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Ornamental plants on sale to the public are a significant source of pesticide residues with implications for the health of pollinating insects☆

A. Lentola [a], A. David [b], A. Abdul-Sada [b], A. Tapparo [a], D. Goulson [b, *], E.M. Hill [b]

[a] Department of Chemical Sciences, University of Padova, Via Marzolo 1 - 35131, Padova, Italy
[b] School of Life Sciences, Sussex University, Falmer BN1 9QG, UK

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ABSTRACT

Garden centres frequently market nectar- and pollen-rich ornamental plants as “pollinator-friendly”, however these plants are often treated with pesticides during their production. There is little information on the nature of pesticide residues present at the point of purchase and whether these plants may actually pose a threat to, rather than benefit, the health of pollinating insects. Using mass spectrometry analyses, this study screened leaves from 29 different ‘bee-friendly’ plants for 8 insecticides and 16 fungicides commonly used in ornamental production. Only two plants (a *Narcissus* and a *Salvia* variety) did not contain any pesticide and 23 plants contained more than one pesticide, with some species containing mixtures of 7 (*Ageratum houstonianum*) and 10 (*Erica carnea*) different agrochemicals. Neonicotinoid insecticides were detected in more than 70% of the analysed plants, and chlorpyrifos and pyrethroid insecticides were found in 10% and 7% of plants respectively. Boscalid, spiroxamine and DMI-fungicides were detected in 40% of plants. Pollen samples collected from 18 different plants contained a total of 13 different pesticides. Systemic compounds were detected in pollen samples at similar concentrations to those in leaves. However, some contact (chlorpyrifos) and localised penetrant pesticides (iprodione, pyroclastrobin and prochloraz) were also detected in pollen, likely arising from direct contamination during spraying. The neonicotinoids thiamethoxam, clothianidin and imidacloprid and the organophosphate chlorpyrifos were present in pollen at concentrations between 6.9 and 81 ng/g and at levels that overlap with those known to cause harm to bees. The net effect on pollinators of buying plants that are a rich source of forage for them but simultaneously risk exposing them to a cocktail of pesticides is not clear. Gardeners who wish to gain the benefits without the risks should seek uncontaminated plants by growing their own from seed, plant-swapping or by buying plants from an organic nursery.

1. Introduction

In many countries there is widespread concern regarding the health of populations of certain insect pollinators including honey bees (*Apis mellifera*) and bumble bees (*Bombus sp*). As a result numerous studies have focussed on the impact of environmental stressors, including exposure to pesticides, on the health of wild bees. In particular, exposure to neonicotinoid insecticides has been cited as one of a number of causes for concern as they are widely used systemic agrochemicals which have been shown to contaminate pollen and nectar of crop plants and nearby wildflowers (Fairbrother et al., 2014; Botías et al., 2015; Goulson et al., 2015), and consequently can be detected in bees (Botias et al., 2017), their hives or nests (e.g. David et al., 2016). In addition, environmentally relevant concentrations of some neonicotinoids can have deleterious effects on bee mortality, foraging, homing, navigation, and queen survival (Pisa et al., 2015; Godfray et al., 2015; Stanley et al., 2016). There is now a consensus that bee declines are the result of the combined effects of multiple stressors (Goulson et al., 2015), within which exposure to pesticides plays a significant role (Arena and Sgolastra, 2014; Rundlöf et al., 2015; Williams et al., 2015).

The neonicotinoid insecticides are one of many classes of

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* Corresponding author.
E-mail address: d.goulson@sussex.ac.uk (D. Goulson).

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pesticides that can contaminate bees and their colonies. For example, 37 insecticide and fungicide chemicals were detected in honey bees and hive products in France (Lambert et al., 2013) and 121 agrochemicals and their metabolites were detected in hive wax and pollen collected by honey bees in the United States (Mullin et al., 2010). In the UK, pollen collected by bee species also contained a wide range of pesticides, including the fungicides carbendazim, boscalid, flusilazole, metconazole, tebuconazole and trifloxystrobin as well as the neonicotinoids thiamethoxam, thiacloprid and imidacloprid (David et al., 2016). These studies suggest that many bee species are likely to be chronically exposed to mixtures of multiple pesticides, including insecticides and fungicides, throughout their development and adult life, particularly when residing in intensively-managed arable and horticultural landscapes (e.g. Roszko et al., 2016).

