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Short Communication

Beyond neonicotinoids – Wild pollinators are exposed to a range of pesticides while foraging in agroecosystems

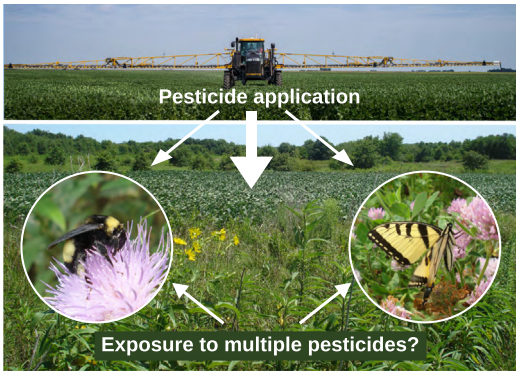
Anson R. Main [a,*,1], Michelle L. Hladik [b], Elisabeth B. Webb [c,a], Keith W. Goyne [a,2], Doreen Mengel [d]

[a] School of Natural Resources, University of Missouri, Columbia, MO, USA
[b] U.S. Geological Survey, California Water Science Center, Sacramento, CA, USA
[c] U.S. Geological Survey, Missouri Cooperative Research Unit, Columbia, MO, USA
[d] Missouri Department of Conservation, Resource Science Division, Columbia, MO, USA

HIGHLIGHTS

- Analyzed wild bees and butterflies for 168 pesticides and degradation products
- 16 pesticides and degradation products were detected in pollinator tissues.
- Bumblebee queens contained fungicides, herbicides, and insecticide degradates.
- Wild pollinators are exposed to a range of pesticides beyond neonicotinoids.

GRAPHICAL ABSTRACT



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ABSTRACT

Pesticide exposure is a growing global concern for pollinator conservation. While most current pesticide studies have specifically focused on the impacts of neonicotinoid insecticides toward honeybees and some native bee species, wild pollinators may be exposed to a broader range of agrochemicals. In 2016 and 2017 we collected a total of 637 wild bees and butterflies from the margins of cultivated agricultural fields situated on five Conservation Areas in mid-northern Missouri. Pollinators were composited by individual genera (90 samples) and whole tissues were then analyzed for the presence of 168 pesticides and degradation products. At least one pesticide was detected (% frequency) in the following wild bee genera: *Bombus* (96%), *Eucera* (75%), *Melissodes* (73%), *Ptilothrix* (50%), *Xylocopa* (50%), and *Megachile* (17%). Similarly, at least one pesticide was detected in the following lepidopteran genera: *Hemaris* (100%), *Hylephila* (75%), *Danaus* (60%), and *Colias* (50%). Active ingredients detected in >2% of overall pollinator samples were as follows: metolachlor (24%), tebuconazole (22%), atrazine (18%), imidacloprid desnitro (13%), bifenthrin (9%), flumetralin (9%), p, p′-DDD (6%), tebupirimfos (4%), fludioxonil (4%), flutriafol (3%), cyproconazole (2%), and oxadiazon (2%). Concentrations of individual pesticides ranged from 2 to 174 ng/g. Results of this pilot field study indicate that wild pollinators are exposed to and are potentially bioaccumulating a wide variety of pesticides in addition to neonicotinoids. Here, we provide evidence that wild bee and butterfly genera may face exposure to a wide range of insecticides, fungicides, and herbicides despite being collected from areas managed for conservation. Therefore, even with the presence of extensive habitat, minimal agricultural activity on Conservation Areas may expose pollinators to a range of pesticides.

* Corresponding author at: Dept. of Pesticide Regulation, 1001 I Street, P.O. Box 4015, Sacramento, CA 95812, USA.
E-mail address: Anson.Main@cdpr.ca.gov (A.R. Main).
[1]Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA, 95812
[2]Department of Forest Resources and Environmental Conservation, Virginia Tech, Blacksburg, VA, USA

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1. Introduction

Insect pollinators provide invaluable ecosystem services through maintenance of global plant diversity and pollination of food and fiber crops (Klein et al., 2007). Although farmers rely on insect pollination via domesticated honey bees, wild pollinators similarly perform ecosystem services that contribute billions of dollars to farmers across the globe with an average of $3000 per hectare in the production of insect-pollinated crops (Kleijn et al., 2015). Moreover, many wild bees and butterflies may be more effective at pollen transfer for specific crops (e.g., pumpkins, cherries) than their domestic counterparts (Artz and Nault, 2011; Holzschuh et al., 2012). However, wild pollinators are in decline. Landscape analyses have demonstrated that pollinator populations are under continuous stress through habitat loss to agricultural production, climate change, reduction of floral resource diversity, and the use of pesticides (Klein et al., 2007; Potts et al., 2016). As some wild pollinators are limited by flight distance (e.g., small bees; Greenleaf et al., 2007), several of the aforementioned factors may lead to a decrease in pollinator abundance and richness with consequences for maintenance of valuable ecosystem services.

