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RESEARCH ARTICLE

Pesticide risk during commercial apple pollination is greater for honeybees than other managed and wild bees

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Abstract

1. Most pesticide research has focussed on risk to managed honeybees, but other managed and wild bees are also exposed to pesticides. Critically, we know little about the magnitude and sources of risk to honeybees compared with other bees during crop pollination.
2. To compare pesticide exposure and risk across wild and managed bees, we sampled the main bee groups present during bloom in 20 apple orchards, including managed honeybees (*Apis mellifera*), managed bumblebee workers (*Bombus impatiens*), wild mining bees (*Andrena* spp. and *Andrena [Melandrena]* spp.), bumblebee foundress queens (*Bombus impatiens*) and eastern carpenter bees (*Xylocopa virginica*). We screened all bees for 92 pesticides and computed a Risk Quotient using available toxicity data (honeybee LD_{50}s), adjusting for differences in toxicity known to scale with body mass. To gain insight into exposure origin, we compared residues in bees to those in focal orchard apple and dandelion flowers.
3. Nearly all bee samples contained pesticides (95%), with the average contamination level ranging from 7.1 ± 2.8 parts per billion (ppb) in *B. impatiens* workers to 388.4 ± 146.2 ppb in *Andrena*. Exposure profiles were similar for all bees except *A. mellifera*, whose unique exposure profile included high levels of the neonicotinoid insecticide thiamethoxam.
4. All bee groups except wild *B. impatiens* queens had at least one sample exceeding a US Environmental Protection Agency or European Food Safety Authority exposure level of concern. *Apis mellifera* experienced significantly greater risk than other bee groups, with 63% and 81% of samples exceeding an acute or chronic exposure level of concern, respectively. Risk to honeybees was driven primarily by high thiamethoxam levels not found in focal orchard flowers and likely originating outside the orchard.
5. *Synthesis and applications*: We find that pesticide exposure and risk differ between honeybees and other managed and wild bees during apple pollination. Furthermore, pesticide exposure is a landscape-scale phenomenon and therefore measures to reduce exposure must consider the surroundings beyond focal farms. Limiting orchard sprays, while reducing on-farm exposures, will not protect

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far-foraging bees from off-farm exposures such as thiamethoxam, which we hypothesize is coming from nearby seed-treated corn fields planted during apple bloom.

KEYWORDS
fungicide, Hazard Quotient, insecticide, pesticide exposure, pollinator health, risk quotient, thiamethoxam

1 | INTRODUCTION

Pollination benefits over 70% of the major food crops across the globe (Klein et al., 2007), contributing more than $170 billion and $15 billion to the global and US economies annually, respectively (Calderone, 2012; Gallai et al., 2009), with the majority of pollination supplied by bees (Willmer, 2011). While crop pollination is often attributed to commercially managed bees, particularly the western honeybee (*Apis mellifera*), an equally large portion of pollination services come from unmanaged wild pollinators (Garibaldi et al., 2013; Reilly et al., 2020; Russo et al., 2015; Winfree et al., 2008).

Wild and managed bee pollinators are experiencing documented range contractions, extirpations and unsustainable losses (Goulson et al., 2015). In 2019–2020, the loss rate of US honeybee hives was over 40% (Bruckner et al., 2023). Similarly, many non-managed pollinators are endangered or threatened; several recent studies have found global or regional declines in wild bee diversity and abundance (Cameron et al., 2011; LeCroy et al., 2020; White et al., 2022; Zattara & Aizen, 2021). There are many stressors that are driving these trends, including pathogens, land use change and the impacts of invasive species, among others. One stressor that has received considerable interest is the use of pesticides (Boyle et al., 2019; Goulson et al., 2015).

Estimating pesticide risk to bees involves assessing both exposure to and toxicity of pesticides (EPA, 2014). The majority of research on pesticide exposure has focussed on managed bees, especially the western honeybee due to its status as a model species for pollinators, and comparatively little work has assessed exposure across assemblages of non-honeybees (Raine & Rundlöf, 2024; Sgolastra et al., 2019). Many of these wild bees vary drastically in their ecology, including foraging habits, nesting location and sociality among other traits (Danforth et al., 2019). Since agricultural fields do not exist in isolation but are surrounded by a network of other crops and land uses, each with their own pesticide application regimes, the foraging patterns and range of bees can dictate the spatial scale at which they interact with their surroundings and experience on-farm and off-farm pesticide exposures (Graham et al., 2021, 2022; Kopit & Pitts-Singer, 2018; Zioga et al., 2023). In addition, traits such as nesting location are likely to shape exposure profiles; ground-nesting bees face exposure to pesticides in the soil, which cavity-nesting bees that interact minimally with soils are not exposed to (Willis Chan & Raine, 2021). Yet, much of how different species of bees with varying life-history traits are exposed to pesticides in the same crop pollination contexts is still relatively unknown (Boyle et al., 2019; Sgolastra et al., 2019).

Just as most exposure data are over-represented with studies of honeybees, toxicity is often extrapolated from managed honeybees (Lewis & Tzilivakis, 2019), which act as a surrogate for other bee species (EPA, 2014). This is problematic as it assumes that all bee species have the same response to a pesticide, however, studies show there is substantial interspecific variation in the sensitivity of bees to pesticides (Arena & Sgolastra, 2014; Heard et al., 2017; Rundlöf et al., 2015). Interestingly, it has been shown that pesticide sensitivity correlates significantly with bee body mass, with heavier species generally being more resistant to pesticides than lighter bees (Pamminger, 2021). This is potentially useful since performing toxicity tests on dozens of bee species for hundreds of pesticides is impractical, whereas using a quantitative mass–toxicity relationship could facilitate extrapolation of honeybee toxicity data to other bee species simply by knowing their mass. Toxicity predictions could then be combined with exposure data to estimate differences in risk among species.