Although fungicides exhibit low toxicity to invertebrates, some laboratory studies have shown that simultaneous exposure to demethylation-inhibiting (DMI) fungicides can increase the toxicity of some neonicotinoids by up to 1000-fold (Iwasa et al., 2004; Schmuck et al., 2003). DMI fungicides such as tebuconazole and metconazole inhibit cytochrome P450 (CYP P450) mediated ergosterol biosynthesis in fungi and are thought to inhibit P450 enzymes in insects which are important for detoxification of insecticides (Schmuck et al., 2003). Synergistic effects of DMI fungicides with the cyanoguanidine neonicotinoids, thiacloprid and acetamiprid, are most apparent as these insecticides are (in the absence of the fungicide) rapidly metabolised in insects to less toxic metabolites (Johnson, 2015). Other pesticide combinations, e.g. neonicotinoids and pyrethroids, have been reported to affect bee mortality and colony performance (Gill et al., 2012) possibly due to additive actions on cholinergic signalling (Palmer et al., 2013). Sublethal concentrations of some fungicides and neonicotinoids can also cause immune suppression in bee species resulting in increased susceptibility to pathogens (reviewed in Sánchez-Bayo et al., 2016). The interaction of exposure to more complex pesticide mixtures and other stressors, such as pathogen infections, on bee health have yet to be studied.

Most studies of exposure of bees to pesticides have focussed on agricultural environments. However, recent studies have revealed that pollen and nectar collected by wild bees (*Bombus sp*) located in gardens in urban environments also often contained a complex mixture of pesticides, including neonicotinoids and fungicides (Botias et al., 2017; David et al., 2016). One source of pesticide use in urban areas may arise from spraying horticultural chemicals to protect ornamental plants prior to or after flowering. However, many ornamental plants are also treated with systemic pesticides prior to purchase and there is little information as to whether these pesticides persist in plant tissues long enough to contaminate pollen during flowering after purchase. However, a recent report published by Greenpeace described the pesticides found in the leaves of 35 popular ornamental garden plants sourced from garden centre in 10 European (but not UK) countries; pesticide residues were found in 97% of these flowering plants (Reuter, 2014).

The aim of this study was to determine whether bee attractive flowering plants purchased from major retailers in the UK were a source of toxic pesticides with the potential to contaminate bees and other pollinators via exposure to their pollen or nectar. Analytical methods were developed to quantify a complex mixture of insecticides and fungicides in plant tissues. Where possible, we analyse levels of pesticides separately in leaves, pollen and nectar. Levels of pesticides in leaves and pollen were compared to identify compounds which were either readily translocated to pollen or had directly contaminated it during recent pesticide applications. This is the first study to provide data on the potential for exposure of bees to pesticides arising from the purchase of ornamental plants intended for UK gardens or parks.

2. Materials and methods

2.1. Chemicals and reagents

Certified standards of carbendazim, thiamethoxam, thiamethoxam-d3, clothianidin, clothianidin-d3, imidacloprid, imidacloprid-d4, acetamiprid, thiacloprid, carboxin, boscalid, spiroxamine, silthiofam, epoxiconazole, tebuconazole, flusilazole, prochloraz, metconazole, pyraclostrobin, trifloxystrobin, fluoxastrobin, λ-cyhalothrin, iprodione, propiconazole, chrysene, pyrene, α-cypermethrin and also formic acid, ammonium formate, magnesium sulphate, sodium chloride and Supel™ QuE PSA/C18/ENVI-Carb™ (ratio 1/1/1) were obtained from Sigma-Aldrich UK. Certified standards of chlorpyrifos, chlorothalonil, carbendazim-d3, tebuconazole-d6 and trans-permethrin-d6 were purchased from LGC standards UK and prochloraz-d7 and carbamazepine-d10 from QMX Laboratories Limited UK. Spin filters (PVDF membrane, pore size 0.2 μm) were purchased from Fisher Scientific UK. All pesticide standards were >99% compound purity (except spiroxamine, 98.5%; λ-cyhalothrin, 97.8%; chlorothalonil, 98.5%; propiconazole, 98.4%; chrysene, 98.5%) and deuterated standards were >97% isotopic purity. HPLC-grade acetonitrile, toluene, methanol and water were obtained from Rathburn Chemicals, Walkerburn, UK. Individual standard pesticide (native and deuterated) stock solutions (1 mg/ml) were prepared in acetonitrile. Calibration points were prepared weekly from stock solutions in H_2O/ACN (70:30) for LC analysis and in toluene for GC analysis. All solutions were stored at −20 °C in the dark.

2.2. Choice of plants and analytes

Popular bee-attractive ornamental plants were purchased from local garden centres located in the East Sussex area (Table 1). Foliage, nectar and pollen samples were collected during flowering, which varied between May and July according to plant species. Foliage samples were obtained for 29 different species or varieties, and pollen and nectar for 18 and 11 of these species/varieties respectively.

Pesticides for analysis were chosen as the most widely used in the UK, based on data from the Department for Food, Environment and Rural Affairs, (DEFRA) and also from a reports of pesticides commonly detected in glasshouse crops grown or exported to the UK (Garthwaite et al., 2009; Goulds, 2012; Reuter, 2014). These included five neonicotinoid, two pyrethroids and one organophosphate insecticide as well as 16 fungicides (see Supplementary Table S1).

