



Wild bees are exposed to low levels of pesticides in urban grasslands and community gardens



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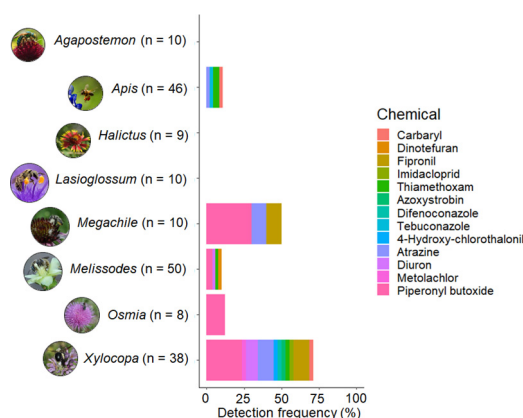
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HIGHLIGHTS

- Wild bees provide important pollination services, but many species are in decline.
- Urban environments often provide floral rich habitat for bees.
- Bees in urban areas can be exposed to pesticides at level known that have sub-lethal effects.
- Limiting pesticides in urban environments will reduce anthropogenic stress on wild bees.

GRAPHICAL ABSTRACT



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ABSTRACT

Globally documented wild bee declines threaten sustainable food production and natural ecosystem functioning. Urban environments are often florally abundant, and consequently can contain high levels of pollinator diversity compared with agricultural environments. This has led to the suggestion that urban environments are an increasingly important habitat for pollinators. However, pesticides, such as commercial bug sprays, have a range of lethal and sub-lethal impacts on bees and are widely available for public use, with past work indicating that managed bees (honeybees and bumblebees) are exposed to a range of pesticides in urban environments. Despite this, we still have a poor understanding of (i) whether wild bees foraging in urban environments are exposed to pesticides and (ii) if exposure differs between genera. Here we assessed pesticide exposure in 8 bee genera foraging across multiple urban landscapes. We detected 13 different pesticides, some at concentrations known to have sub-lethal impacts on pollinators. Both the likelihood of pesticides being detected, and the concentrations observed, were higher for larger bees, likely due to their greater foraging ranges. Our results suggest that restricting agrochemical use in urban environments, where the economic benefits are limited, is a simple way to reduce anthropogenic stress on wild bees.

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1. Introduction

Bees are vital pollinators of crops and wildflowers, but many species are in decline (Powney et al., 2019; Zattara and Aizen, 2021). Whilst the drivers

of bee declines are numerous (Goulson et al., 2015; Siviter et al., 2021a; Vanbergen and Insect Pollinators Initiative, 2013), human-induced landscape change is undoubtedly one of the most severe threat to pollinators (Baude et al., 2016; Potts et al., 2016). Conventional agriculture reduces floral resources for bees and is heavily reliant on pesticides (insecticides, herbicides, fungicides) for controlling crop pests (Potts et al., 2016; Siviter and Muth, 2020; Tilman et al., 2002). In contrast, growing evidence suggests that urban landscapes provide rich floral habitat which benefits bees (Samuelson et al., 2021, 2020, 2018; Tew et al., 2021, but see Milano et al., 2019). For example, green spaces such as parks, wildlife refuges, and residential and community gardens in urban areas are 'hot spots' for bee diversity (Baldock et al., 2019) and urban environments are often associated with a greater diversity of bees than surrounding rural locations (Theodorou et al., 2020). This suggests that cities could be an important refuge for bee diversity (Hall et al., 2017) and, as urbanisation increases globally, understanding potential threats to bees in urban environments is of utmost importance.

One anthropogenic stressor bees may encounter in urban environments is pesticides (David et al., 2016; Longing et al., 2020; Nicholls et al., 2018). Commercial 'bug sprays' (insecticides), herbicides, and fungicides are widely available and can be used in urban environments and, in contrast to agricultural use, the applicator will likely have no training in how to use or dispose of the product. Indeed, wildflowers in urban environments, which are often planted to promote bee diversity, may become contaminated with pesticide drift from gardens or other sources (Halsch et al., 2020; Nicholls et al., 2018). Similarly, the nectar and pollen of ornamental flowers purchased from garden centres often contain a cocktail of different pesticides (Halsch et al., 2022; Lentola et al., 2017). Furthermore, veterinary flea treatments often contain insecticides, such as imidacloprid and fipronil, which are thought to contaminate fresh water sources (Perkins et al., 2021). Public health spraying in urban areas to control mosquitoes and other pests is another source of pesticide exposure (Long and Krupke, 2016). This means that bees foraging in urban areas may be simultaneously exposed to complex mixtures of different pesticides.

While it is clear that wild bees can be exposed to pesticides in urban environments, the majority of pesticide residue studies have been conducted with domesticated honeybees (*Apis mellifera*) or commercial bumblebees (*Bombus* spp.) (Lawrence et al., 2016; Longing et al., 2020; Nicholls et al., 2018). Honeybees and bumblebees are social, and many aspects of their ecology differ significantly from the majority of other bee species, including their degree of sociality, nesting strategy, and body size. These differences can result in different exposure routes (Sgolastra et al., 2019). For example, systemic insecticides can contaminate soil and thus ground-nesting or cavity-nesting bees that collect soil may be exposed (Willis Chan et al., 2019; Willis Chan and Raine, 2021; Fortuin and Gandhi, 2021; Rondeau et al., 2022). Similarly, smaller bees (e.g. *Lasioglossum* spp.) have limited foraging ranges compared with bumblebees and honeybees (Greenleaf et al., 2007), and so if systemic pesticides are applied locally, these bees may be forced to feed exclusively on contaminated flowers. On the other hand, larger bees, such as carpenter bees (*Xylocopa* spp.), which have greater foraging ranges (Gathmann and Tscharntke, 2002; Greenleaf et al., 2007), consume more nectar (Peat and Goulson, 2005), potentially increasing their exposure relative to smaller-bodied bees. While there have been a few studies addressing pesticide exposure in wild native bees, these have typically: (i) focused on agricultural environments (Botías et al., 2016; Hladik et al., 2016; Main et al., 2020); (ii) used pooled samples from blue vane traps, making it impossible to compare between different genera (Hladik et al., 2016); or (iii) been limited to a small number of insecticides (e.g. neonicotinoids) (Botías et al., 2017; Longing et al., 2020). As such, we know little about wild bee exposure to pesticides in urban environments.

Here we assessed pesticide exposure in wild bees foraging in community gardens and grassland patches within an urban environment. We netted bees from 10 sites across Travis country, Texas, USA and used liquid chromatography-mass spectrometry (LC-MS) to identify and quantify the presence of 92 pesticides. Specifically, we collected bees from 8 genera

that varied in their size, nesting type, and degree of sociality. The aim of the project was to (i) document if wild bees are indeed exposed to pesticides in urban areas and to compare (ii) the detected pesticides and (iii) their concentrations across different bee genera.

2. Methods

2.1. Sample collection

Between 22/05/2020 and 09/06/2020, we sampled 10 sites in Travis Country, Texas, USA, where 5 of the sites were grasslands and 5 were community gardens, both containing an abundance of flowers (Ballare et al., 2019). Pesticide use was banned in all community gardens, and grassland sites were largely managed as prairie restorations and were not treated with any pesticides to our knowledge. All sites were chosen such that they were a minimum of 1 km from each other to increase data independence (Ballare et al., 2019; Greenleaf et al., 2007). The community gardens ranged in size from 1885.82 m² - 17,710.10 m² and the grasslands ranged in size from 3867.13 m² - 740,893.67 m².

