	Adults	Imidacloprid	26	12.84 (48 h; SB)*	**2.02**	Agricultural pest	North America	Prabhaker et al., 2006
			Thiamethoxam	106	674.35(48 h; SB)**	**0.16**			
	Myzus persicae	Adults	Imidacloprid	26	73 (48 h; O)	**0.36**	Agricultural pest	Cosmopolitan	Nauen and Elbert, 1997
	Myzus nicotianae	Adults	Imidacloprid	26	14,000 (48 h; O)	1.86E-03	Agricultural pest	Cosmopolitan	
	Orius laevigatus	5th instar nymphs	Imidacloprid	26	40 (72 h; RB)	**0.65**	Biocontrol of pests	Europe	Delbeke et al., 1997
					1,100 (72 h; O)	**0.02**			
		Adults			300 (72 h; RB)	**0.09**			
					2,100 (72 h; O)	**0.01**			
	Hyaliodes vitripennis	Nymphs	Thiamethoxam	106	1,430 (24 h; RB)	**0.07**	Biocontrol of pests	North America	Bostanian et al., 2005
		Adults			500 (24 h; RB)	**0.21**			
	Greocoris punctipes	Adults	Imidacloprid	26	5,180,000 (96 h; SB)	5.02E-06	Biocontrol of pests	North and Central America	Prabhaker et al., 2011
			Thiamethoxam	106	2,170,000 (96 h; SB)	4.88E-05			
	Orius insidiosus	Adults	Imidacloprid	26	2,780,000 (96 h; SB)	9.35E-06	Biocontrol of pests	North and South America	
			Thiamethoxam	106	1,670,000 (96 h; SB)	6.35E-05		Europe†	
	Podisus nigrispinus	2nd instar nymphs	Imidacloprid	26	130 (5 days; O)	**0.20**	Biocontrol of pests	South and Central America	Torres and Ruberson, 2004
		5th instar nymphs			440 (5 days; O)	**0.06**			
		2nd instar nymphs	Thiamethoxam	106	50 (5 days; O)	**2.12**			
		5th instar nymphs			60 (5 days; O)	**1.77**			
	Bemisia tabaci	Adults	Imidacloprid	26	264,000 (48 h; SB)	9.85E-05	Agricultural pest	Cosmopolitan	Prabhaker et al., 2005
			Thiamethoxam	106	108,000 (48 h; SB)	9.81E-04			
Coleoptera	*Anoplophora glabripennis*	Adults	Imidacloprid	26	1,900 (72 h; O + RB)	**0.01**	Agricultural pest	Eastern Asia	Wang et al., 2005
					5,900 (72 h; O)	4.41E-03		North America†	
			Thiamethoxam	106	1,000 (72 h; O + RB)	**0.11**		Europe†	
			Clothianidin	11	1,100 (72 h; O + RB)	**0.01**			

Supplementary Information

Table S1. Neonicotinoid concentrations in foliage and pollen collected from three sites in five oilseed rape field crops. (TMX: thiamethoxam, CLO: clothianidin, IMC: imidacloprid, THC: thiacloprid, ACT: acetamiprid). Concentrations at detectable levels are outlined in bold numbers.