2.3. Sample collection

Replicate foliage samples consisted of 10 g of leaves manually gathered from either individual or several plants depending on leaf size and stored at −70 °C for later analyses. Prior to extraction, leaves were ground with liquid nitrogen followed by manual homogenisation using a micro-spatula. Pollen samples from the same plants were isolated from flowers which had been frozen at −70 °C. Flowers were gently defrosted and dried in an incubator at 37 °C for 24 h to facilitate pollen release from the anthers. After drying, flowers were brushed over food strainers to separate pollen from anthers and sifted through multiple sieves of decreasing pore size (from 250 to 45 μm). For some species where pollen was difficult to isolate from flowers, it was manually sampled by tweezers or both pollen and anthers were analysed together in order to obtain a sufficient amount of sample material. Collection of nectar from

Table 1
Number of pesticides detected in leaves of different ornamental plants.

Common name	Species and variety	Retailer	Insecticides	Fungicides
Achillea	*Achillea millefolium* 'Desert Eve Deep Rose'	B&Q	1	3
Ageratum	*Ageratum houstonianum*	Aldi	3	4
Allium	*Allium hollandicum*	Wyevale	2	1
Bellflower	*Campanula portenschlagiana*	Wyevale	0	2
Catmint	*Nepeta cataria* 'Six Hill Giant'	Wyevale	2	3
Catmint	*Nepeta cataria* 'Walkers low'	Wyevale	1	2
Coreopsis	*Coreopsis grandiflora* 'Early Sunrise'	B&Q	1	3
Cosmos	*Cosmos bipinnatus* 'Casanova Violet'	Homebase	4	1
Crocus	*Crocus vernus* 'Golden Yellow'	Wyevale	1	1
Daffodil	*Narcissus jonquilla* 'Tete-a-Tete'	Wyevale	0	0
Dahlia	*Dahlia x hybrida* 'Gallery Art Fair'	Staverton's	0	1
Dahlia	*Dahlia x hortensis 'Bishop of Llandaff'*	Wyevale	1	0
Dahlia	*Dahlia x hybrida* 'Mystic Dreamer'	B&Q	2	2
Dutch iris	*Iris tingitana* × *I. xiphium*	Wyevale	1	3
Foxgloves	*Digitalis purpurea* 'Dalmatian White'	Wyevale	1	1
Grape hyacinith	*Muscari armeniacum*	Wyevale	1	5
Heathers	*Erica carnea*	Wyevale	5	5
Lavender	*Lavandula stoechas* 'Victory'	Wyevale	0	3
Lavender	*Lavandula angustifolia*	Wyevale	0	1
Lavender	*Lavandula stoechas* 'Papillon'	Wyevale	0	3
Salvia	*Salvia longispicata x S. farinacea* 'Mystic Spires'	Staverton's	1	0
Salvia	*Salvia nemerosa* 'Sensation Deep Rose'	Homebase	0	0
Scabious	*Scabiosa columbaria* 'Pink Mist'	Wyevale	1	1
Scabious	*Scabiosa columbaria* 'Butterfly Blue'	Homebase	3	2
Strawberry	*Fragaria* × *ananassa* 'Toscana F1′	Homebase	2	2
Thistles	*Cirsium atropurumeum*	Wyevale	2	1
Verbena	*Verbena x hybrida*	Aldi	3	3
Veronica	*Veronica spicata*	Staverton's	2	4
Wallflower	*Erysimum linifolium* 'Bowles's Manve'	Wyevale	1	1

flowers was performed through capillary action into glass 5 μl calibrated micropipettes, which were then sealed with putty and stored at −70 °C until analysis. Where there was not enough nectar and pollen material to analyse three replicates per species/variety, then composite samples were collected from the same plants sampled for leaf foliage.

2.4. Sample extraction

A QuEChERS method suitable for analysis of multiple pesticides in plant tissues was adapted from David et al. (2015) in order to extract pyrethroids, organophosphate and fungicides alongside neonicotinoids.

Leaves: 100 mg of ground leaves were spiked with 250 pg of a mix of the LC internal standards in ACN (carbendazim-d3, thiamethoxam-d3, clothianidin-d3, imidacloprid-d4, carbamazepine-d10, tebuconazole-d6 and prochloraz-d7) and 5 ng of a mix of the GC internal standards (pyrene, chrysene and trans-permethrin-d6) in toluene. 500 μL of acetonitrile with acetic acid 1% was added and the samples vortexed. After addition of 400 μL of water, the analytes were extracted by mixing on a multi axis rotator for 10 min. Then, 250 mg of a salt mixture (MgSO4 and sodium chloride; 4:1) was added and the samples quickly mixed to prevent salt clumping. After centrifugation, the organic phase was transferred to an Eppendorf vial containing 50 mg of a dispersive solid phase extraction (d-SPE) phase (PSA/C18/ENVI-Carb). The extract was mixed on a multi axis rotator for 10 min and centrifuged. The supernatant was removed, and the d-SPE phase further extracted with 200 μL of a solution of ACN/toluene (1/3, vortex 15 s). After centrifugation, the supernatants were combined and spin filtered. For GC analyses, 200 μL of the extract were transferred to an injection vial, evaporated with a nitrogen flow and reconstituted with 10 μL of toluene. For LC analysis, 400 μL of the extract was transferred to a glass tube, evaporated to dryness under vacuum and reconstituted with 50 μL of ACN/water (30:70).