We visited sites in the morning (between 7:00–12:00) when bee activity was highest. Bees were randomly caught at sites with aerial nets while actively foraging on flowers and immediately placed into individual vials, which were then chilled on icepacks. We focused our collections on the non-native managed bee *Apis mellifera* and 7 wild bee genera: *Agapostemon*, *Halictus*, *Lasioglossum*, *Megachile*, *Melissodes*, *Osmia*, and *Xylocopa* (Table S1). We chose these genera because: (i) these bees were abundant enough for us to have genus-level replication per site for pesticide residue analysis (see below); (ii) because they exhibited a range of sociality (e.g., social vs solitary), nesting types (e.g., ground and cavity nesters), and body sizes (see Table 1); and (iii) they are representative of bee communities in Travis County (Ballare et al., 2019). We did not collect *Bombus pensylvanicus* due to its ongoing range decline (Cameron et al., 2011). We aimed to collect at least 5 bees per genus at each site per sampling day. If this was not possible, we returned the following day and resampled the site (maximum sampling days was 2 per site). After returning to the lab, on their collection day, all bees were placed in a -80 °C freezer.

We collected 322 bees across all sites from 8 genera (*Agapostemon*, $n = 34$, *Apis mellifera* $n = 46$, *Halictus* $n = 39$, *Lasioglossum* $n = 55$, *Megachile* $n = 32$, *Melissodes* $n = 50$, *Osmia* $n = 28$, *Xylocopa* $n = 38$; see Table S1). Bees from different genera varied in both their mass and abundance at different sites. To ensure we had enough material for pesticide residue analysis, we pooled samples of the smaller, less numerous bees within each site (see Table S1). This resulted in final sample sizes of: *Agapostemon*, $n = 10$; *Halictus* $n = 9$; *Lasioglossum* $n = 10$; *Megachile* $n = 10$; *Osmia* $n = 8$ (note, n refers to the number of pooled samples, not the number of bees which is stated above, see Table S1). Bees from the other genera were analyzed individually (*Apis mellifera* $n = 46$, *Melissodes* $n = 50$, *Xylocopa* $n = 38$). After pooling samples (see above), bees were transported to Cornell Chemical Ecology Core Facility (CCECF) on dry ice and refrozen at -80 °C.

2.2. Chemicals and reagents

Acetonitrile and water of HPLC grade were purchased from EMD Millipore. LC-MS grade formic acid was purchased from Thermo Scientific. The 5 M ammonium formate solution, the QuEChERS extraction packets (4 g MgSO₄; 1 g NaCl; 1 g sodium citrate tribasic dihydrate; 0.5 g sodium citrate dibasic sesquihydrate) and the d-SPE kits (150 mg MgSO₄, 25 mg PSA and 25 C18EC) were purchased from Agilent Technologies. The deuterated internal standards were purchased from Sigma-Aldrich International.

2.3. Sample preparation

The samples were extracted using a modified version of the EN 15662 QuEChERS procedure (Standardization, 2018) and screened for 92 pesticides (including some metabolites and breakdown products) by liquid chromatography mass spectrometry (LC-MS/MS) (See Table S2 for a complete

Table 1
The mean and maximum concentration (ppb) of compounds detected in 8 bee genera across all sites and samples. ND = No detection.

| Active ingredient | Aggostemon (n = 10) | | Apis (n = 46) | | Halictus (n = 9) | | Lasiglossum (n = 10) | | Megachile (n = 10) | | Melissodes (n = 50) | | Osmia (n = 8) | | Xylocopa (n = 38) | |
|--------------------------|---------------------|---------|---------------|---------|------------------|---------|----------------------|---------|--------------------|---------|---------------------|---------|---------------|---------|-------------------|---------|
| | Mean | Maximum | Mean | Maximum | Mean | Maximum | Mean | Maximum | Mean | Maximum | Mean | Maximum | Mean | Maximum | Mean | Maximum |
| Insecticide | | | | | | | | | | | | | | | | |
| Carbaryl | ND | ND | 0.01 | 0.52 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.01 | 0.34 |
| Dinotefuran | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 | 2.74 | ND | ND | ND | ND |
| Fipronil | ND | ND | ND | ND | ND | ND | ND | ND | 0.04 | 0.36 | ND | ND | ND | ND | 0.81 | 18.51 |
| Imidacloprid | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.02 | 0.81 |
| Thiamethoxam | ND | ND | 0.02 | 0.51 | ND | ND | ND | ND | ND | ND | 0.03 | 1.31 | ND | ND | 0.02 | 0.88 |
| Fungicide | | | | | | | | | | | | | | | | |
| Azoxystrobin | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.00 | 0.04 |
| Difenoconazole | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.01 | 0.52 |
| Tebuconazole | ND | ND | 0.04 | 1.94 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 4-Hydroxy-chlorothalonil | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.59 | 22.25 |
| Herbicide | | | | | | | | | | | | | | | | |
| Atrazine | ND | ND | 0.00 | 0.20 | ND | ND | ND | ND | 0.00 | 0.03 | ND | ND | ND | ND | 0.02 | 0.38 |
| Diuron | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.02 | 1.21 | ND | ND | 0.07 | 1.90 |
| Metolachlor | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.04 | 1.45 |
| Synergist | | | | | | | | | | | | | | | | |
| Piperonyl butoxide | ND | ND | ND | ND | ND | ND | ND | ND | 0.01 | 0.05 | 0.02 | 1.08 | 0.01 | 0.06 | 0.05 | 0.73 |

list of the 92 chemicals screened) (Graham et al., 2022, 2021; Halsch et al., 2020; Urbanowicz et al., 2019).

Whole frozen bees (0.079–0.561 g) were hydrated with 300 μ L of water and then mixed with 700 μ L of acetonitrile. The samples were homogenized for 1 min using ceramic beads (2.8 mm diameter) and a Bead Ruptor 24 (OMNI International, USA). Specimens of *Xylocopa* and *Megachile* that weighed >600 mg ($n = 8$) were instead ground using mortar and pestle in liquid nitrogen, and 500 mg ($\pm 1\%$) of the ground material was transferred to a 2 mL tube and subsequently mixed with 300 μ L of water and 700 μ L of acetonitrile using a vortex shaker for 1 min.

After complete homogenization, 330 mg of EN 15662 salts were added (203 mg MgSO₄; 51 mg NaCl; 51 mg sodium citrate tribasic dihydrate; 25 mg sodium citrate dibasic sesquihydrate). Samples were then thoroughly vortexed and centrifuged at 7300 $\times g$ for 5 min. One mL of supernatant was collected and transferred into a d-SPE (dispersive solid phase extraction) tube containing 150 mg MgSO₄, 25 mg PSA and 25 mg C18EC. After the d-SPE step, 297 μ L of supernatant was collected and 3 μ L of internal standard solution (d₄-fluopyram 0.15 μ g/mL; d₃-pyraclostrobin 0.3 μ g/mL; ¹³C₆-metalxyl 0.3 μ g/mL) was added. The samples were filtered through 0.22 μ m PTFE and analyzed immediately thereafter.