		FOLIAGE OILSEED RAPE PLANTS					POLLEN OILSEED RAPE PLANTS				
FIELD	SITES	NEONICOTINOID RESIDUES (ng/g)					NEONICOTINOID RESIDUES (ng/g)				
		TMX	CLO	IMC	THC	ACT	TMX	CLO	IMC	THC	ACT
1	S1	**2.63**	**2.09**	**≤ 0.60**	≤ 0.02	≤ 0.02	**4.08**	**1.93**	≤ 0.16	**3.03**	≤ 0.04
	S2	**1.73**	**2.17**	≤ 0.20	≤ 0.02	≤ 0.02	**3.40**	**1.45**	≤ 0.16	**0.49**	≤ 0.04
	S3	**1.63**	**1.80**	**≤ 0.60**	≤ 0.02	≤ 0.02	**2.12**	**1.48**	≤ 0.16	≤ 0.04	≤ 0.04
2	S1	**1.04**	**2.01**	≤ 0.20	≤ 0.02	≤ 0.02	**1.72**	**1.23**	≤ 0.16	≤ 0.04	≤ 0.04
	S2	**≤ 0.30**	**2.33**	≤ 0.20	≤ 0.02	≤ 0.02	**1.10**	**1.21**	≤ 0.16	**2.67**	≤ 0.04
	S3	**0.41**	**2.89**	≤ 0.20	≤ 0.02	≤ 0.02	**1.02**	**0.99**	≤ 0.16	≤ 0.04	≤ 0.04
3	S1	**≤ 0.30**	**1.60**	≤ 0.20	≤ 0.02	≤ 0.02	**3.42**	**1.79**	≤ 0.16	**1.06**	≤ 0.04
	S2	**≤ 0.30**	**1.41**	≤ 0.20	≤ 0.02	≤ 0.02	**1.55**	**0.21**	≤ 0.16	**3.16**	≤ 0.04
	S3	**0.79**	**2.94**	≤ 0.20	≤ 0.02	≤ 0.02	**1.30**	**≤ 0.36**	≤ 0.16	**≤ 0.12**	≤ 0.04
4	S1	**≤ 0.30**	**1.34**	≤ 0.20	≤ 0.02	≤ 0.02	**3.16**	**2.52**	≤ 0.16	**1.54**	≤ 0.04
	S2	**≤ 0.30**	**1.49**	≤ 0.20	≤ 0.02	≤ 0.02	**2.03**	**≤ 0.36**	≤ 0.16	**7.25**	≤ 0.04
	S3	**1.04**	**1.90**	≤ 0.20	≤ 0.02	≤ 0.02	**3.07**	**≤ 0.36**	≤ 0.16	**5.48**	≤ 0.04
5	S1	**1.56**	**5.49**	≤ 0.20	≤ 0.02	≤ 0.02	**11.01**	**9.78**	≤ 0.16	**1.32**	≤ 0.04
	S2	**2.34**	**8.72**	≤ 0.20	≤ 0.02	≤ 0.02	**4.70**	**1.91**	≤ 0.16	**1.27**	≤ 0.04
	S3	**1.88**	**5.57**	**3.10**	≤ 0.02	≤ 0.02	**3.50**	**3.61**	≤ 0.16	**0.67**	≤ 0.04

775 Tables S2a-S2e. Concentrations of neonicotinoid residues in foliage collected from wild plants
776 growing in the four margins of five oilseed rape fields.

777 Table S2a. Field 1.

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FIELD	MARGIN	SPECIES	PLANT TYPE	LIFE HISTORY STRATEGY	NEONICOTINOID RESIDUES (ng/g)				
					TMX	CLO	IMC	THC	ACT
1	M1	*Lamium purpureum*	H	A	**19.49**	≤ 0.20	**≤ 0.60**	≤ 0.02	≤ 0.02
		Glechoma hederacea	H	P	**22.94**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Lamium album	H	P	**88.50**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Vicia sativa	H	A	**20.24**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Trifolium pratense	H	P	**11.47**	**0.97**	≤ 0.20	≤ 0.02	≤ 0.02
		Dactylis glomerata	H	P	≤ 0.10	≤ 0.20	**25.20**	≤ 0.02	≤ 0.02
	M2	*Cardamine pratensis*	H	P	**37.59**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Papaver rhoeas	H	A	**41.76**	**1.99**	**≤ 0.60**	≤ 0.02	**≤ 0.06**
		Ranunculus repens	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Trifolium repens	H	P	≤ 0.10	≤ 0.20	**14.52**	≤ 0.02	≤ 0.02
		Galium aparine	H	A	**35.63**	≤ 0.20	**10.16**	≤ 0.02	≤ 0.02
	M3	*Crataegus monogyna*	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Trifolium repens	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Rubus fruticosus	W	P	**65.13**	**≤ 0.60**	≤ 0.20	≤ 0.02	≤ 0.02
		Papaver rhoeas	H	A	**6.72**	**0.75**	**0.87**	≤ 0.02	≤ 0.02
		Viola arvensis	H	A	**1.29**	**≤ 0.60**	**1.63**	≤ 0.02	≤ 0.02
		Glechoma hederacea	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M4	*Calystegia sylvatica*	H	P	≤ 0.10	≤ 0.20	**1.18**	≤ 0.02	≤ 0.02
		Malva sylvestris	H	P	**≤ 0.30**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Matricaria recutita	H	A	**≤ 0.30**	**≤ 0.60**	**≤ 0.60**	≤ 0.02	≤ 0.02
		Sonchus oleraceus	H	A	≤ 0.10	≤ 0.20	**14.79**	≤ 0.02	≤ 0.02
		Silene latifolia	H	P	**1.14**	**5.93**	≤ 0.20	≤ 0.02	≤ 0.02
		Dactylis glomerata	H	P	≤ 0.10	≤ 0.20	**6.23**	≤ 0.02	≤ 0.02