Pollen and nectar: The amount of pollen and nectar used for the extraction was variable depending on sample availability and ranged between 5 and 90 mg pollen/sample and 10–50 μL nectar/sample. Samples were extracted as described above, except that the water (400 μL) was added prior to the initial acetonitrile extraction.

2.5. GC-MS/MS analysis

GC-MS/MS analysis were carried out using a Trace GC Ultra, Thermo Scientific linked to an ion trap mass spectrometer (ITQ1100, Thermo Scientific) operating in splitless mode. Compounds were separated on an Agilent DB-5MS UI column (30 m × 0.25 mm, 0.25-μm film thickness) using helium as the carrier gas (99.996% purity) at a flow rate of 1.3 ml/min. The injector and transfer line were set at 250 °C and 300 °C respectively, the source at 250 °C. The column was held at 95 °C for 6 min after injection and then programmed at 12 °C/min to 320 °C and held for 4 min. The mass spectrometer was operated in the electron ionization mode (EI, 70 eV) and analytes were detected using MS/MS mode. Analyte precursor and fragment ions and their associated IS used for quantitation are reported in Table S2. GC-MS/MS spectra were analysed on Xcalibur v1.2 software (Thermoquest-Finningan). Concentrations were determined using a least-square linear regression analysis of the peak area ratio (analyte to IS) versus the analyte concentration using a matrix-matched calibration curve.

2.6. UHPLC-MS/MS analysis

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 × 5 mm, Waters, Manchester, UK) and maintained

at 24 °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). The initial ratio (A/B) was 90:10 and separation was achieved using a flow rate of 0.15 ml/min with the following gradient: 90:10 to 70:30 in 10 min, from 70:30 to 45:55 at 11 min, from 45:55 to 43:57 at 20 min, from 43:57 to 0:100 at 22 min and held for 8 min prior to return to initial conditions and equilibration for 5 min.

MS/MS was performed in the multiple reaction monitoring (MRM) using ESI in the positive mode, and two characteristic fragmentations of the protonated molecular ion $[M+H]^+$ were monitored (Table S2). The declustering potential (DP, 0–40 V) and collision energy (CE, 10–40 eV) were optimised for each analyte. Other parameters were optimised as follows: capillary voltage −3.3 kV, extractor voltage 8 V, multiplier voltage 650 V, source temperature 100 °C, desolvation temperature 300 °C. Argon was used as collision gas (P collision cell, 3×10^{-3} mbar), and nitrogen as desolvation gas (600 l/h). Mass calibration of the spectrometer was performed with sodium iodide. Data were acquired using MassLynx 4.1 and the quantification was carried out by calculating the response factor of pesticides to their respective IS. Concentrations were determined using a least-square linear regression analysis of the peak area ratio versus the concentration ratio (analyte to IS).

2.7. Method validation

For method validation, daffodil leaves were chosen as a test matrix as an initial analysis revealed that no pesticides were detected in this species. Method recoveries and precision were evaluated by spiking control leaves, and the method performance acceptability criteria from EU guidelines were used for assessment (EU, SANCO/12571/2013). Leaf samples (100 mg) were used for the recovery experiments and to prepare matrix-matched standard solutions for calibration. For recovery experiments, leaves samples (four replicates) were spiked at two concentration levels of the analytes: 1 and 10 ng/g for UHPLC-MS/MS and 100 and 1000 ng/g for GC-MS/MS analyses. After extraction of the analytes from the spiked samples, 250 pg of the IS mix used for UHPLC-MS/MS plus 5 ng of the IS mix used for GC-MS/MS analyses were added. Calibration solutions were prepared using non-spiked leaf extracts and consisted of six points of each test analyte equivalent to 0.5, 1, 5, 10, 25 and 50 ng/g together with 2.5 ng/g of IS mixture for UHPLC-MS/MS and 10, 50, 100, 250, 500 and 1000 ng/g together with 50 ng/g of IS mixture for GC-MS/MS. The repeatability of the method was determined as the intra-day relative standard deviation (RSD %) of repeated extractions ($n = 4$) of a matrix extract spiked at the two concentrations used in recovery studies. The sensitivity of the method was calculated in terms of method detection and quantification limits (MDL and MQL, respectively) which were determined from spiked samples which had been extracted using the QuEChERS method. MDLs were determined as the minimum amount of analyte detected with a signal-to-noise ratio of 3, and MQLs as the minimum amount of analyte detected with a signal-to-noise ratio of 10.

