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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☆

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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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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