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791 Table S2b. Field 2.

FIELD	MARGIN	SPECIES	PLANT TYPE	LIFE HISTORY STRATEGY	NEONICOTINOID RESIDUES (ng/g)				
					TMX	CLO	IMC	THC	ACT
2	M1	*Cirsium vulgare*	H	B	**106.16**	≤ 0.20	**≤ 0.60**	≤ 0.02	≤ 0.02
		Rubus fruticosus	W	P	**43.83**	**11.45**	≤ 0.20	≤ 0.02	≤ 0.02
		Hieracium agg.	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Sonchus arvensis	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Crataegus monogyna	W	P	**1.03**	**≤ 0.60**	≤ 0.20	≤ 0.02	≤ 0.02
		Galium aparine	H	A	≤ 0.10	**5.12**	**≤ 0.60**	≤ 0.02	≤ 0.02
	M2	*Rubus fruticosus*	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Silene vulgaris	H	P	**14.94**	**≤ 0.60**	**≤ 0.60**	≤ 0.02	≤ 0.02
		Cirsium vulgare	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Anthriscus sylvestris	H	P	≤ 0.10	≤ 0.20	**≤ 0.60**	≤ 0.02	≤ 0.02
		Heracleum sphondylium	H	P	≤ 0.10	≤ 0.20	**0.72**	≤ 0.02	≤ 0.02
		Stachys sylvatica	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Crataegus monogyna	W	P	≤ 0.10	**3.26**	≤ 0.20	≤ 0.02	≤ 0.02
	M3	*Matricaria recutita*	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Cirsium vulgare	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Papaver rhoeas	H	A	**39.05**	**5.59**	≤ 0.20	≤ 0.02	≤ 0.02
		Veronica persica	H	A	**32.93**	**≤ 0.60**	**2.60**	≤ 0.02	≤ 0.02
		Senecio jacobaea	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Sonchus oleraceus	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Viola arvensis	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M4	*Matricaria recutita*	H	A	**≤ 0.30**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Sonchus oleraceus	H	A	**22.05**	**≤ 0.60**	**5.06**	≤ 0.02	≤ 0.02
		Cirsium vulgare	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Carduus sp.	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Lamium purpureum	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Fallopia convolvulus	H	A	**2.22**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02

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805 Table S2c. Field 3.

