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Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes☆

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ABSTRACT

The increased use of pesticides has caused concern over the possible direct association of exposure to combinations of these compounds with bee health problems. There is growing proof that bees are regularly exposed to mixtures of agrochemicals, but most research has been focused on managed bees living in farmland, whereas little is known about exposure of wild bees, both in farmland and urban habitats. To determine exposure of wild bumblebees to pesticides in agricultural and urban environments through the season, specimens of five different species were collected from farms and ornamental urban gardens in three sampling periods. Five neonicotinoid insecticides, thirteen fungicides and a pesticide synergist were analysed in each of the specimens collected. In total, 61% of the 150 individuals tested had detectable levels of at least one of the compounds, with boscalid being the most frequently detected (35%), followed by tebuconazole (27%), spiroxamine (19%), carbendazim (11%), epoxiconazole (8%), imidacloprid (7%), metconazole (7%) and thiamethoxam (6%). Quantifiable concentrations ranged from 0.17 to 54.4 ng/g (bee body weight) for individual pesticides. From all the bees where pesticides were detected, the majority (71%) had more than one compound, with a maximum of seven pesticides detected in one specimen. Concentrations and detection frequencies were higher in bees collected from farmland compared to urban sites, and pesticide concentrations decreased through the season. Overall, our results show that wild bumblebees are exposed to multiple pesticides when foraging in agricultural and urban landscapes. Such mixtures are detected in bee tissues not just during the crop flowering period, but also later in the season. Therefore, contact with these combinations of active compounds might be more prolonged in time and widespread in the environment than previously assumed. These findings may help to direct future research and pesticide regulation strategies to promote the conservation of wild bee populations.

1. Introduction

Bees are exposed to environmental pollutants via contaminated food resources such as pollen, nectar or water (Bonmatin et al., 2015), and through external contact with aerosols during spraying and contaminated dust emitted during the sowing of dressed seeds as their hairy bodies trap particulate residues (Greig-Smith et al., 1994; Pistorius et al., 2015). Many studies have used honeybees as relevant organisms to monitor environmental pollution (Celli and Maccagnani, 2003; Porrini et al., 2003). Bumblebees also forage in a great diversity of places and strongly interact with the environment, mainly the flora, surrounding their nests in a range of maximum foraging distances of 363–1650 m depending on the species (Walther-Hellwig and Frankl, 2000; Wood et al., 2015), and are thus also suitable organisms for monitoring landscape-based ecological pollution.

While most pesticide research has been focused on managed bees, there has been less work on wild bee populations. For instance, the only European bumblebee that has been studied in relation to pesticide exposure and toxicology is *Bombus terrestris*, simply because this species is easy to rear in captivity and commercially reared colonies are readily available (Baron et al., 2014; Gill et al., 2012; Rundlöf et al., 2015; Whitehorn et al., 2012).

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There is increasing evidence that managed bees living in agricultural landscapes are routinely exposed to mixtures of agrochemicals (David et al., 2016; Lambert et al., 2013; Long and Krupke, 2016; Mullin et al., 2010; Pettis et al., 2013), but little is known about the exposure of wild bees in these environments (Hladik et al., 2016). Nevertheless, bees constitute a highly diverse group where different taxonomic groups differ widely in their vulnerability to pesticide exposure (Biddinger et al., 2013; Devillers et al., 2007; Piiroinen and Goulson, 2016; Thompson and Hunt, 1999). Furthermore, bee species exhibit pronounced differences in floral preferences and foraging habits, collecting pollen and nectar more frequently from particular plant species according to their morphological traits (e.g. tongue length, body shape and size) and nutritional needs (Goulson et al., 2008; Vanderplanck et al., 2014; Vaudo et al., 2016). Such foraging choices may profoundly influence the probability of bees to be more or less exposed to some active compounds (Woodcock et al., 2016). Treated crop plants growing in agricultural landscapes have often been regarded as the only source of exposure to agrochemicals for pollinators, but recent research revealed their presence in wild plants growing near crops (Botías et al., 2015; David et al., 2016; Long and Krupke, 2016; Mogren and Lundgren, 2016). We would expect bees that visit flowering arable crops to have higher exposure than those that do not, but also those that visit wild plant species may have varying exposure depending on the ecology, physiology and morphology of their preferred flowers (Botías et al., 2016). Therefore, it is essential to understand the possible differences in levels of exposure among bee species, since this could reveal which are the most likely exposed and the most frequent mixtures of agrochemicals that they are exposed to.