2.4. Liquid chromatography and mass spectrometry

The analysis was performed with a Vanquish Flex UHPLC system (Dionex Softron GmbH, Germering, Germany) coupled with a TSQ Quantis mass spectrometer (Thermo Scientific, San Jose, CA). The UHPLC was equipped with an Acquity UPLC BEH C18 column (100 mm \times 2.1 mm, 1.7 μ m particle size). The mobile phase consisted of (A) Water with 2 mM ammonium formate and 0.1 % formic acid and (B) Acetonitrile/Water (98:2, v/v) with 2 mM ammonium formate and 0.1 % formic acid. The temperature of the column was set at 40 $^{\circ}$ C and the flow rate of the LC was 300 μ L/min. The elution program was the following: 1.5 min equilibration (2 % B) prior to injection, 0–0.5 min (2 % B, isocratic), 0.5–15 min (2 % \rightarrow 70 % B, linear gradient), 15–17 min (70 % \rightarrow 100 % B, linear gradient), 17–20 min (100 % B, column wash), 20–20.2 min (100 % \rightarrow 2 % B, linear gradient), 20.2–23 min (2 % B, re-equilibration). The flow from the LC was directed to the mass spectrometer through a Heated Electrospray probe (H-ESI). The settings of the H-ESI were: spray voltage 2000 V for positive mode and 2000 V for negative mode, Sheath gas 55 (arbitrary unit), Auxiliary gas 25 (arbitrary unit), Sweep gas 2 (arbitrary unit), Ion transfer tube temperature 325 $^{\circ}$ C, and Vaporizer temperature 350 $^{\circ}$ C.

The MS/MS detection was carried out using the Selected Reaction Monitoring (SRM) mode. Two transitions were monitored for each compound: one for quantification and the other for confirmation. The SRM parameters for each individual compound are summarized in Table 1. The resolution of both Q1 and Q3 was set at 0.7 FWHM, the cycle time was 0.4 s, and the pressure of the collision gas (argon) was set at 2 mTorr.

2.5. Data handling and analysis

The residues identified were classified into three groups based on level of detection. (i) When sample chemicals were below the level of detection, the residue level was considered to be zero. (ii) When a chemical was detected, but the concentration was too low to be quantified, we used the lowest known level of detection (LOD) as our estimated concentration (aka trace levels). (iii) When the chemical was detected and quantifiable, we used the quantified value. To compare the likelihood of detection across pooled samples, we used an information theoretic model selection approach and selected models when Δ AICc < 2. We ran a generalised linear model, with a binomial error structure (Bates et al., 2015), and the full model contained genus, site, and the mean bee mass per sample. The selected model contained only bee genus. *P* values were obtained using the function anova.

3. Results

We detected 13 pesticides across 322 bees (181 samples, see methods and Table S1), from 8 genera across 10 sites. These included 5 insecticides, 4 fungicides, 3 herbicides, and one synergist (Table 1, Figs. 1, 2). Piperonyl butoxide (8.3 %), a synergist, was the most commonly detected chemical across all samples, followed by atrazine (3.3 %), fipronil (2.8 %), diuron (2.2 %), thiamethoxam (2.2 %), carbaryl (1.1 %), dinotefuran (0.6 %), imidacloprid (0.6 %), 4-hydroxy-chlorothalonil (0.6 %), azoxystrobin (0.6 %), tebuconazole (0.6 %), metolachlor (0.6 %), and difenoconazole (0.6 %).

We found differences in the likelihood of pesticides being detected between genera (Fig. 2, $p < 0.001$), with larger bees being more likely to have detectable levels of pesticide (Fig. 3). 52 % of *Xylocopa* samples, 50 % *Megachile*, 12.5 % *Osmia*, 10.8 % *Apis mellifera*, and 10 % *Melissodes* contained at least one pesticide. No pesticides were detected in *Agapostemon*, *Halictus*, and *Lasioglossum*. A total of 11 different pesticides were detected in *Xylocopa*, 4 were detected in honeybees (*Apis mellifera*) and *Melissodes*, 3 in *Megachile*, and 1 in *Osmia* (Fig. 2). Only 6 samples contained >1 pesticide, all of which were *Xylocopa* (5 samples contained 2 pesticides, and 1 contained 3).

Across all compounds, the concentrations detected ranged from less than 1 ppb to 22 ppb, but were relatively low across all sites (Table 1, Fig. 1A, Figs. 1B, 4). In some cases, the concentrations were too low to be detected, and we used the limit of detection as the estimated concentrations (see methods and Fig. 1A). The 4 highest concentrations were found in *Xylocopa*, where 4-hydroxy-chlorothalonil (22.4 ppb), fipronil (18.5 ppb), diuron (1.9 ppb), and metolachlor (1.4 ppb) were detected. This was followed by dinotefuran (2.7 ppb), thiamethoxam (1.3 ppb), and diuron (1.2 ppb), which were detected in *Melissodes* (Fig. 4). All other compounds detected contained residue levels lower than 1 ppb (Table 1, Fig. 4).

4. Discussion

Urban environments are an increasingly important habitat for wild bees as a consequence of their high floral resources, but exposure to pesticides has remained a critical concern (Hall et al., 2017; Nicholls et al., 2018). In this study, we detected 13 different pesticides in wild bees foraging in urban environments, some at concentrations with known sub-lethal impacts on bees (Bonmatin et al., 2015; Siviter et al., 2021c). We also found that (i) the likelihood of pesticides being detected and (ii) observed concentrations were higher for larger bees, with *Xylocopa* containing the highest concentrations across a range of chemicals. However, both detection rates and observed concentrations were relatively low when compared to agricultural

environments (Hladik et al., 2016; Main et al., 2020). This suggests that urban environments, and specifically community gardens and grasslands, can provide important habitat for wild bees with relatively low levels of pesticides.

Pesticide residue data for bees beyond honeybees and bumblebees are few, but our data suggest that exposure differs between genera. Specifically, we found that larger bees were more likely to contain pesticides. For example, 52 % of *Xylocopa* (~355 mg) and 50 % of *Megachile* (~105 mg) were exposed to at least one pesticide, while no pesticides were detected in *Agapostemon*, *Halictus*, and *Lasioglossum* (~33 mg, ~20 mg, and ~4mg respectively). Given that all samples with few to no pesticides were detected in small-bodied genera with limited foraging ranges (Gathmann and Tschardt, 2002; Greenleaf et al., 2007; Griffin and Haddad, 2021), our results suggest that larger bees were exposed outside of the sample sites. We collected data over a limited time period, and thus future research conducted across multiple seasons is required to determine if our observations might be consistent over the whole year. However, our data indicates that community gardens and grasslands in urban environments could offer a vital, relatively pesticide-free environment for smaller bees with limited foraging ranges.

Bees' nesting environment did not appear to explain the variation we found in pesticide exposure. Ground-nesting bees (*Agapostemon*, *Halictus*, *Lasioglossum* and *Melissodes*, see Table S1) were typically exposed to fewer pesticides, but they were also smaller, with reduced foraging ranges, which likely drove this result for a few reasons. First, *Melissodes* is the largest ground nesting genus included in our analysis and was also the only member of this group where we detected pesticides (see Table S1 for mass comparison). Second, the pesticide concentrations in *Melissodes* were similar to those found in cavity nesting bees of equivalent sizes (e.g., *Megachile*, see Fig. 4, Table S1). Sociality also did not appear to explain our results, with solitary, communal nesting, and eusocial bees all exposed to pesticides at similar detection levels and concentrations. We posit that detailed studies examining pesticide concentrations in bees that differ in nesting type and sociality, but forage on the same flowers in a controlled experiment are required to better understand how nesting type and sociality influence pesticide exposure (Willis Chan and Raine, 2021).