Linearity was evaluated both in solvent and matrix, using matrix-matched calibration curves prepared as described above. The effect of the matrix was evaluated by comparison of the slopes of the calibration curves in solvent only (ACN/H_2O; 30:70 for UHPLC-MS/MS and toluene for GC-MS/MS) and in the matrix. The percent increase or decrease of the matrix-matched calibration curve was measured in relation to the solvent-only curve as described in other studies (Bueno et al., 2014; Walorczyk, 2014).

2.8. Quality control

One workup sample (i.e. using extraction methods without the matrix) per batch was injected at the beginning of the analytical run to ensure that no contamination occurred during the sample preparation. Solvent samples (ACN/H_2O (30:70) and toluene for UHPLC-MS/MS and GC-MS/MS respectively) were also injected between sample batches to ensure that there was no carryover. Identification of pesticides in samples was determined by comparing expected retention time and the ratio of the two transitions (primary/secondary) with standard solutions. Quality control samples (QCs, i.e. standard solutions) were injected every 10 samples to monitor the sensitivity changes during the analysis of each batch.

2.9. Statistical analyses

The relationship between pesticide concentrations in leaves and pollen were determined using Pearson's correlation coefficient after a $\log_{10}$ transformation of the data.

3. Results and discussion

3.1. Performance of the analytical methods

The developed analytical method allowed the quantification of pesticides belonging to many different agro-chemical classes (Table S3). The d-SPE sorbents were effective in removing matrix interferences but required an additional toluene extraction to avoid retention of planar analytes. Care was taken to ensure extraction solvents were acidic or neutral to avoid losses of chlorothalonil, which is sensitive to an alkaline environment. To avoid losses of chlorpyrifos via volatilisation, extracts for GC analyses were concentrated in a nitrogen stream at atmospheric pressure rather than using a vacuum. The linearity, precision and bias of the method were all satisfactory and recoveries of analytes were between 71 and 124%. A significant matrix effect was observed for three GC-MS/MS analytes (chlorothalonil, chlorpyrifos and iprodione) and a matrix-matched calibration curve was used for an accurate quantification of these compounds. Other analytes were quantified using standards prepared in solvents. The MQL values for the compounds analysed with UHPLC-MS/MS were between 0.14 and 5.9 ng/g, and for GC-MS/MS compounds were between 44 and 230 ng/g. Overall, these results show that this method can be used to efficiently recover mixtures of insecticides and fungicides in leaf samples with high precision.

3.2. Identity of pesticide residues in leaves

Plants supplied by all 5 retailers contained pesticide residues. Of the 29 different ornamental plants that were analysed, only two varieties (*Narcissus* and a *Salvia* variety) did not contain any residues of the pesticides targeted in this study (Table 1). Of the remainder, 23 varieties contained more than one pesticide with some varieties containing a mixture of 7 (*Ageratum houstonianum*) and 10 (*Erica carnea*) different insecticides and fungicides. Within the insecticides, neonicotinoids were detected in more than 70% of the analysed plants, whereas chlorpyrifos and pyrethroids were detected in 10% and 7% of plants respectively (Fig. 1). It is likely that the higher prevalence of neonicotinoids is at least in part due to their higher persistence compared to the other insecticide classes currently in use (Bonmatin et al., 2015). Our results also indicate that neonicotinoids are widely used for treatment of ornamental plants and their residues could contaminate gardens and parks. In addition, boscalid, spiroxamine and DMI-fungicides were detected

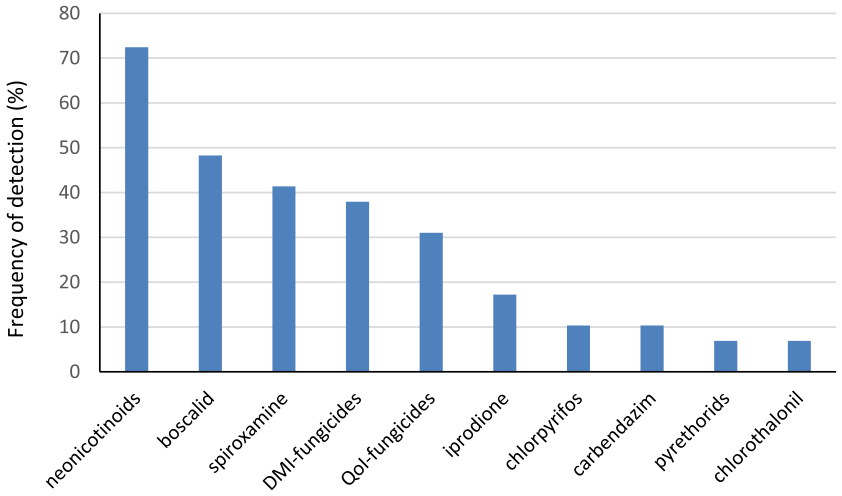

Fig. 1. Frequency of detection of different agro-chemical classes in leaves of ornamental plants.
Individual pesticides are named when just one pesticide was detected in a particular class.

in more than 38% of plants indicating widespread treatment of ornamentals with these pesticides.