Over the last decade researchers have more closely studied the impacts of pesticide use on global pollinator communities (Blacquière et al., 2012; Woodcock et al., 2017). While much of the literature has focused on pesticide impacts to honey bees specifically (Johnson et al., 2010; Woodcock et al., 2017), other studies have analyzed the effects that agrochemicals may have on wild bees and butterflies (Arena and Sgolastra, 2014; Muratet and Fontaine, 2015). Although insecticides are likely the most directly toxic to wild pollinators (Mulé et al., 2017; Scott-Dupree et al., 2009), herbicides and fungicides may interact with insecticides (e.g., additive, synergistic effects) or lead to indirect impacts (e.g., removal of habitat or loss of flowering plants by herbicides) on pollinators (Biddinger et al., 2013; Russell and Schultz, 2010). Pesticides are applied to agricultural crops and urban landscapes through a variety of ways including foliar sprays, seed treatments, soil applications (e.g., drenches), and chemigation (Hladik et al., 2018; Jeschke et al., 2011), which could influence the level of contact that pollinators may have with pesticide residues. Additionally, pesticide routes of exposure may differ depending on pollinator life history and include interactions with soil, floral resources (e.g., pollen, flowers) or contact with leafy material (Kopit and Pitts-Singer, 2018).

In the agricultural environment, one class of insecticides, the neonicotinoids, have received increased scrutiny for their association with global honey bee declines and sublethal effects on wild pollinators, including reduced wild bee density, reproductive success, and species richness (Main et al., 2020; Rundlöf et al., 2015; Woodcock et al., 2017). Currently, neonicotinoids are the most widely used insecticide in the world and one active ingredient, imidacloprid, is registered for use on >140 crops in 120 countries (Jeschke et al., 2011). Because they are applied to mass flowering crops such as canola, almonds, soybeans, cotton, and cucumbers, neonicotinoids may pose an increased risk to wild pollinator communities (Rundlöf et al., 2015). Due to their high water solubility and persistence, neonicotinoids may also move into adjacent margin habitat or be detected in various environmental media years after first application (Hladik et al., 2018). However, neonicotinoids are not the only pesticide used in the agricultural environment and depending on the region or crop, wild pollinators may be exposed to a suite of agrochemicals with differing toxicities and modes of action (Arena and Sgolastra, 2014; Hladik et al., 2016). This may be especially true for migrant species (e.g., monarch butterflies – *Danaus plexippus*; (Olaya-Arenas and Kaplan, 2019)) or wild bees that forage across a greater landscape area (e.g., bumblebees, *Bombus* spp. (Thompson, 2001)). Nevertheless, questions remain as to how smaller species that forage in more localized settings or travel less distances may be impacted by pesticide use and whether certain pollinator species are exposed to a wider range of agrochemicals.

Although many studies have evaluated the direct or indirect impacts of agricultural pesticides on wild pollinators, few studies have evaluated the level to which wild bees and butterflies are exposed to current-use pesticides (Hladik et al., 2016) or have focused only on a small suite of chemicals (e.g., neonicotinoids; Botías et al., 2017; Longing et al., 2020). To better capture total pesticide exposure to pollinators, in 2016 and 2017, we conducted a small-scale study to evaluate pesticide concentrations in field-collected wild bees, butterflies (incl. moths and caterpillars), and queen bumblebees. The study was conducted across several public lands managed for conservation that include a matrix of forested areas, agricultural crops, and herbaceous zones. These lands are managed with a focus on providing diverse habitat for wildlife. As such, we hypothesized that local pollinators are largely buffered from pesticides. However, due to their large foraging area, we further hypothesized that bumblebee queens would likely be more exposed to a range of pesticides compared to smaller bees. Determining the extent to which wild pollinators are exposed to a variety of pesticides will help inform land manager decisions concerning conservation efforts that may mitigate effects of pesticide use on local wild pollinator communities.