To better understand exposure and risk from pesticides among different bee groups, we focussed on an apple orchard system. Apple is heavily dependent on animal-mediated pollination to set fruit with nearly all varieties requiring the pollen of a different cultivar for successful pollination (Ramírez & Davenport, 2013). Apple is cultivated worldwide, producing over 87 million tons of fruit in 2019 (FAO, 2019), and is dependent on a broad range of both managed and wild pollinators (Blitzer et al., 2016; Gardner & Ascher, 2006). Studies in New York, USA, have found over 100 bee species present in apple orchards (Russo et al., 2015) and research in the UK has shown most visitors to apple flowers are wild bees (Garratt et al., 2014). We sampled the main bee groups present in 20 New York apple orchards during bloom, including managed honeybees (*Apis mellifera*), managed bumblebee workers (*Bombus impatiens*), as well as wild mining bees (*Andrena* spp.), grouped into the subgenus *Melandrena* or not, bumblebee foundress queens (*Bombus impatiens*) and eastern carpenter bees (*Xylocopa virginica*) to answer two main questions: (1) Do managed and wild bees differ in their exposure to pesticides during crop pollination? (2) Does pesticide risk vary across bee groups? Finally, we sampled each orchard for apple flowers and dandelions and compared their pesticide residues to the sampled bees to answer (3) are bees being exposed to pesticides from flowers within focal apple orchards or outside of orchards?

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2 | MATERIALS AND METHODS

2.1 | Field collections

We collected female bees from 20 apple orchards in western and central New York State during bloom in May 2019. Orchards were located in the state's main apple growing region near Lake Ontario, and in the Finger Lakes region (Figure S1 in the Supporting Information). Immediately prior to bloom, we placed one honeybee (*A. mellifera*) colony at each orchard between 30 April and 1 May, and one bumblebee (*B. impatiens*) colony between May 9 and 14. Prior to placement at orchards, the honeybee colonies had been located at the Cornell Dyce Lab for Honeybee Studies, which is primarily surrounded by forested and University land. The bumblebee colonies were obtained from Biobest (Leamington, Ontario, Canada), screened for pathogens in the laboratory via microscopy, then immediately placed in the field.

During peak apple bloom between May 15 and 24, which varied among sites, we sampled 11 returning honeybee workers (*A. mellifera*) and 11 common eastern bumblebee workers (*B. impatiens*) from the entrances of their hives. These collections occurred 15–24 days after the honeybee colonies had been placed in orchards and 7–10 days after bumblebee colonies had been placed in orchards. One worker from each collection was randomly selected for pesticide analysis, and the remaining 10 workers were saved for pathogen analysis (to be summarized in a different paper). We also collected wild mining bees (*Andrena*) split into two groups (those in the subgenus *Melandrena* and all others; see Table S1 for a list of which species comprise these two groupings in New York apple orchards), eastern carpenter bees (*Xylocopa virginica*), and wild *B. impatiens* foundress queens netted in the orchard. Three bees from each species/group were sampled per orchard, and one bee was randomly selected for pesticide analysis while the remaining two bees were saved for pathogen analysis. Two samples each of apple flowers and dandelion flowers were collected from the lanes between apple rows at each orchard. Each flower sample was a composite of petals, stamens and anthers from 2 to 3 plants, and each sample was obtained from randomly selected plants spread throughout each orchard. All samples were immediately placed on dry ice in the field before being stored at −80°C until processing for pesticide analysis. No ethics approval or collecting licences were required for this work.

2.2 | Pesticide analysis

Full methods are identical to those in (Siviter et al., 2023), summarized in Appendix S1, with MS/MS detection parameters shown in Table S2. Briefly, we extracted each sample via a modified QuEChERS extraction protocol and screened them for 92 pesticides, including insecticides, fungicides, herbicides, a synergist and some metabolites, via LC-ESI-MS/MS (Vanquish UHPLC coupled with a TSQ Quantis Mass Spectrometer; Thermo Scientific) equipped with a C18 reversed-phase column (Accucore aQ 2.6 μm, 100 × 2.10 mm; Thermo Scientific) in the Cornell Chemical Ecology Core Facility (https://blogs.cornell.edu/ccecf/). Each sample consisted of either the whole bee or a composite of 3–5 whole flowers.

2.3 | Risk quotient

We obtained oral and contact LD_{50} values for each pesticide for adult honeybee workers (*A. mellifera*) from the British Crop Protection Council Pesticide Manual (MacBean, 2012), the ECOTOX database of the US Environmental Protection Agency (Olker et al., 2022), and the AgriTox Database of the French government (Maniere et al., 2011). There are little data on the toxicity of most pesticides to non-honeybees, and while using honeybee LD_{50} values is a common and defended approximation method (EPA, 2014), it assumes all bees have the same susceptibility to toxins as honeybees.

Some evidence suggests LD_{50} values scale with mass among bee species (Medrzycki et al., 2013; Pamminger, 2021), which would allow for an extrapolation of any bee's LD_{50} from known honeybee LD_{50} data. To quantitatively assess the relationship between bee mass and LD_{50} among bee species, we plotted a regression of $\log_{10}(LD_{50})$ as a function of $\log_{10}$(bee mass) based on a data set of 55 toxicology studies, representing 340 endpoint values across 27 bee species (13 genera across 5 families) and 61 active ingredients, initially assembled in Pamminger (2021). We used this quantitative relationship to extrapolate LD_{50} values for each pesticide–bee combination (Appendix S2, Figure S2). Next, we used mass-adjusted LD_{50} values and pesticide residue data to calculate a Risk Quotient (RQ) for each sample by dividing the pesticide residues detected by the LD_{50} values of each chemical, summed across all detected pesticides, performed separately for oral LD_{50} and contact LD_{50}, as follows: $RQ = \sum_{i=1}^{n} \frac{\text{Residue}_i}{LD_{50i}}$.

Our Risk Quotient is identical to a Hazard Quotient (Stoner & Eitzer, 2013) and toxicity-weighted concentration (Nicholson et al., 2023) except the adjusted LD_{50} values facilitate comparative assessments of risk among bee species that differ in mass. The values can be directly compared with US Environmental Protection Agency (EPA) and European Food Safety Authority (EFSA) levels of concern (LOCs) because the pesticides are in the bees themselves, not other matrices such as pollen or nectar. Thus, no assumptions are necessary concerning dietary intake rates to estimate oral exposure or active ingredient physical transfer rates to estimate contact exposure. We note that while the EPA's BeeREX tool facilitates risk assessment from oral exposure using empirical residue data, it does not currently facilitate risk assessment from contact exposure using empirical residue data; instead, the tool assesses risk from contact exposure via predictive models that extrapolate from pesticide application rates. Empirical residue data are superior to predictive models because they represent empirical exposure values rather than predicted exposure values. Thus, we suggest our use of empirical residue data is justified when calculating risk and comparing to the EPA LOC for contact exposure as well as oral exposure. Further details are contained in Supporting Information Appendix S2.