Mean neonicotinoid concentrations in leaves of the different plants varied from (mean ± SD) 1.7 ± 1.9 ng/g for thiacloprid to 25 ± 34 ng/g for thiamethoxam (Table 2). Mean concentrations of other insecticides were far higher, at 121 ± 27 and 844 ± 205 ng/g for the pyrethroids cyhalothrin and cypermethrin respectively, and 207 ± 93 ng/g for the organophosphate chlorpyrifos. Of the fungicides, mean leaf concentrations of boscalid, prochloraz, pyraclostrobin and carbendazim were between 46 ± 64 and 88 ± 83 ng/g and iprodione was 2344 ± 3550 ng/g. In general, concentrations of individual pesticides varied widely between the different plant varieties which was likely due to variations in timing and types (foliar or soil applied) of treatment applied. However, the data indicates that leaves of ornamental plants are contaminated with complex mixtures of insecticides and fungicides which were present from ng/g to μg/g concentrations.

3.3. Pesticides residue in pollen and nectar

Pollen samples from 18 plant varieties were collected and these contained a total of 13 different pesticides (Table 3 and Table S4). Compared to contact and penetrant pesticides, systemic compounds were detected in pollen samples with higher frequency and, with the exception of acetamiprid, were present in pollen at similar concentrations to leaves. There was a significant correlation between the concentrations of all the systemic pesticides quantified in the leaves and pollen of individual plants (Pearson's $r = 0.780$, $p < 1.1 \times 10^{-9}$ $n = 42$ plant replicates). These results suggest that systemic pesticides, such as carbendazim and the

Table 2
Concentration of pesticides detected in leaves of different ornamental plant species or varieties.

Pesticide	Number of plant species/varieties where the pesticide was detected (% of total plants analysed)[a]	Mean ± SD (ng/g)	Median (ng/g)	Range (ng/g)
Thiacloprid	14 (48)	1.0 ± 1.8	0.28	0–6.4
Boscalid	14 (48)	37 ± 61	7.7	0–223
Spiroxamine	12 (41)	0.65 ± 0.85	0.34	0–3.5
Imidacloprid	11 (38)	3.9 ± 8.4	0.36	0–29
Prochloraz	9 (31)	59 ± 99	3.5	0–308
Pyroclastrobin	7 (24)	39 ± 66	3.1	0–257
Acetamiprid	6 (21)	7.5 ± 21	0.04	0.04–85
Iprodione	5 (17)	1966 ± 3549	327	3.7–10593
Thiamethoxam	4 (14)	16 ± 35	0.77	0.09–119
Carbendazim	3 (10)	54 ± 79	9.6	1.2–213
Chlorpyrifos	3 (10)	108 ± 127	19	19–328
Chlorothalonil	2 (7)	486 ± 416	364	0–1190
Fluoxastrobin	2 (7)	8.0 ± 17	0.19	0.09–41
Tebuconazole	2 (7)	0.16 ± 0.23	0.09	0–0.60
Clothianidin	1 (3)	9.3 ± 4.9	11	3.8–13
λ-Cyhalothrin	1 (3)	121 ± 33	105	99–158
Cypermethrin[b]	1 (3)	844 ± 251	805	616–1113
Propiconazole	1 (3)	0.65 ± 1.1	0	0–2.0
Trifloxystrobin	1 (3)	0.27 ± 0.04	0.24	0.24–0.32

Mean, median and range value were calculated using the concentrations measured in all the plant species/varieties where a specific compound was detected. The concentrations over the MDL but below the MQL were assigned the MDL value, whilst concentrations below the MDL were considered to be zero.
The concentrations of the fungicides carboxin, epoxyiconazole, flusilazole, metconazole and siltiofam were all below MDL.
[a] For each species/varieties 3 leaf replicates were analysed.
[b] Detected 3 isomers, quantified as sum of the three peaks on calibration curve obtained from α-cypermethrin.

Table 3
Comparison between the mean concentration of pesticides in leaves and pollen of different ornamental plant species or varieties.