We only found pesticides in bees with large foraging ranges, suggesting that exposure occurred in the areas surrounding collection sites, most of which were residential gardens. While pesticide use was restricted in the community gardens we sampled, previous studies with honeybees and bumblebees have shown that bees are routinely exposed to pesticides in residential gardens (David et al., 2016; Nicholls et al., 2018; Šlachta et al., 2020). Pesticides, including insecticides, fungicides, and herbicides, are regularly used in residential gardens, and the co-formulants included

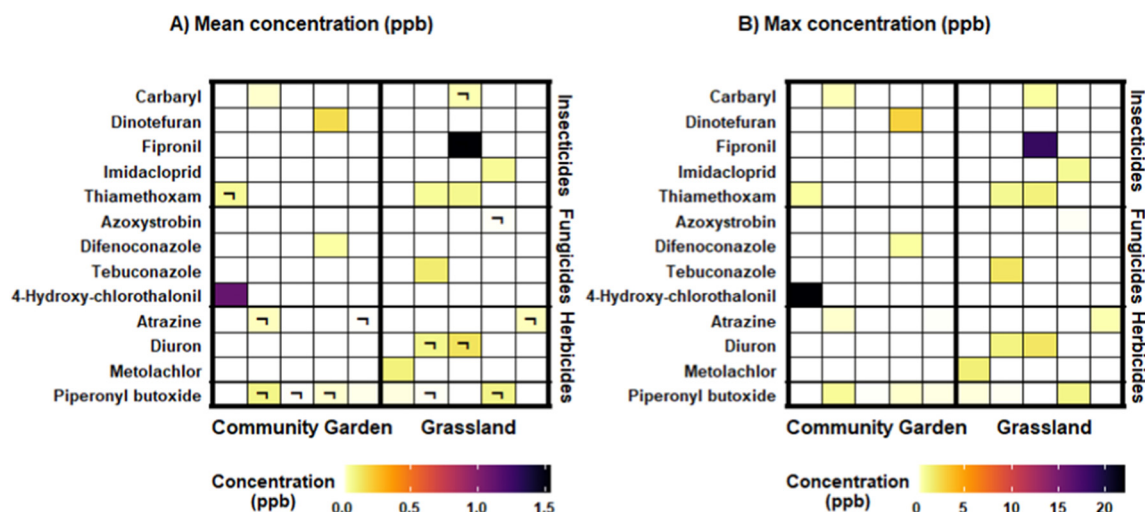


Fig. 1. Mean (A) and max (B) concentrations of compounds at each site across all bee genera sampled. Values are shown in ppb and - indicates that the compound was only detected at trace amounts (see methods for details).

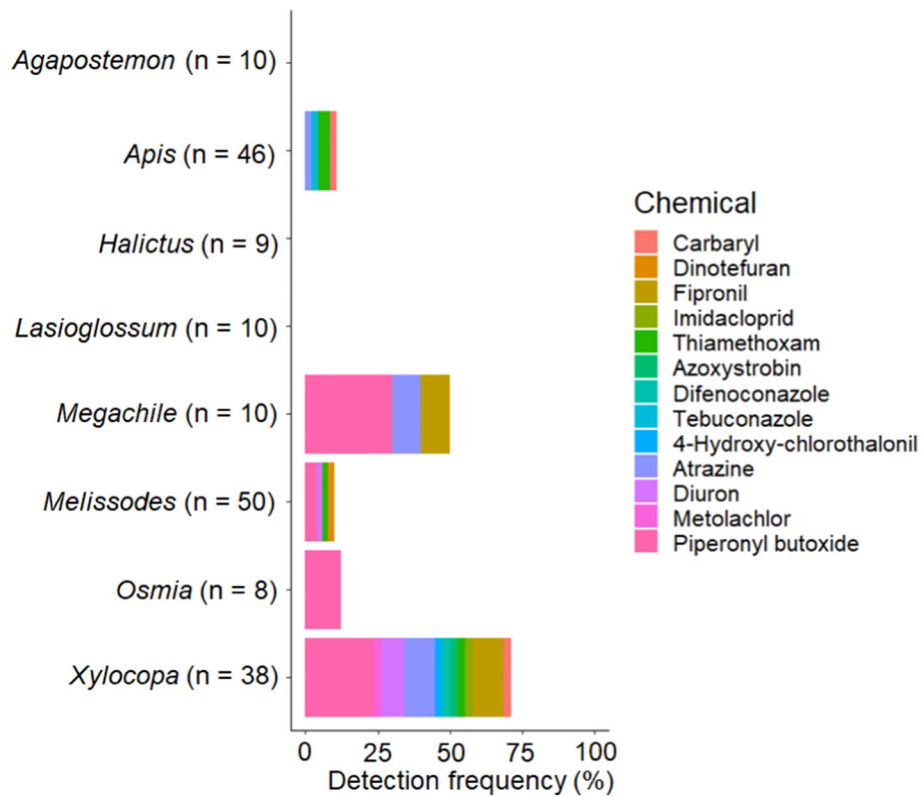


Fig. 2. The detection frequency (% of samples containing compounds) across bee genera. N refers to the number of samples (for number of bees see Table S1). Note that each stacked bar graph refers to each compound. 52 % of all *Xylocopa* samples contained at least one chemical compound, but the total percentage here is higher as multiple chemicals were detected in 5 samples (see Results for further details).

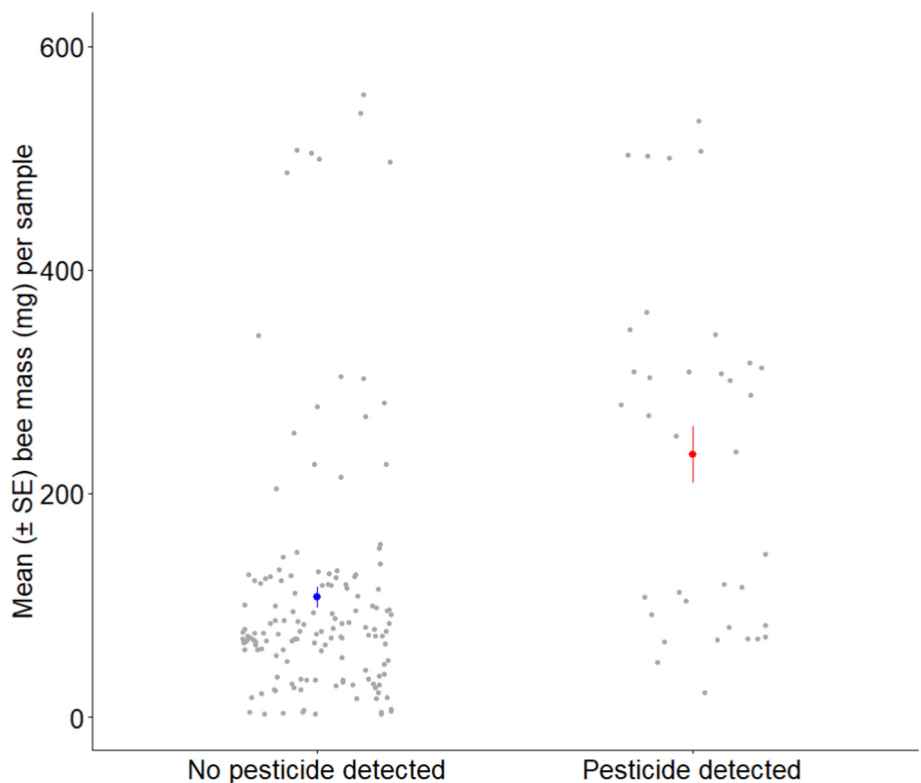


Fig. 3. The average mass (mg) of bees within a sample where no pesticides were detected vs. samples where at least one pesticide was detected. Grey dots = one individual sample. Bee mass for pooled samples (*Agapostemon*, *Halictus*, *Lasioglossum*, *Megachile* and *Osmia*) was calculated by dividing the total mass of the sample by the number of bees in the sample. Bee mass for individual samples (*Apis mellifera*, *Melissodes*, *Xylocopa*) was the mass of the whole sample.