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2.4 | Data analysis

Data analysis was performed in RStudio, using R 4.3.1 (R Core Team, 2021; RStudio Team, 2020). The packages rstatix (Kassambara, 2021), vegan (Oksanen et al., 2022) and pairwiseAdonis (Martinez Arbizu, 2020) were used for analysis. Data manipulation and visualization was performed using the Tidyverse group of packages (Wickham et al., 2019).

To assess differences in total pesticide count and RQ between bee groups, we ran Kruskal–Wallis tests followed when significant by post hoc Dunn's tests with a Holm–Bonferroni correction to control for multiple comparisons. Nonparametric Kruskal–Wallis and Dunn tests were used to account for non-normal data.

To assess differences in the composition of pesticide residues among each bee group, we ran PERMANOVAs with 999 permutations and Bray–Curtis dissimilarity. Significant differences were followed by pairwise PERMANOVAs to see which specific sample groups differed in their pesticide composition. The same analysis was performed to assess dissimilarity between the composition of pesticides contributing to RQ. Residue and RQ data were plotted using NMDS, with the default axis number of $k=3$ to keep stress under 0.2. All 3 NMDS axes are graphed as each axis may explain equal amounts of variance, unlike other ordinations such as PCA in which some component axes explain more than others. To further understand which specific pesticides contributed most to differences between groups, we ran SIMPER analyses on both residue and RQ data with 999 permutations. All pesticide residue PERMANOVAs and SIMPERs were run with the inclusion of apple and dandelion flowers to assess whether bees and orchard flowers experienced similar pesticide exposure. Finally, because we found the neonicotinoid insecticide thiamethoxam at uniquely high concentrations in honeybees (Figure 1) and this pesticide contributed the most to driving differences in RQ between honeybees and other bee groups (Figure 4, Table S5), a Pearson's correlation coefficient was calculated to assess whether thiamethoxam levels in honeybees were correlated with levels in flowers across the 20 orchards.

3 | RESULTS

3.1 | Exposure among bee groups

Almost all bees sampled were contaminated with at least one of the 92 pesticides, with 5 out of 103 (less than 5% of bee samples) having no pesticides detected (Figure 1). All *A. mellifera*, *B. impatiens* queens, *Melandrena* and *X. virginica* individuals collected were found to have detectable pesticide residues and only four *B. impatiens* workers and one *Andrena* sp. were found to have no detectable pesticides. There was a significant difference in the number of unique pesticides detected among bee groups ($X^2(5, N=103)=29.47$, $p<0.001$), ranging from an average of 3.1 ± 0.4 in *B. impatiens* workers to 7.9 ± 0.6 in *B. impatiens queens* (Figure 2A), as well as in the average contamination level ($X^2(5, N=103)=24.77$, $p<0.001$) ranging from 7.1 ± 2.8 parts per billion (ppb) in *B. impatiens* workers to 388.4 ± 146.2 ppb in *Andrena* (Figure 2B).

When looking at the composition of pesticides, bees were significantly different in their exposure profiles ($F_{5,\ 92}=2.38$, $p<0.001$). When comparing across all bee groups, difference in residue composition was primarily driven by difenoconazole, which explained 27% of the variation (Table S3) and was present primarily in *Andrena* and to a lesser extent in *Melandrena*, *B. impatiens* queens and *X. virginica*, while being nearly absent in *B. impatiens* workers and *A. mellifera*. In pairwise comparisons, *A. mellifera* had a significantly different exposure profile than all other bee groups ($p<0.05$; Figure S3, Table S4), while no other pairs of bees were significantly different from one another. When assessing why *A. mellifera* specifically had a unique exposure profile, high levels of thiamethoxam was always the most significant contributor to dissimilarity in pairwise comparisons between *A. mellifera* and other bees, contributing on average 27.9% to dissimilarity (range 23.1%–37.7%; Table S5).

3.2 | Risk among bee groups

Bee groups differed significantly in their oral RQ ($X^2(5, N=103)=36.1$, $p<0.001$) and contact RQ ($X^2(5, N=103)=35.8$, $p<0.001$; Figure 3). In pairwise comparisons, *A. mellifera* had a significantly higher oral and contact RQ than all other bee groups sampled ($p<0.05$; Figure 3). Meanwhile, all other bee groups were not significantly different from one another in either oral or contact RQ. All bee groups except *B. impatiens* queens had at least one sample that exceeded an EPA or EFSA LOC. 81% of sampled *A. mellifera* and approximately 10% of other bee groups exceeded the EFSA chronic oral exposure LOC (Table S6). *A. mellifera* also had samples exceeding every EPA and EFSA acute exposure threshold, with 25% of *A. mellifera* exceeding the EPA and EFSA acute contact exposure LOC, 56% exceeding the EPA acute oral exposure LOC, and 63% exceeding the EFSA acute oral exposure LOC. Using mass-unadjusted RQ values, *A. mellifera* still had the highest RQ, but was no longer significantly different from larger bees such as *X. virginica* (oral and contact RQ) and *B. impatiens* queens (oral RQ), which decreased in RQ when accounting for their larger mass (Figure S4).

The pesticides driving contact RQ were also significantly different among bee groups ($F_{5,\ 92}=2.6$, $p<0.001$; Figure 4), with pairwise comparisons showing *A. mellifera* significantly different from all other bee groups. This indicates that *A. mellifera* had a unique collection of pesticides contributing to risk that were not shared by other bees sampled ($p<0.05$, Table S7). Additionally, *B. impatiens* workers had a significantly different composition of pesticides contributing to risk compared with *Andrena* ($p<0.05$), while all other comparisons were non-significant ($p>0.05$).