Pesticides grouped by translocation properties in the plant	Leaves (ng/g)	Pollen (ng/g)	LD_{50} honey bee[a] (ng/g)		Mass of pollen to give
	Mean ± SD	Mean ± SD	Oral	Contact	LD50[d]
Systemic					
acetamiprid	8.6 ± 23	0.45 ± 0.23	14,000	7900	31,111
imidacloprid	3.8 ± 9.1	6.9 ± 16	13	61	1.9
thiacloprid	1.2 ± 1.9	0.78 ± 1.1	17,000	36,000	21,794
thiamethoxam	17 ± 35	11.0 ± 16	5	25	0.45
clothianidin	9.3 ± 4.9	11.0 ± 9.3	3.5	39	0.32
carbendazim	54 ± 79	57 ± 98	NA	>50,000	NA
spiroxamine	0.54 ± 0.82	<0.20[b]	92,000	42,00	5×10^5
Acropetal penetrant					
boscalid	30 ± 66	0.53 ± 1.1	166,000	>200,000	3×10^5
fluoxastrobin	8.0 ± 17	<MDL[c]	843,000	>200,000	0
propiconazole	0.65 ± 1.1	<MDL[c]	77,000	50,000	0
tebuconazole	0.16 ± 0.23	<MDL[c]	83,000	>200,000	0
Localised penetrant					
iprodione	2743 ± 4459	252 ± 496	25,000	400,000	99
pyroclastrobin	38 ± 85	9.8 ± 14	73,000	>100,000	7449
trifloxystrobin	0.27 ± 0.04	<MDL[c]	>200,000	>200,000	0
prochloraz	55 ± 104	4.9 ± 12	60,000	50,000	12,245
Contact					
chlorothalonil	485 ± 416	<MDL[c]	63,000	135,000	0
chlorpyrifos	146 ± 142	81 ± 115	240	72	3.2
cyhalothrin	121 ± 33	<MDL[c]	NA	22	0
cypermethrin	844 ± 251	<111[b]	64	34	0

Mean concentrations of pesticides were calculated for samples from all plant species/varieties where there were matching leaf and pollen samples. The concentrations over the MDL but below the MQL were assigned the MDL value, whilst concentrations below the MDL were considered to be zero. The number of replicates analysed and the mean values for each plant species/varieties are reported in Supplementary Table S4.

[a] Data from Sanchez-Bayo and Goka (2014).
[b] Below the MQL in all the analysed samples.
[c] Below the MDL in all the analysed samples.
[d] Mass of pollen (g) a bee would need to consume to obtain the LD50.

neonicotinoid insecticides, easily contaminate the plant pollen and their residues are still available to pollinator insects when ornamental plants reach the gardens. In addition, some contact (chlorpyrifos) and localised penetrant pesticides (iprodione, pyroclastrobin and prochloraz) were also detected in pollen (Table 3). However, these pesticides may have been applied by spray and some of the plants were already in flower when purchased (Table S4) so pollen may have already been directly contaminated during pesticide application. No significant correlation ($p < 0.05$) were observed between leaf and pollen concentrations of pesticides classified as local penetrants ($n = 19$), acropetal penetrants ($n = 12$) or as contact action ($n = 6$).

The finding of residues of imidacloprid, carbendazim and pyroclastrobin in pollen samples supports recent work where these pesticides were frequently detected in pollen collected from bumble-bees nests located in the same urban area of S.E UK where our samples were purchased (David et al., 2016) and suggests that ornamental plants are a potential source of contaminated pollen to pollinator insects.

Nectar samples from only 11 different plant species/varieties were collected, due to the difficulty of collecting enough volume for the chemical analysis. However, concentrations of all target analytes were below MDL except for the neonicotinoids where acetamiprid was detected in just one species below MQL of 0.14 ng/g, and thiacloprid detected in one species below MQL of 0.15 ng/g (Table S4). Imidacloprid was detected in five species/varieties, but only in one plant at concentration higher than MQL (1.2 ng/g) of 5.7 ng/g. The data confirms that nectar concentrations of some neonicotinoids were low in this study, likely due, in part, to the small quantities of nectar available for analysis. Previous studies have found that concentrations of neonicotinoids in nectar are often (but not always) lower than those found in pollen (Bonmatin et al., 2015; Mogren and Lundgren, 2016).

3.4. Implications for toxicity to non-target insects

The presence of pesticides residues in ornamental plants could be a threat to non-target insects such as insect pollinators, which may be exposed to pesticides by ingestion of contaminated pollen and nectar or through contact with residues on pollen and leaves after spraying. Many ornamental plants are a rich source of flowers in urban environments and bees and other pollinator insects are usually highly attracted to these plants and therefore could be exposed to a complex mixture of different agrochemicals. Indeed, many gardeners are keen to encourage wildlife such as pollinators in their garden and may deliberately purchase plants such as those we tested to provide forage for bees, butterflies and hoverflies.