FIELD	MARGIN	SPECIES	PLANT TYPE	LIFE HISTORY STRATEGY	NEONICOTINOID RESIDUES (ng/g)				
					TMX	CLO	IMC	THC	ACT
3	M1	*Matricaria recutita*	H	A	**≤ 0.30**	**≤ 0.60**	**≤ 0.60**	≤ 0.02	≤ 0.02
		Fumaria officinalis	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Matricaria recutita	H	A	**≤ 0.30**	**≤ 0.60**	**≤ 0.60**	≤ 0.02	≤ 0.02
		Sonchus arvensis	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Cirsium arvense	H	P	**62.40**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Sherardia arvensis	H	A	**0.59**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Fallopia convolvulus	H	A	**≤ 0.30**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Galium aparine	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M2	*Anthriscus sylvestris*	H	P	**2.46**	**≤ 0.60**	**1.72**	≤ 0.02	≤ 0.02
		Matricaria recutita	H	A	≤ 0.10	**3.56**	**≤ 0.60**	≤ 0.02	≤ 0.02
		Pimpinella saxifraga	H	P	**≤ 0.30**	≤ 0.20	**≤ 0.60**	≤ 0.02	≤ 0.02
		Avena fatua	H	A	≤ 0.10	**≤ 0.60**	**≤ 0.60**	≤ 0.02	≤ 0.02
		Euphorbia helioscopia	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Polygonum aviculare	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M3	*Senecio jacobaea*	H	B	**40.65**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Convolvulus arvensis	H	P	≤ 0.10	≤ 0.20	**≤ 0.60**	≤ 0.02	≤ 0.02
		Solanum dulcamara	W	P	≤ 0.10	**5.47**	≤ 0.20	≤ 0.02	≤ 0.02
		Crataegus monogyna	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Ligustrum vulgare	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M4	*Urtica dioica*	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Sisymbrium vulgare	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Cirsium vulgare	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Galium aparine	H	A	≤ 0.10	≤ 0.20	**≤ 0.60**	≤ 0.02	≤ 0.02
		Calystegia sepium	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Cirsium arvense	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Convolvulus arvensis	H	P	≤ 0.10	**4.47**	≤ 0.20	≤ 0.02	≤ 0.02
		Crataegus monogyna	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02

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807 Table S2d. Field 4.

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FIELD	MARGIN	SPECIES	PLANT TYPE	LIFE HISTORY STRATEGY	NEONICOTINOID RESIDUES (ng/g)				
					TMX	CLO	IMC	THC	ACT
4	M1	*Crataegus monogyna*	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Silete latifolia	H	P	**55.78**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Cirsium vulgare	H	B	**≤ 0.30**	≤ 0.20	**26.06**	≤ 0.02	≤ 0.02
	M2	*Heracleum sphondylium*	H	P	**92.79**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Cirsium vulgare	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Sonchus arvensis	H	P	≤ 0.10	≤ 0.20	**5.13**	≤ 0.02	≤ 0.02
	M3	*Centaurea nigra*	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Sonchus arvensis	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Crataegus monogyna	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Heracleum sphondylium	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Rubus fruticosus	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M4	*Heracleum sphondylium*	H	P	≤ 0.10	≤ 0.20	**≤ 0.60**	≤ 0.02	**≤ 0.06**
		Silene latifolia	H	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Cirsium vulgare	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02

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Table S2e. Field 5.

FIELD	MARGIN	SPECIES	PLANT TYPE	LIFE HISTORY STRATEGY	NEONICOTINOID RESIDUES (ng/g)				
					TMX	CLO	IMC	THC	ACT
5	M1	*Hedera helix*	W	P	**1.50**	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Ligustrum vulgare	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Crataegus monogyna	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M2	*Papaver rhoeas*	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Senecio jacobaea	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M3	*Papaver rhoeas*	H	A	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Ligustrum vulgare	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
	M4	*Hedera helix*	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Ligustrum vulgare	W	P	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02
		Senecio jacobaea	H	B	≤ 0.10	≤ 0.20	≤ 0.20	≤ 0.02	≤ 0.02

Table S3. Absolute recoveries (%) of neonicotinoids from spiked foliage samples (1 ng/g dw, n=4 and 5 ng/g dw, n=4) extracted with the QuEChERS method. TMX = thiamethoxam, CLO = clothianidin, IMC = imidacloprid, ACT = acetamiprid and THC = thiacloprid.

	1 ng/g dw		*5 ng/g dw*	
	Av	SD	Av	SD
TMX	80	15	91	2
CLO	89	14	105	9
IMC	101	6	115	6
ACT	82	8	94	9
THC	72	15	84	11