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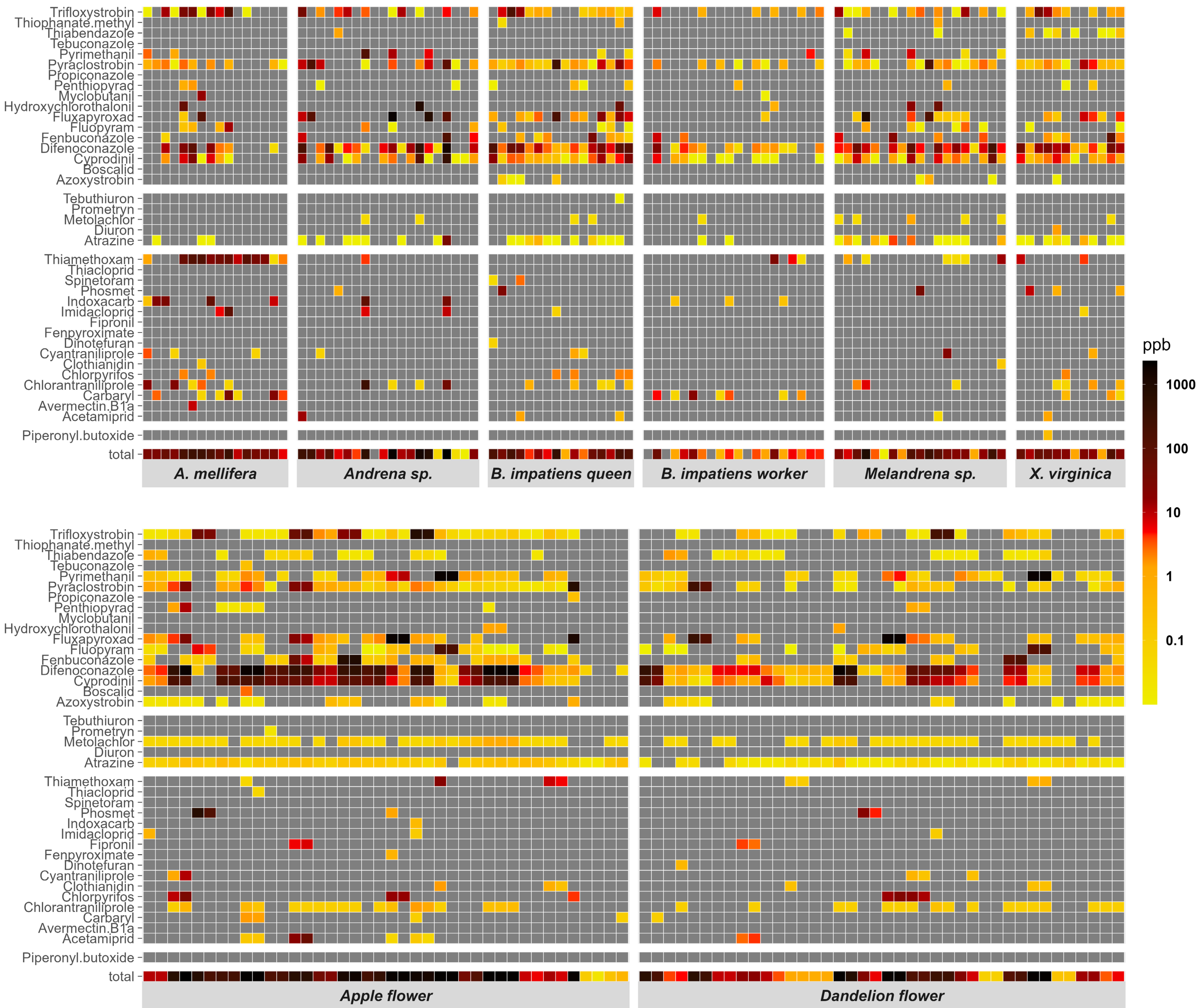

FIGURE 1 Many pesticides were present across all sample types, with 95% of bee samples and all flower samples contaminated with pesticides measured in ng/g (= parts per billion; ppb). Darker colours indicate greater concentrations of a pesticide and grey indicates the pesticide was absent or below the limit of detection. Each column represents a single bee or flower sample from one of the 20 apple orchards. One bee per type and two flowers per type were sampled per orchard when possible. Rows are grouped from top to bottom by (1) fungicides, (2) herbicides, (3) insecticides and (4) synergists. The bottom row (total) represents the additive quantity of all pesticides measured in that sample.

Thiamethoxam was the main driver of dissimilarity among bee groups for contact RQ, explaining 30% of dissimilarity, followed by difenoconazole explaining 10% (Table S8). When assessing which pesticides were driving the dissimilarity in contact RQ between *A. mellifera* and other bee groups, high levels of thiamethoxam and indoxacarb in *A. mellifera* were the top drivers in each pairing, contributing 57%–63% and 15%–18% of variation in dissimilarity, respectively (Table S9). The dissimilarity between *B. impatiens* workers and *Andrena* were primarily driven by carbaryl found only in *B. impatiens* and higher exposure levels of difenoconazole found in *Andrena*, explaining 19% and 18% of dissimilarity, respectively. See Tables S10–S13 for full results of unadjusted RQ analyses.

3.3 | Pesticide residue differences between bees and flowers

When analysing pairwise differences between bee groups and apple and dandelion flowers' pesticide residue profiles, apples and dandelions were not different from one another (Table S14). Apple flowers had a significantly different exposure profile from all bees ($p < 0.05$) and dandelion flowers were significantly different only from *A. mellifera* and *B. impatiens* workers ($p < 0.05$; Figure 5). Overall, orchard flowers were high in fungicides such as difenoconazole, cyprodinil and fluxapyroxad, which were less prevalent in bees and much less prevalent in *A. mellifera* and *B. impatiens* workers specifically (Tables S15 and S16). *A. mellifera* was also much higher in