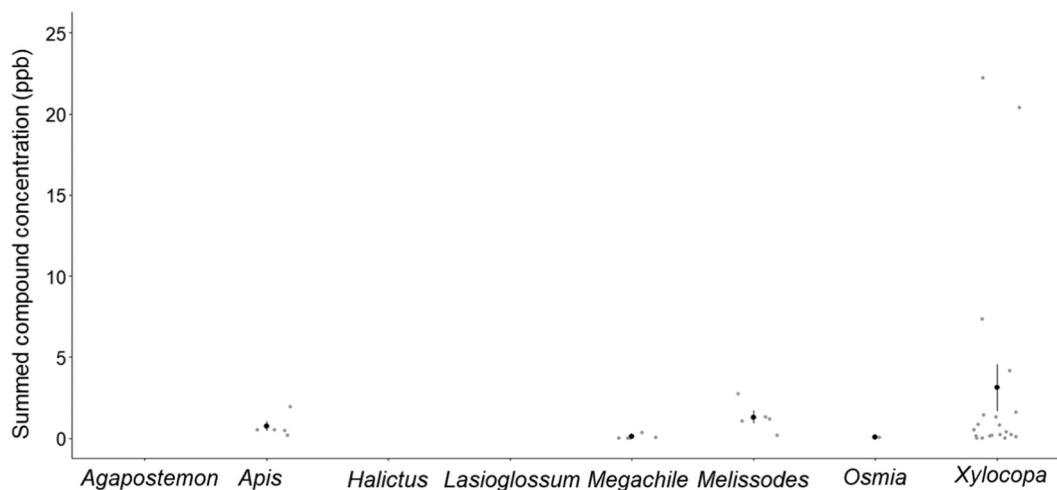


Fig. 4. The mean (\pm SE) concentration (ppb) of all compounds summed from each sample across 8 bee genera. Grey dots indicate individual samples. Samples that contained no compounds are not shown in this figure to aid with presentation (see Table 1).

with these commercial products can often increase the impact of their active ingredients (Fine et al., 2017; Straw et al., 2021; Straw and Brown, 2021). Interestingly, the most commonly observed chemical in our results was the synergist, piperonyl butoxide. Piperonyl butoxide is not acutely toxic to bees, but can increase the efficacy of insecticides including neonicotinoids (Darriet and Chandre, 2013; Moores et al., 2012), which could lead to unintended synergistic interactions between chemicals (Siviter et al., 2021a; Siviter and Muth, 2020). Piperonyl butoxide is often combined with pyrethroids, which were not assessed in our analysis, but can be purchased from garden centres for residential garden use. Piperonyl butoxide is also used in pet flea treatments and thus dog and cat visitation could potentially contaminate flowering plants in gardens. To our knowledge, no previous studies have considered this potential exposure route, but antiparasitic drugs and other veterinary pharmaceuticals used on cattle have been found in wildflowers (Peterson et al., 2021, 2017). While determining the routes of exposure was beyond the scope of the current study, past work has indeed documented exposure routes within residential gardens (Longing et al., 2020; Nicholls et al., 2018), and thus educating gardeners about alternative pest management practices is clearly vital to reduce bee pesticide exposure in urban areas.

We found that pesticide concentrations observed in urban environments were low compared with other data collected in agricultural environments (Hladik et al., 2016; Main et al., 2020). However, these relatively low concentrations may still have negative sub-lethal effects on bees (Pisa et al., 2017; Siviter et al., 2021c, 2018). The insecticide fipronil was present in *Xylocopa* at 18.5ppb. While our analysis cannot determine whether bees were exposed via direct consumption of the insecticide or via topical exposure, behavioural effects on honeybees are evident even at relatively low topical doses (Aliouane et al., 2009). Sub-lethal concentrations of fipronil can also influence honeybee learning and memory (El Hassani et al., 2009) and stingless bee neurology (Jacob et al., 2015). We also found the neonicotinoids dinotefuran (at 2.7 ppb) and thiamethoxam (at 1.3 ppb) in *Melissodes*. Low concentrations of neonicotinoids can have a plethora of sub-lethal effects on bees including reduced reproduction (Klaus et al., 2021; Stuligross and Williams, 2020; Whitehorn et al., 2012), colony growth (Siviter et al., 2021c), foraging (Feltham et al., 2014; Gill et al., 2012; Muth and Leonard, 2019; Siviter et al., 2021b) and cognition (Muth et al., 2019; Samuelson et al., 2016; Siviter et al., 2018).

Bees were also exposed to fungicides and herbicides. *Xylocopa* were exposed to the fungicides 4-Hydroxy-chlorothalonil (18.5 ppb) and honeybees to tebuconazole (1.94 ppb). At present there is no available data on the sub-lethal impacts of 4-Hydroxy-chlorothalonil on bees, but exposure to field realistic levels of other fungicides can have sub-lethal impacts on honeybee learning (DesJardins et al., 2021), lifespan (Fisher et al., 2021),

and colony development (Fisher et al., 2021). The fungicide tebuconazole can also act as a synergist with insecticides, increasing their potential impact (Willow et al., 2019). *Xylocopa* were also exposed to the herbicides diuron (1.9 ppb) and metolachlor (1.4 ppb). Herbicide exposure can have sub-lethal effects on bee behaviour (Balbuena et al., 2015; Herbert et al., 2014), but to our knowledge the sub-lethal impacts of diuron and metolachlor have not been explored at the concentrations observed in our data. Further studies that determine the sub-lethal impacts of fungicides and herbicides on non-*Apis* bees are vital, however, our results confirm that pesticide exposure in urban areas is likely having sub-lethal impacts on wild bees.

Urban environments can provide a floral-rich habitat that often supports a greater diversity of bees than agricultural environments (Baldock et al., 2019; Samuelson et al., 2021; Tew et al., 2021; Theodorou et al., 2020). As urbanisation increases globally, and wild bees continue to decline (Powney et al., 2019; Zattara and Aizen, 2021), urban environments could play an increasingly critical role in wild bee conservation (Hall et al., 2017). Our results show that wild bees are exposed to low levels of pesticides in sites that are thought to be 'pesticide free', some at concentrations which can have negative sub-lethal impacts on pollinators. Restricting pesticide use in urban environments, where the economic gains of pesticide application are limited, is a clear and simple way of protecting bees and other beneficial non-target organisms from the unintended impacts of agrochemical use.

CRedit authorship contribution statement

HS, GP, SJ & FM designed the experiment. HS and GP collected the bees. NB conducted the pesticide analysis. HS wrote the first version of the manuscript. All authors contributed to subsequent versions.