The widespread occurrence of mixtures of agrochemicals in bee tissues (Hladik et al., 2016) increases concerns regarding the possible detrimental effects of simultaneous exposure to a cocktail of compounds. In general, only the effects of single active substances are studied in toxicity studies both for research and pesticide registration protocols, and exposure to mixtures are only evaluated in risks assessments when they are part of the same formulation. However, the application of two or more plant protection products during the same cropping season is a common practice in conventional farming (Botías et al., 2015; Garthwaite et al., 2013), and hence complex mixtures of agrochemicals which are not co-formulants of a single product can be simultaneously detected in bee forage and bee tissues (David et al., 2016; Hladik et al., 2016; Long and Krupke, 2016). This issue is worrisome given that exposure to mixtures might pose higher risks for animal health than the single impact of a specific class of compounds (Cedergreen, 2014; Rizzati et al., 2016). For example, some combinations of insecticides (*e.g.* pyrethroids with neonicotinoids) and of insecticides with fungicides can lead to additive and synergistic toxicity for bees at the individual and the colony level (Gill et al., 2012; Iwasa et al., 2004; Schmuck et al., 2003; Sgolastra et al., 2016). The scarcity of information on the field-relevant mixtures of agrochemicals and levels of exposure for bees could lead us to overlook the possible additive or synergistic effects of pesticide mixtures when risk assessment studies are performed, some of which have been designed to evaluate the hazards of such combinations (Sánchez-Bayo and Goka, 2014).

Another major gap in knowledge regarding exposure of bees to pesticides is the potential uptake and contact with these compounds in urban areas, where ornamental nursery plants can also be treated with pesticides (Brown et al., 2013; Fevery et al., 2016) and no information is available on their use in domestic gardens. The possible exposure of bees to harmful pesticides through forage collected in gardens is of high ecological concern, since these habitats are of great value for bees, providing nectar, pollen and nest sites, and sustaining a remarkably high pollinator species richness and abundance (Baldock et al., 2015; Kaluza et al., 2016; Samnegård et al., 2011), including bumblebees (Fetridge et al., 2008; Goulson et al., 2010). If foraging resources and nesting sites in urban habitats are contaminated with pesticide residues, it is likely that exposure to certain active compounds could be more widely spread in the landscape and more prolonged in time than previously assumed.

The aim of this study was to evaluate and compare exposure in different wild bumblebee species. To do this, we analysed the levels of five neonicotinoid insecticides, thirteen currently-used fungicides and a pesticide synergist in tissues of five bumblebee species (*B. hortorum*, *B. pascuorum*, *B. terrestris*, *B. lapidarius* and *B. pratorum*). These wild bumblebee samples were collected in agricultural and urban habitats to compare levels of exposure in both environments and to study distribution of agrochemicals in the landscape. The bees were gathered in three different periods (late spring, early summer and midsummer) in order to monitor the length of exposure to agrochemicals through the season.

Our results show evidence that wild bumblebees are frequently exposed to mixtures of agrochemicals when they forage in arable and urban habitats, with peak concentrations decreasing through the season.

2. Materials and methods

2.1. Sampling sites and field collection

Wild bumblebees were collected in five farms and five urban landscapes in East Sussex (South-East England, UK), all sites being at least 2 km apart from each other (Fig. 1) (Table S1). The sites selected to collect bees in agricultural land consisted of arable fields within mixed farms, where the predominant crops were oilseed rape, winter wheat and spring barley, and part of the land was pasture. The urban sampling sites consisted of ornamental public gardens and parks surrounded by houses that had private gardens in most cases. Foraging bumblebees were collected using insect nets and kept in individual labeled tubes and put on ice during transport back to the lab, and then kept at −80 °C until pesticide analysis was performed. Specimens of five bumblebee species with different ranges of tongue length were sampled (Brodie, 1996; Prys-Jones and Corbet, 2011): short-tongued bumblebees were *B. pratorum* (6.4–7.1 mm), *B. lapidarius* (6–8.1 mm), *B. terrestris* (5.8–8.2 mm); medium-tongued was *B. pascuorum* (7.6–8.6 mm); and long-tongued was *B. hortorum* (12–13.5 mm) (Table 1). The flowers where the bees were foraging at the time of capture were recorded (Tables S2a–S1c), since bumblebees exhibit a high degree of floral constancy (Wilson and Stine, 1996), and this may help predict exposure.

Bumblebee individuals were gathered during three sampling periods, spring (27/04/14–14/05/14), early summer (5/06/14–23/06/14) and midsummer (15/07/14–2/08/14), and 150 bee individuals were collected in total. Oilseed rape crops were in bloom during the first sampling period (late spring), and 18 out of the 25 individuals gathered in arable sites during that period were foraging in oilseed rape crops when collected (Table S2a). The pesticide usage information of the crops where bees were foraging was not provided by the farmers. The EU moratorium on the use of neonicotinoid insecticides started on the 1st December 2013, but the oilseed rape crops that were in bloom in the 2014 spring were sown at the end of August-beginning of September 2013, so these crops were still allowed to be seed-treated with neonicotinoids.