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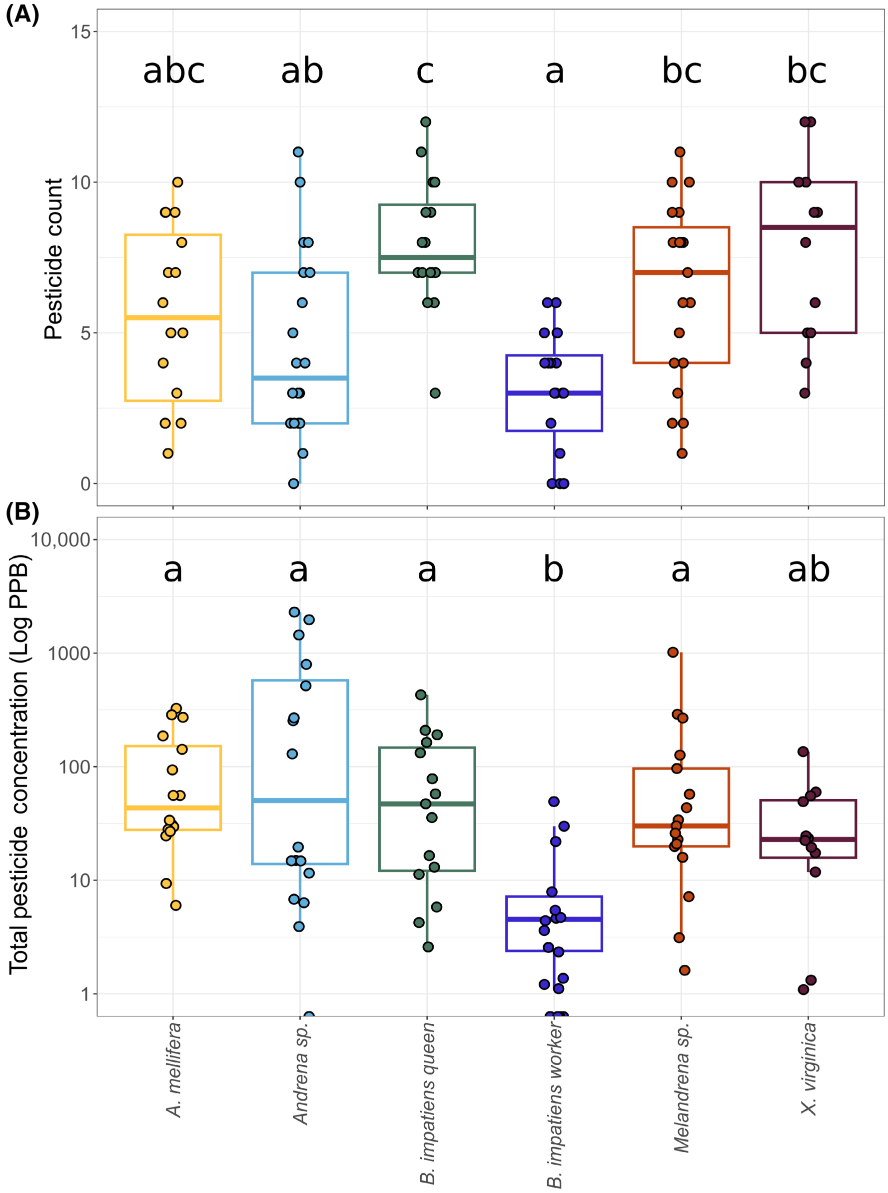

FIGURE 2 Bee samples averaged 7 unique pesticides per sample (A), with an average total concentration of 130.28 ppb (B). Each point represents a single sample with horizontal jittering to help differentiate points. Boxplots extend from first to third quantile with a line at the median. Whiskers extend to the largest and smallest values within 1.5 times the interquartile range. Letters indicate significance in post-hoc pairwise comparisons ($p < 0.05$).

thiamethoxam than orchard flowers. The levels of thiamethoxam in *A. mellifera* did not correlate with the levels in orchard flowers ($t = -1.429$, df = 14, $p = 0.175$ for apple flowers and $t = -1.0207$, df = 14, $p = 0.325$ for dandelions; Figure S5).

4 | DISCUSSION

While almost all of the bees we sampled in apple orchards showed evidence of pesticide exposure, exactly *which* pesticides they had been exposed to, and the resultant risk they faced, varied among species. Honeybees had by far the highest RQ, with 81% exceeding the EFSA chronic oral exposure LOC, 56% and 63% exceeding the EPA and EFSA acute oral exposure LOCs, respectively, and 25% exceeding the acute contact exposure LOC from both the EPA and EFSA. This high level of risk in honeybees was primarily driven by uniquely high levels of thiamethoxam, a systemic neonicotinoid insecticide, which was uncorrelated with infrequent detections in focal orchard flowers and therefore likely originating outside the orchard. Compared with other surveyed bees, honeybees forage at a greater radius (Beekman & Ratnieks, 2000; Gathmann & Tscharntke, 2002; Grüter & Hayes, 2022) and collect pollen from many non-crop flowers during apple bloom (McArt et al., 2017), which may explain the different composition of pesticides they harboured.

Thiamethoxam, while approved for use in apple orchards, is illegal to spray during bloom when pollinators are present. As a systemic insecticide, however, applications shortly before bloom can remain detectable in the nectar and pollen during bloom (Heller et al., 2020), which explains the residues in a few orchard flowers (10%). While much pesticide exposure is certainly occurring within the orchard, as evidenced by some degree of overlap in exposure profiles between bees and orchard flowers (Figure 5), the main drivers of honeybee risk appear to be coming from outside the orchard, mirroring results from other studies (Graham