Are the concentrations we describe sufficient to cause harm to pollinators? Calculation of the amount of pollen a honey bee would need to consume to receive the LD50 (Table 3) suggests that honeybees are unlikely to receive a lethal dose, at least in the short term. For example, to receive a lethal dose a honeybee would need to consume 0.32 g of pollen containing the mean concentration of clothianidin found in samples. Given that a honeybee weighs approximately 0.1 g, and consumes up to 29 mg per day (Schmidt et al., 1987), it would take at least ten days to receive a lethal dose. However, the concentrations found here overlap with those found to cause significant sublethal effects on bees, something that has been studied extensively in neonicotinoids. Where detected, the mean concentrations of imidacloprid, clothianidin and thiamethoxam in pollen where 6, 11 and 11 ng/g, respectively. These values are similar to or slightly higher than residues typically found in pollen of treated crops (Bonmatin et al., 2015) that have been found to have measureable impacts on pollinators. For example, bumblebees nests fed on imidacloprid in pollen at 6 ng/g (plus in nectar at 0.7 ng/g) grew more slowly and produced 85% fewer queens than control nests (Whitehorn et al., 2012). This same

concentration significantly reduced pollen collection in bumblebees (Feltham et al., 2014). Following field exposure to thiamethoxam at up to 1.6 ng/g in pollen, bumblebee nests grew less and produced significantly fewer queens (Goulson, 2015). In honeybees, exposure to just 1 ng/g of clothianidin significantly impaired the immune response allowing viruses to replicate more quickly (Di Prisco et al., 2013). Thus the concentrations of individual neonicotinoids found in our study are certainly well within the range found to have measurable impacts on bees, and at worst exceed concentrations that cause harm by an order of magnitude.

Unlike neonicotinoids, chlorpyrifos is more toxic via contact rather than consumption (honeybee LD50s 72 ng for contact exposure and 240 ng for oral consumption, Table 3). Thus pollinators may be exposed via contact with foliage and petals as well as contact with and consumption of pollen. Some residues in foliage and pollen were relatively high (up to 273 and 163 ng/g), but how this would translate into total exposure of a foraging bee is not clear. The same is true of the pyrethroids, which were found in few plants but at high concentrations, and are also more toxic via contact exposure (Table 3).

Pollinators feeding on the flowers we studied are likely to be simultaneously exposed to a cocktail of chemicals. A recent study on the effects of exposure of bees to pairs of pesticides concluded that most pesticides act additively (Spurgeon et al., 2016), so we might attempt to assess the total effect of exposure to a pesticide cocktail by summing the individual effects of each chemical. However, there is evidence that DMI fungicides, which were detected in 38% our samples, act synergistically with insecticides (Iwasa et al., 2004; Schmuck et al., 2003). Residues of the DMI fungicide prochloraz as well as five other fungicide structures were detected in pollen samples and the effect of exposure to these complex mixtures is currently unknown.

4. Conclusion

The results of our screening reveal that ornamental plants are widely treated with a mixture of insecticides and fungicides and that significant residues of these chemicals are still present in the plant tissues when they reach retailers and gardens. In particular, the neonicotinoid insecticides and the fungicides boscalid, spiroxamine and prochloraz were frequently detected while pyrethroid and organophosphate insecticides were found infrequently but sometimes at high concentrations. The concentrations of individual chemicals found overlap with and sometimes considerably exceed those known to do measureable harm to bees. Residues of pesticides in plants bought by members of the public will decline over time, and unless large numbers of contaminated plants are bought and planted together, it is likely that the total residues to which pollinators are exposed will be diluted by their also feeding on other, uncontaminated plants nearby. Many ornamental plants are bought in spring, which may provide a pulse of exposure of bees to pesticides at a critical time in the early development of bumblebee colonies and when honey bees colonies are normally undergoing rapid growth. With the current state of knowledge, we are not able to evaluate whether the net effect of planting 'pollinator-friendly' flowers contaminated with pesticides is likely to be positive or negative. However, it is clear that levels of pesticides found in some plants may well be sufficient to do harm, and the purchaser currently has no way of knowing what residues are in the different plants on sale. All of the retailers we tested were selling plants containing highly variable combinations of potentially harmful chemicals, so that any purchaser is playing 'Russian roulette' with their garden pollinators. In these circumstances, the safest option for a gardener wishing to encourage pollinators would be to buy plants from an organic nursery, grow plants from seed, or plant-swap with friends and neighbours that do not use pesticides. Alternatively, the horticultural industry might consider adding data on pesticide exposure to plant labels so that consumers could make an informed choice.

Recently, most attention has been focussed on the negative effects of environmental pesticide pollution as a result of agricultural uses. However, our results suggest that applications of pesticides to ornamental plants are also contributing to the exposure of pollinating insects to harmful chemicals.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.03.084.

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