Data availability

All data is available online at OSF (<https://osf.io/k6bjx/>).

Declaration of competing interest

The authors declare they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.159839>.

References

- Aliouane, Y., El Hassani, A.K., Gary, V., Armengaud, C., Lambin, M., Gauthier, M., 2009. Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior. *Environ. Toxicol. Chem.* 28, 113–122. <https://doi.org/10.1897/08-110.1>.
- Balbuena, M.S., Tison, L., Hahn, M.-L., Greggers, U., Menzel, R., Farina, W.M., 2015. Effects of sublethal doses of glyphosate on honeybee navigation. *J. Exp. Biol.* 218, 2799–2805. <https://doi.org/10.1242/jeb.117291>.
- Baldock, K.C.R., Goddard, M.A., Hicks, D.M., Kunin, W.E., Mitschunas, N., Morse, H., Osgathorpe, L.M., Potts, S.G., Robertson, K.M., Scott, A.V., Staniczenko, P.P.A., Stone, G.N., Vaughan, I.P., Memmott, J., 2019. A systems approach reveals urban pollinator hotspots and conservation opportunities. *Nat. Ecol. Evol.* 3, 363–373. <https://doi.org/10.1038/s41559-018-0769-y>.
- Ballare, K.M., Neff, J.L., Ruppel, R., Jha, S., 2019. Multi-scalar drivers of biodiversity: local management mediates wild bee community response to regional urbanization. *Ecol. Appl.* 29. <https://doi.org/10.1002/eap.1869>.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 51. <https://doi.org/10.18637/jss.v067.i01>.
- Baude, M., Kunin, W.E., Boatman, N.D., Conyers, S., Davies, N., Gillespie, M.A.K., Morton, R.D., Smart, S.M., Memmott, J., 2016. Historical nectar assessment reveals the fall and rise of floral resources in Britain. *Nature* 530, 85–88. <https://doi.org/10.1038/nature16532>.
- Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreuzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environ. Sci. Pollut. Res.* 22, 35–67. <https://doi.org/10.1007/s11356-014-3332-7>.
- Botías, C., David, A., Hill, E.M., Goulson, D., 2016. Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects. *Sci. Total Environ.* 566–567, 269–278. <https://doi.org/10.1016/j.scitotenv.2016.05.065>.
- Botías, C., David, A., Hill, E.M., Goulson, D., 2017. Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes. *Environ. Pollut.* 222, 73–82. <https://doi.org/10.1016/j.envpol.2017.01.001>.
- Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., Griswold, T.L., 2011. Patterns of widespread decline in north american bumble bees. *Proc. Natl. Acad. Sci.* 108, 662–667. <https://doi.org/10.1073/pnas.1014743108>.
- Darriet, F., Chandre, F., 2013. Efficacy of six neonicotinoid insecticides alone and in combination with deltamethrin and piperonyl butoxide against pyrethroid-resistant *Aedes aegypti* and *Anopheles gambiae* (Diptera: Culicidae). *Pest Manag. Sci.* 69, 905–910. <https://doi.org/10.1002/ps.3446>.
- David, A., Botías, C., Abdul-Sada, A., Nicholls, E., Rotheray, E.L., Hill, E.M., Goulson, D., 2016. Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. *Environ. Int.* 88, 169–178. <https://doi.org/10.1016/j.envint.2015.12.011>.
- DesJardins, N.S., Fisher, A., Ozturk, C., Fewell, J.H., DeGrandi-Hoffman, G., Harrison, J.F., Smith, B.H., 2021. A common fungicide, Pristine®, impairs olfactory associative learning performance in honey bees (*Apis mellifera*). *Environ. Pollut.* 288, 117720. <https://doi.org/10.1016/j.envpol.2021.117720>.
- El Hassani, A.K., Dupuis, J.P., Gauthier, M., Armengaud, C., 2009. Glutamate and GABAergic effects of fipronil on olfactory learning and memory in the honeybee. *Invertebr. Neurosci.* 9, 91–100. <https://doi.org/10.1007/s10158-009-0092-z>.
- Feltham, H., Park, K., Goulson, D., 2014. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* 23, 317–323. <https://doi.org/10.1007/s10646-014-1189-7>.
- Fine, J.D., Cox-Foster, D.L., Mullin, C.A., 2017. An inert pesticide adjuvant synergizes viral pathogenicity and mortality in honey bee larvae. *Sci. Rep.* 7, 40499. <https://doi.org/10.1038/srep40499>.
- Fisher, A., DeGrandi-Hoffman, G., Smith, B.H., Johnson, M., Kaftanoglu, O., Cogley, T., Fewell, J.H., Harrison, J.F., 2021. Colony field test reveals dramatically higher toxicity of a widely-used Mito-toxic fungicide on honey bees (*Apis mellifera*). *Environ. Pollut.* 269, 115964. <https://doi.org/10.1016/j.envpol.2020.115964>.
- Fortuin, C.C., Gandhi, K.J.K., 2021. Mason bees (*Hymenoptera: Megachilidae*) exhibit no avoidance of imidacloprid-treated soils. *Environ. Entomol.* <https://doi.org/10.1093/ee/nvab083>.
- Gathmann, A., Tschardt, T., 2002. Foraging ranges of solitary bees. *J. Anim. Ecol.* 71, 757–764. <https://doi.org/10.1046/j.1365-2656.2002.00641.x>.
- Gill, R.J., Ramos-Rodriguez, O., Raine, N.E., 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* 491, 105–108. <https://doi.org/10.1038/nature11585>.
- Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347, 1255957. <https://doi.org/10.1126/science.1255957>.
- Graham, K.K., Milbrath, M.O., Zhang, Y., Soehnen, A., Baert, N., McArt, S., Isaacs, R., 2021. Identities, concentrations, and sources of pesticide exposure in pollen collected by managed bees during blueberry pollination. *Sci. Rep.* 11, 16857. <https://doi.org/10.1038/s41598-021-96249-z>.
- Graham, K.K., Milbrath, M.O., Zhang, Y., Baert, N., McArt, S., Isaacs, R., 2022. Pesticide risk to managed bees during blueberry pollination is primarily driven by off-farm exposures. *Sci. Rep.* 12, 7189. <https://doi.org/10.1038/s41598-022-11156-1>.
- Greenleaf, S.S., Williams, N.M., Winfree, R., Kremen, C., 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153, 589–596. <https://doi.org/10.1007/s00442-007-0752-9>.
- Griffin, S.R., Haddad, N.M., 2021. Connectivity and edge effects increase bee colonization in an experimentally fragmented landscape. *Ecography (Cop.)* 44, 919–927. <https://doi.org/10.1111/ecog.05299>.