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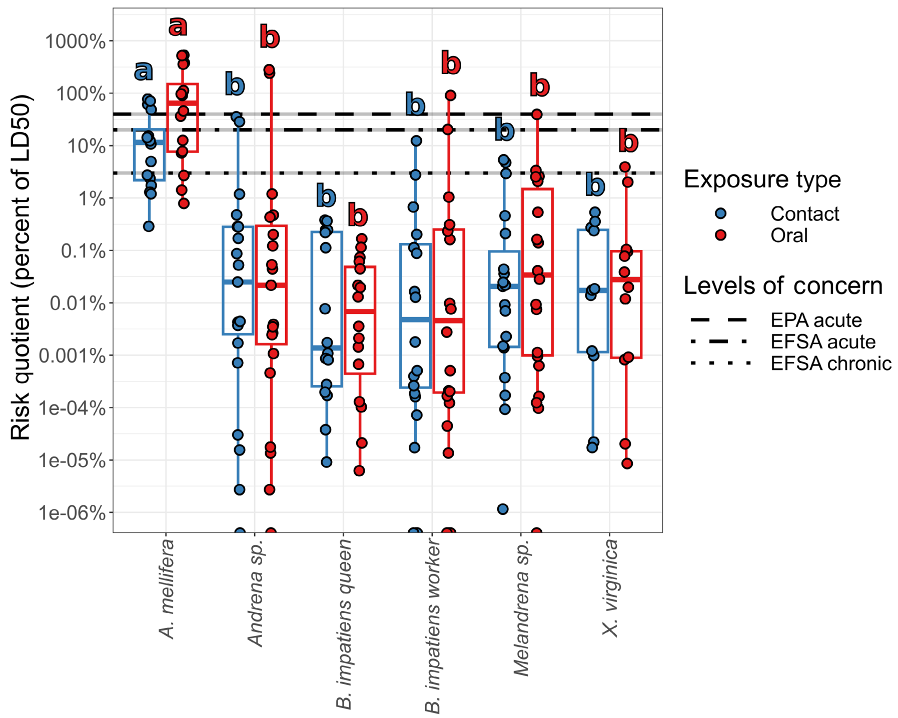
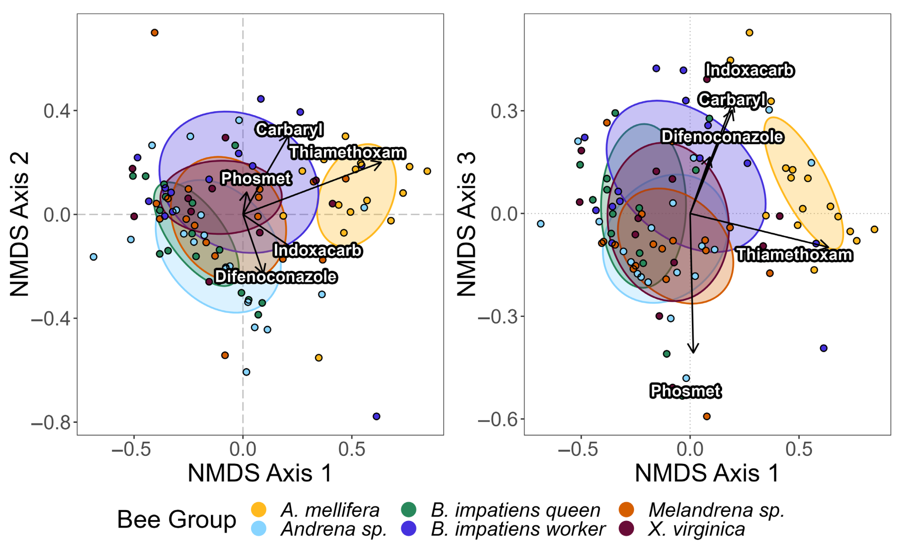


FIGURE 3 *Apis mellifera* samples had a significantly higher oral (red) and contact (blue) Risk Quotient than other bee groups, and all bees except *Bombus impatiens* queens had at least one sample exceeding regulatory agency a level of concern. Lines represent the levels of concern set by the U.S. Environmental Protection Agency (EPA) for acute contact exposure (Tier 1 risk quotient = 40%; dashed line), European Food Safety Authority (EFSA) for acute contact exposure (20%; dash dot line), and EFSA 10-day chronic oral exposure (3%; dotted line). Each point represents a single sample. Boxes extend from first to third quartile with a line at the median. Whiskers extend to the largest and smallest values within 1.5 times the interquartile range. Letters indicate significance in post-hoc pairwise comparisons ($p < 0.05$), calculated separately for contact and oral exposures.

FIGURE 4 *Apis mellifera* had a unique set of pesticides contributing to their Risk Quotient (RQ), with high levels of thiamethoxam exposure being the main driver. Each panel shows 2 of the 3 axes of the NMDS ($k = 3$, stress = 0.15) plotting the Bray–Curtis dissimilarity of RQ among bee groups. Each point represents a single sample and shaded ovals represent 1 standard deviation. Arrows show the direction of the top 5 drivers of dissimilarity, calculated via multigroup SIMPER. Gradients of drivers may not be linear. Arrows length shows the relative strength of each driver.



et al., 2022; McArt et al., 2017). We suspect that much of the thiamethoxam in honeybees is coming from pesticide-treated corn and/or soybean seeds that are planted concurrently with apple bloom. Thiamethoxam is one of the main corn and soybean seed treatments used in New York (Grout et al., 2020) and studies have shown that 2–3% of the active ingredient is lost as dust during planting (Krupke et al., 2012; Schaafsma et al., 2018), spreading the pesticide within and outside of fields (Krupke et al., 2017). Additionally, the plant takes up less than 5% of the active ingredient (Alford & Krupke, 2017) with the majority leaching into soils, surface water, and groundwater (Alford & Krupke, 2019). We hypothesize thiamethoxam dust is landing on flowers, in turn being picked up by far-foraging honeybees, causing high levels of exposure. In agreement with this hypothesis, researchers have found

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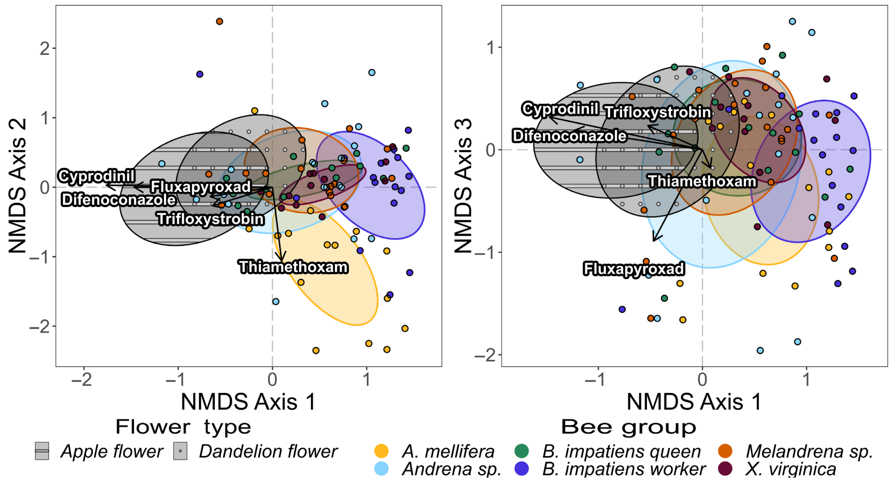

FIGURE 5 Apple flowers had a significantly different pesticide composition from all bee samples while dandelion samples were only different from *Apis mellifera* and *Bombus impatiens* workers. Each panel shows 2 of the 3 axes used in the NMDS ($k=3$, stress $=0.16$) plotting the Bray–Curtis dissimilarity of the pesticide composition found in bees and apple and dandelion orchard flowers. Each point represents a single bee sample, with individual flowers not shown as points to aid in readability. Shaded ovals represent 1 standard deviation. Arrows show the direction of the top 5 drivers of dissimilarity, calculated via multigroup SIMPER. Gradients of drivers may not be linear. Arrow length shows the relative strength of each driver.

high levels of thiamethoxam in flowers and honeybees surrounding recently planted corn fields (Krupke et al., 2012). While it cannot be known from this study exactly where or when honeybees are picking up thiamethoxam, the lack of clothianidin, the metabolite of thiamethoxam, in the honeybee samples indicates that the thiamethoxam exposure occurred shortly before sampling and well after the hives were placed in the orchards (Coulon et al., 2018).

While honeybees had a unique composition of pesticide residues, the other bee groups had no significant difference in their pesticide exposure profiles despite having varying life histories and foraging patterns. It should be noted, however, that wild bees were netted while visiting flowers inside the apple orchard, and therefore likely actively foraging on apple or weedy flowers within orchards, while managed *A. mellifera* and *B. impatiens* workers were netted while returning to their hive in apple orchards. Thus, it is unknown whether returning foragers were returning from distant or within-orchard foraging trips. Interestingly, all bees were significantly different from apple flowers in their pesticide profile, suggesting that while non-*Apis* bee groups are exposed to similar pesticides, these are not solely coming from the focal crop. Other exposure venues could be weedy flowers, including dandelions, or other matrices such as soil, surface water, or off-farm venues.

The levels of pesticide exposure found in this study are not unique to the apple system and previous studies have found similarly frequent and high levels of pesticides in bees. Graham et al. (2022) found a comparable number of pesticides in honeybees sampled in blueberry fields, although they found a lower overall RQ. McArt et al. (2017) quantified residues in honeybee beebread in New York state apple orchards and found lower concentrations of thiamethoxam but higher concentrations of most other compounds compared with findings reported here. Ward et al. (2022) found a similar number and concentration of pesticides in bees netted in flower strips between large agricultural fields. In the UK, David et al. (2016) found thiamethoxam to be the most common pesticide detected in *Bombus terrestris* pollen loads, with 100% of pollen samples positive, while bee samples themselves more often contained fungicides such as carbendazim and boscalid. Nicholson et al. (2023) placed *Bombus terrestris* colonies in apple orchards across Europe and found Indoxacarb to be the greatest driver of risk. Few studies, however, have compared wild bee exposure levels (however, see Hladik et al., 2016 and Siviter et al., 2023). To our knowledge, this is the first study to compare exposure and risk across several managed and wild bee species, although previous studies have looked at other matrices such as bee collected pollen and nectar (Knapp et al., 2023; Zioga et al., 2023). We hypothesize this level of risk is not unique to this system and suggest more comparative studies are needed to better understand how risk to different bees varies across systems and landscapes.

Two previous studies have found that pesticide risk to solitary bees and bumblebees is greater than risk to honeybees during crop pollination (Rundlöf et al., 2015; Woodcock et al., 2017). On the surface, these results seem contrary to our findings. However, there are two major differences between those studies and ours that may account for this discrepancy. First, the route of exposure to high-risk neonicotinoids is likely different. Rundlöf et al. (2015) and Woodcock et al. (2017) focussed on risk from neonicotinoid-treated oilseed rape fields in Europe. Oilseed rape is a pollinator-attractive crop and the neonicotinoid residues they found in hives/nests in their studies corresponded to residues in pollen and nectar sampled directly from the focal crop, suggesting exposure from the focal crop. In our study, the uniquely high residues of thiamethoxam in honeybee workers did not correspond to residues in the focal crop or weedy flowers at the orchards. Thus, exposure likely came from outside of the orchard in our study, making it more likely that far-foraging honeybees would

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be exposed while near-foraging solitary bees could avoid exposure. Second, Rundlöf et al. (2015) and Woodcock et al. (2017) monitored bee health metrics, whereas here we estimate risk using the Hazard Quotient and do not quantify direct health impacts. Honeybee colonies possess multiple social detoxification strategies that buffer them from the impacts of pesticide exposure while in solitary bees, exposure occurs directly to the reproductive females and offspring (Straub et al., 2015), potentially increasing the likelihood of negative effects on solitary bees compared to honeybees at a similar level of exposure.

LD_{50} values used in this study were adjusted from toxicity experiments performed on honeybees and without a full factorial experiment of every pesticide on every bee species it is difficult to understand to what degree different bee species are impacted by each pesticide. To account for the difference in toxicity across bees we analysed data using both unadjusted LD_{50}s, which assumes that all bees have the same LD_{50} (μg pesticide/bee) as well as a novel LD_{50} adjustment based on the previous findings that not all bees have a consistent LD_{50} (Medrzycki et al., 2013; Pamminger, 2021). This novel adjustment represents our best attempts to extrapolate toxicity values to the bees in our study for which no data exists. This adjustment decreased the RQ of bees heavier than 100 mg (the assumed weight of a honeybee in LD_{50} studies) while increasing the RQ of smaller bees. It should be noted though, that the regression used to adjust toxicity is built on limited available data and does not include any of the bees used in our study except *A. mellifera*. While the regression-based adjustment does a better job fitting to available toxicity data (Figure S2), it still makes the assumption that toxicity and weight scale consistently across all bee species. It is likely that different bee groups scale differently, possibly in non-linear ways, due to differences in physiological traits.