- Hall, D.M., Camilo, G.R., Tonietto, R.K., Ollerton, J., Ahnér, K., Arduser, M., Ascher, J.S., Baldock, K.C.R., Fowler, R., Frankie, G., Goulson, D., Gunnarsson, B., Hanley, M.E., Jackson, J.L., Langellotto, G., Lowenstein, D., Minor, E.S., Philpott, S.M., Potts, S.G., Sirohi, M.H., Spevak, E.M., Stone, G.N., Threlfall, C.G., 2017. The city as a refuge for insect pollinators. *Conserv. Biol.* 31, 24–29. <https://doi.org/10.1111/cobi.12840>.
- Halsch, C.A., Code, A., Hoyle, S.M., Fordyce, J.A., Baert, N., Forister, M.L., 2020. Pesticide contamination of milkweeds across the agricultural, urban, and open spaces of low-elevation northern California. *Front. Ecol. Evol.* 8. <https://doi.org/10.3389/fevo.2020.00162>.
- Halsch, C.A., Hoyle, S.M., Code, A., Fordyce, J.A., Forister, M.L., 2022. Milkweed plants bought at nurseries may expose monarch caterpillars to harmful pesticide residues. *Biol. Conserv.* 273, 109699. <https://doi.org/10.1016/j.biocon.2022.109699>.
- Herbert, L.T., Vázquez, D.E., Arenas, A., Farina, W.M., 2014. Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour. *J. Exp. Biol.* 217, 3457–3464. <https://doi.org/10.1242/jeb.109520>.
- Hladik, M.L., Vandever, M., Smalling, K.L., 2016. Exposure of native bees foraging in an agricultural landscape to current-use pesticides. *Sci. Total Environ.* 542, 469–477. <https://doi.org/10.1016/j.scitotenv.2015.10.077>.
- Jacob, C.R., Soares, H.M., Nocelli, R.C., Malaspina, O., 2015. Impact of fipronil on the mushroom bodies of the stingless bee *Scaptotrigona postica*. *Pest Manag. Sci.* 71, 114–122. <https://doi.org/10.1002/ps.3776>.
- Klaus, F., Tschardt, T., Bischoff, G., Grass, I., 2021. Floral resource diversification promotes solitary bee reproduction and may offset insecticide effects – evidence from a semi-field experiment. *Ecol. Lett.* 24, 668–675. <https://doi.org/10.1111/ele.13683>.
- Lawrence, T.J., Culbert, E.M., Felsot, A.S., Hebert, V.R., Sheppard, W.S., 2016. Survey and risk assessment of *Apis mellifera* (Hymenoptera: Apidae) exposure to neonicotinoid pesticides in urban, rural, and agricultural settings. *J. Econ. Entomol.* 109, 520–528. <https://doi.org/10.1093/ee/tov397>.
- Lentola, A., David, A., Abdul-Sada, A., Tapparo, A., Goulson, D., Hill, E.M., 2017. Ornamental plants on sale to the public are a significant source of pesticide residues with implications for the health of pollinating insects. *Environ. Pollut.* 228, 297–304. <https://doi.org/10.1016/j.envpol.2017.03.084>.
- Long, E.Y., Krupke, C.H., 2016. Non-cultivated plants present a season-long route of pesticide exposure for honey bees. *Nat. Commun.* 7, 11629. <https://doi.org/10.1038/ncomms11629>.
- Longing, S.D., Peterson, E.M., Jewett, C.T., Rendon, B.M., Discua, S.A., Wooten, K.J., Subbiah, S., Smith, P.N., McIntyre, N.E., 2020. Exposure of Foraging Bees (*Hymenoptera*) to neonicotinoids in the U.S. Southern High Plains. *Environ. Entomol.* 49, 528–535. <https://doi.org/10.1093/ee/nvaa003>.
- Main, A.R., Hladik, M.L., Webb, E.B., Goyné, K.W., Mengel, D., 2020. Beyond neonicotinoids – wild pollinators are exposed to a range of pesticides while foraging in agroecosystems. *Sci. Total Environ.* 742, 140436. <https://doi.org/10.1016/j.scitotenv.2020.140436>.
- Milano, N.J., Iversen, A.L., Nault, B.A., McArt, S.H., 2019. Comparative survival and fitness of bumble bee colonies in natural, suburban, and agricultural landscapes. *Agric. Ecosyst. Environ.* 284, 106594. <https://doi.org/10.1016/j.agee.2019.106594>.
- Moore, G.D., Węgorzek, P., Zamojska, J., Field, L., Philippou, D., 2012. The effect of a piperonyl butoxide/tau-fluvalinate mixture on pollen beetle (*Meligethes aeneus*) and honey bees (*Apis mellifera*). *Pest Manag. Sci.* 68, 795–800. <https://doi.org/10.1002/ps.2328>.
- Muth, F., Leonard, A.S., 2019. A neonicotinoid pesticide impairs foraging, but not learning, in free-flying bumblebees. *Sci. Rep.* 9, 4764. <https://doi.org/10.1038/s41598-019-39701-5>.
- Muth, F., Francis, J.S., Leonard, A.S., 2019. Modality-specific impairment of learning by a neonicotinoid pesticide. *Biol. Lett.* 15, 20190359. <https://doi.org/10.1098/rsbl.2019.0359>.
- Nicholls, E., Botías, C., Rotheray, E.L., Whitehorn, P., David, A., Fowler, R., David, T., Feltham, H., Swain, J.L., Wells, P., Hill, E.M., Osborne, J.L., Goulson, D., 2018. Monitoring neonicotinoid exposure for bees in rural and peri-urban areas of the U.K. During the transition from pre- to post-moratorium. *Environ. Sci. Technol.* 52, 9391–9402. <https://doi.org/10.1021/acs.est.7b06573>.
- Peat, J., Goulson, D., 2005. Effects of experience and weather on foraging rate and pollen versus nectar collection in the bumblebee, *Bombus terrestris*. *Behav. Ecol. Sociobiol.* 58, 152–156. <https://doi.org/10.1007/s00265-005-0916-8>.
- Perkins, R., Whitehead, M., Civil, W., Goulson, D., 2021. Potential role of veterinary flea products in widespread pesticide contamination of english rivers. *Sci. Total Environ.* 755, 143560. <https://doi.org/10.1016/j.scitotenv.2020.143560>.
- Peterson, E.M., Wooten, K.J., Subbiah, S., Anderson, T.A., Longing, S., Smith, P.N., 2017. Agrochemical mixtures detected on wildflowers near cattle feed yards. *Environ. Sci. Technol. Lett.* 4, 216–220. <https://doi.org/10.1021/acs.estlett.7b00123>.
- Peterson, E.M., Thompson, K.N., Shaw, K.R., Tomlinson, C., Longing, S.D., Smith, P.N., 2021. Use of nest bundles to monitor agrochemical exposure and effects among cavity nesting pollinators. *Environ. Pollut.* 286, 117142. <https://doi.org/10.1016/j.envpol.2021.117142>.
- Pisa, L., Goulson, D., Yang, E.-C., Gibbons, D., Sánchez-Bayo, F., Mitchell, E., Aebi, A., van der Sluijs, J., MacQuarrie, C.J.K., Giorio, C., Long, E.Y., McField, M., Bijleveld van Lexmond, M., Bonmatin, J.-M., 2017. An update of the worldwide integrated assessment (WIA) on

- systemic insecticides. Part 2: impacts on organisms and ecosystems. *Environ. Sci. Pollut. Res.* 28, 1–49. <https://doi.org/10.1007/s11356-017-0341-3>.
- Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., Dicks, L.V., Garibaldi, L.A., Hill, R., Settele, J., Vanbergen, A.J., 2016. Safeguarding pollinators and their values to human well-being. *Nature* 540, 220–229. <https://doi.org/10.1038/nature20588>.
- Powney, G.D., Carvell, C., Edwards, M., Morris, R.K.A., Roy, H.E., Woodcock, B.A., Isaac, N.J.B., 2019. Widespread losses of pollinating insects in Britain. *Nat. Commun.* 10, 1018. <https://doi.org/10.1038/s41467-019-08974-9>.
- Rondeau, S., Baert, N., McArt, S., Raine, N.E., 2022. Quantifying exposure of bumblebee (*Bombus* spp.) queens to pesticide residues when hibernating in agricultural soils. *Environ. Pollut.* 309, 119722. <https://doi.org/10.1016/j.envpol.2022.119722>.
- Samuelson, A.E., Gill, R.J., Brown, M.J.F., Leadbeater, E., 2018. Lower bumblebee colony reproductive success in agricultural compared with urban environments. *Proc. R. Soc. B Biol. Sci.* 285, 20180807. <https://doi.org/10.1098/rspb.2018.0807>.
- Samuelson, A.E., Gill, R.J., Leadbeater, E., 2020. Urbanisation is associated with reduced *Nosema* sp. infection, higher colony strength and higher richness of foraged pollen in honeybees. *Apidologie* 51, 746–762. <https://doi.org/10.1007/s13592-020-00758-1>.
- Samuelson, A.E., Schürch, R., Leadbeater, E., 2021. Dancing bees evaluate central urban forage resources as superior to agricultural land. *J. Appl. Ecol.* <https://doi.org/10.1111/1365-2664.14011>.
- Samuelson, E.E.W., Chen-Wishart, Z.P., Gill, R.J., Leadbeater, E., 2016. Effect of acute pesticide exposure on bee spatial working memory using an analogue of the radial-arm maze. *Sci. Rep.* 6, 38957. <https://doi.org/10.1038/srep38957>.
- Sgolastra, F., Hinarejos, S., Pitts-Singer, T.L., Boyle, N.K., Joseph, T., Lückmann, J., Raine, N.E., Singh, R., Williams, N.M., Bosch, J., 2019. Pesticide exposure assessment paradigm for solitary bees. *Environ. Entomol.* 48, 22–35. <https://doi.org/10.1093/ee/nvy105>.
- Siviter, H., Muth, F., 2020. Do novel insecticides pose a threat to beneficial insects? *Proc. R. Soc. B Biol. Sci.* 287, 20201265. <https://doi.org/10.1098/rspb.2020.1265>.
- Siviter, H., Koricheva, J., Brown, M.J.F., Leadbeater, E., 2018. Quantifying the impact of pesticides on learning and memory in bees. *J. Appl. Ecol.* 55, 2812–2821. <https://doi.org/10.1111/1365-2664.13193>.
- Siviter, H., Bailes, E.J., Martin, C.D., Oliver, T.R., Koricheva, J., Leadbeater, E., Brown, M.J.F., 2021a. Agrochemicals interact synergistically to increase bee mortality. *Nature* 596, 389–392. <https://doi.org/10.1038/s41586-021-03787-7>.
- Siviter, H., Johnson, A.K., Muth, F., 2021b. Bumblebees exposed to a neonicotinoid pesticide make suboptimal foraging decisions. *Environ. Entomol.* 50, 1299–1303. <https://doi.org/10.1093/ee/nvab087>.
- Siviter, H., Richman, S.K., Muth, F., 2021c. Field-realistic neonicotinoid exposure has sublethal effects on non-apis bees: a meta-analysis. *Ecol. Lett.* 24, 2586–2597. <https://doi.org/10.1111/ele.13873>.
- Šlachta, M., Erban, T., Votavová, A., Bešta, T., Skalský, M., Václavíková, M., Halešová, T., Edwards-Jonášová, M., Včeláková, R., Cudlín, P., 2020. Domestic gardens mitigate risk of exposure of pollinators to pesticides—an urban-rural case study using a red Mason bee species for biomonitoring. *Sustainability* 12, 9427. <https://doi.org/10.3390/su12229427>.
- Standardization, E.C., 2018. Multimethod for the Determination of Pesticide Residues Using GC- and LC-based Analysis Following Acetonitrile Extraction/partitioning and Clean-up by Dispersive SPE - Modular QuEChERS-method. BS EN 156622018.
- Straw, E.A., Brown, M.J.F., 2021. Co-formulant in a commercial fungicide product causes lethal and sub-lethal effects in bumble bees. *Sci. Rep.* 11, 21653. <https://doi.org/10.1038/s41598-021-00919-x>.
- Straw, E.A., Carpentier, E.N., Brown, M.J.F., 2021. Roundup causes high levels of mortality following contact exposure in bumble bees. *J. Appl. Ecol.* 58, 1167–1176. <https://doi.org/10.1111/1365-2664.13867>.
- Stuligross, C., Williams, N.M., 2020. Pesticide and resource stressors additively impair wild bee reproduction. *Proc. R. Soc. B Biol. Sci.* 287, 20201390. <https://doi.org/10.1098/rspb.2020.1390>.
- Tew, N.E., Memmott, J., Vaughan, I.P., Bird, S., Stone, G.N., Potts, S.G., Baldock, K.C.R., 2021. Quantifying nectar production by flowering plants in urban and rural landscapes. *J. Ecol.* 109, 1747–1757. <https://doi.org/10.1111/1365-2745.13598>.
- Theodorou, P., Radzevičiūtė, R., Lentendu, G., Kahnt, B., Husemann, M., Bleidorn, C., Settele, J., Schweiger, O., Grosse, I., Wubet, T., Murray, T.E., Paxton, R.J., 2020. Urban areas as hotspots for bees and pollination but not a panacea for all insects. *Nat. Commun.* 11, 576. <https://doi.org/10.1038/s41467-020-14496-6>.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature* <https://doi.org/10.1038/nature01014>.
- Urbanowicz, C., Baert, N., Blüher, S.E., Bőröczky, K., Ramos, M., McArt, S.H., 2019. Low maize pollen collection and low pesticide risk to honey bees in heterogeneous agricultural landscapes. *Apidologie* 50, 379–390. <https://doi.org/10.1007/s13592-019-00655-2>.
- Vanbergen, A.J., Insect Pollinators Initiative, 2013. Threats to an ecosystem service: pressures on pollinators. *Front. Ecol. Environ.* 11, 251–259. <https://doi.org/10.1890/120126>.
- Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D., 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336, 351–352. <https://doi.org/10.1126/science.1215025>.
- Willis Chan, D.S., Raine, N.E., 2021. Population decline in a ground-nesting solitary squash bee (*Eucera pruinosus*) following exposure to a neonicotinoid insecticide treated crop (*Cucurbita pepo*). *Sci. Rep.* 11, 4241. <https://doi.org/10.1038/s41598-021-83341-7>.
- Willis Chan, D.S., Prosser, R.S., Rodríguez-Gil, J.L., Raine, N.E., 2019. Assessment of risk to hoary squash bees (*Peponapis pruinosus*) and other ground-nesting bees from systemic insecticides in agricultural soil. *Sci. Rep.* 9, 11870. <https://doi.org/10.1038/s41598-019-47805-1>.
- Willow, J., Silva, A., Veromann, E., Smagghe, G., 2019. Acute effect of low-dose thiacloprid exposure synergised by tebuconazole in a parasitoid wasp. *PLoS One* 14, e0212456. <https://doi.org/10.1371/journal.pone.0212456>.
- Zattara, E.E., Aizen, M.A., 2021. Worldwide occurrence records suggest a global decline in bee species richness. *One Earth* 4, 114–123. <https://doi.org/10.1016/j.oneear.2020.12.005>.