In our study we used a common method of calculating risk based on pesticide exposure and toxicity using LD_{50} values (i.e. the Hazard Quotient). Here, we refer to the Hazard Quotient as a Risk Quotient because the residues were found in bees, not other matrices such as pollen or nectar where additional information regarding intake rates are necessary to accurately predict risk, as in the EPA's BeeRex model. However, there remain some limitations to our method of assessing risk in bees using RQ. First, LD_{50} values come from studies that test single compounds and are therefore oversimplified given that real-world exposure is almost always composed of numerous pesticides simultaneously (e.g. Figures 1 and 2). The RQ formula assumes an additive effect of each pesticide, but synergistic interactions among pesticides are known (e.g. Tosi & Nieh, 2019), which could lead to an underestimate of risk. Second, sublethal effects of pesticides are ignored by RQ because it uses LD_{50} as its metric of toxicity. Numerous studies have shown that pesticide exposure can cause sublethal effects in bees, impairing motor control (Tosi & Nieh, 2017) and memory and orientation (Fischer et al., 2014) which impact foraging abilities, reproduction, and survival (Tosi & Nieh, 2019), and in turn reduce bee fitness (Stuligross & Williams, 2021). Finally, it should be noted that this study is limited to pesticide residues in bees that were actively flying. Any bees exposed to lethal doses of pesticides would have died before sampling, inherently biasing the data set towards individuals that were capable of flying and leading to an underestimate of risk.

In summary, we show that different bee groups face widely different risk from pesticides during crop pollination. This is important since the western honeybee is currently a model species for assessing pesticide risk to bees, but here we show that honeybees do not accurately predict exposure and risk to other bee groups. Our results show that current risk assessment methods do not accurately protect pollinators and highlight the need for post-registration risk assessments to quantify the true risk bees face in the field from different pesticides. Such post-release monitoring programs are overseen by the Food and Drug Administration (FDA) for pharmaceutical products released in the United States, but an analogous program for pesticides is not currently present in the United States, Europe, or anywhere else in the world. Such post-registration monitoring of pesticide risk could have obvious benefits for non-target organisms and the environment. In addition, while best management practices might limit within-orchard exposure to bees during bloom, some risks, especially for far-forage bees such as honeybees, arise from outside of the orchard. This highlights that pesticide exposure is a landscape-scale problem. Therefore, landscape-scale communication and/or regulation of pesticide applications is necessary to decrease harmful pesticide exposures to bees.

AUTHOR CONTRIBUTIONS

Tobias G. Mueller analysed the data and wrote the first draft of the manuscript. Nicolas Baert and David E. Sossa helped with bee sampling and performed pesticide analyses. Paige A. Muñiz oversaw bee sampling and provided taxonomic identifications. Bryan N. Danforth helped conceive and design the study. Scott H. McArt conceived the idea for the project, designed the study, and helped with bee sampling. All authors contributed to editing the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

Data and code are available from the Zenodo Digital Repository https://doi.org/10.5281/zenodo.10835332 (Mueller et al., 2024).

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SUPPORTING INFORMATION

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Appendix S1. Pesticide analysis.

Appendix S2. Risk quotient adjustment for species-level differences in bee mass.

Figure S1. The location of apple orchards sampled in the study within New York State.

Figure S2. The correlation between bee weight and averaged LD_{50} across different bee species (see methods section for details).

Figure S3. NMDS ($k=3$, stress = 0.15) plotting the Bray–Curtis dissimilarity of the pesticide composition found in each bee group.

Figure S4. *Unadjusted* oral (in red) and contact (in blue) Hazard Quotient values for pesticide residues found in different bee groups.

Figure S5. Thiamethoxam residue in *A. mellifera* versus apple flower and dandelion flowers at the same orchard. Lines show fitted linear models.

Table S1. *Andrena* species present in New York apple orchards.

Table S2. Retention times and optimized SRM acquisition parameters for HPLC-MS/MS analysis of pesticides (RT, Retention time; CE, Collision Energy).

Table S3. SIMPER analysis showing how each pesticide contributed to Bray–Curtis dissimilarity across all bee groups' pesticide exposure.

Table S4. Pairwise PERMANOVA assessing dissimilarity in composition of pesticide residues between paired bee groups.

Table S5. Pairwise SIMPER analysis showing how each pesticide contributed to Bray–Curtis dissimilarity between sample groups' pesticide exposure.

Table S6. Percent of samples within each bee group that exceeded either the European Food Safety Authority (EFSA) 10-day chronic oral exposure threshold (exposure/toxicity = 3% LD_{50}), the EFSA acute oral and contact exposure threshold (exposure/toxicity = 20% LD_{50}), or the Environmental Protection Agency (EPA) acute oral and contact exposure threshold (Tier 1 risk quotient = 40% LD_{50}).

Table S7. Pairwise PERMANOVA assessing dissimilarity in contact RQ between paired bee groups.

Table S8. SIMPER analysis showing how each pesticide contributed to Bray–Curtis dissimilarity across all bee groups' RQ.

Table S9. Pairwise SIMPER analysis showing how each pesticide contributed to Bray–Curtis dissimilarity between bee groups' RQ.

Table S10. PERMANOVA assessing dissimilarity in unadjusted RQ across bee groups. Unadjusted data assumes that all bees have the same LD_{50} (ug/bee).

Table S11. Pairwise PERMANOVA assessing dissimilarity in unadjusted RQ between paired bee groups.

Table S12. SIMPER analysis showing how each pesticide contributed to Bray–Curtis dissimilarity across all bee groups' unadjusted RQ.

Table S13. Pairwise SIMPER analysis showing how each pesticide contributed to Bray–Curtis dissimilarity between bee groups' unadjusted RQ.

Table S14. Pairwise PERMANOVA assessing dissimilarity in composition of pesticide residues between all paired sample groups (bees and flowers).

Table S15. SIMPER analysis showing how each pesticide contributed to Bray–Curtis dissimilarity across all sample groups' exposure (bees and flowers).

Table S16. Results of pairwise SIMPER analysis showing how each pesticide contributed to Bray–Curtis dissimilarity between all sample groups' exposure (bees and flowers).

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