2. Methods

2.1. Study area

A pilot study to evaluate exposure of wild pollinators to pesticides was conducted in 2016 and 2017 across five Conservation Areas (CAs) situated in the central-northern portion of Missouri. Missouri is an agriculturally productive state and is a major grower of soybeans (*Glycine* max) and corn (maize; *Zea mays*) in the United States. Typically, Missouri experiences hot and humid summers (mean maximum, July = 31 °C) and mild to cold winters (mean low, January = −1 °C) with most precipitation falling as rain showers or thunderstorms. Missouri CAs are public lands that also include areas designated for production agriculture to provide habitat and further grow row crops (e.g., corn, soybeans, sunflowers) to feed a range of wildlife species. While smaller than conventional fields (e.g., <2 ha in size), many agricultural fields on CAs are rented to permittee farmers who actively cultivate crops using a range of conventional growing practices, including pesticide applications. This may include the use of seed treatments or foliar sprays. Typically, permittee farmers will request permission to apply pesticides to control weeds, fungus, or insect pests to yield a commercially viable crop. In contrast, CA land managers mainly use a limited number of herbicides to achieve conservation targets such as eradicating invasive species. Agricultural fields on CAs are often surrounded by >60% native habitat that is a mix of herbaceous and woody material. All CAs are actively managed by the Missouri Department of Conservation, with study CA locations and field descriptions described in Main et al. (2020). This study was part of a larger multi-year project that included a monitoring study in 2016 (Main et al., 2020) and an experimental evaluation of potential impacts of neonicotinoid seed treatments on wild bee communities that occurred during 2017 to 2018. In 2016, study fields were planted to corn, soybean, or left as untreated hayfields/grasslands. Corn and soybeans were sown using clothianidin-treated (Poncho® Bayer CropScience) or imidacloprid-treated seeds (Gaucho® Bayer CropScience). In 2017 and 2018, one third of all study fields were sown to soybeans treated with imidacloprid as above. Seed was ordered from a registered seed dealer and was treated before purchase.

2.2. Wild pollinator collection and identification

Wild pollinators, including native bees and butterflies, were collected from field margins adjacent to cultivated study fields. For this study, most bees and butterflies were caught during June and July as this time-period coincided with peak pollinator emergence and pesticide application (e.g., planted seed treatments, foliar sprays). We used

SpringStar™ blue vane traps (BVTs; SpringStar Inc., Woodinville, WA) to capture wild bees at each of the five CAs. A wide variety of bee species can be caught using BVTs and these traps have been used effectively in other monitoring studies (Hladik et al., 2016). At each field, we installed six traps throughout the field margin with ~30 m between traps. Traps were positioned along the same margin and placed at a vegetation height sufficient to insure visibility by foraging bees. All BVTs were deployed at approximately 0700 h Central Standard Time and left open for a 24 h period. Traps were dry and contained no euthanizing agent of any kind to eliminate potential exposure of bee tissues to a solvent. A detailed description of the BVT installation can be found in Main et al. (2020). Wild bees were collected in both 2016 and 2017.

To capture butterflies, two researchers walked a ~200 m length of the field margin and actively netted butterflies for 30 min intervals at each site. Sweep-net samples were initiated by 0800 h and completed by no later than 1330 h. This may have limited butterfly capture, but as butterflies were not the focus of our larger study, this was a more targeted sampling effort. Researchers used 38 cm diameter polyester mesh butterfly nets (BioQuip 7300 Professional Series) to capture butterflies that were within their immediate field of view (~ 2 m). Any butterflies that were collected were immediately placed in jars containing crumbled paper towel and a 2.54 cm layer of plaster on the bottom, which was wetted with several drops of ethyl acetate. In addition, observers walked the field margins to collect available caterpillars by turning over leaves of forage plants (e.g., common milkweed, *Asclepias syriaca*). However, few caterpillars were collected during this study. Caterpillars and butterflies were only collected during 2016.

All bees and butterflies were placed in individual 50 mL Falcon™ centrifuge tubes and stored in coolers containing ice packs while transported back to the laboratory. Centrifuge tubes were frozen at −20 °C until identification. Bee specimens were identified to species using the Discover Life key (Ascher and Pickering, 2016) and butterflies were identified using the Kaufman Field Guide to Butterflies of North America (Brock and Kaufman, 2006).

2.3. Sample preparation

In 2016, there were 101 wild bees (incl. 16 bumblebee queens), 7 caterpillars, and 62 butterflies and moths collected across four CAs. The following year (2017), we collected an additional 467 wild bees (incl. 16 bumblebee queens) across the same four CAs with the addition of one new CA. The substantial increase in bee numbers in 2017 was due to compositing more bees for analysis compared to the majority of pollinators collected in 2016 being analyzed individually. In each bee sample, approximately three to seven individuals per CA field were composited by species for most bees. Composite numbers depended largely on the size of the specimen, with larger bees (e.g., *Eucera hamata*) requiring fewer individuals (~5–7) to be composited compared to smaller bees (e.g., *Halictus ligatus*; sweat bees) which required up to 40 individuals. Similarly, larger butterflies (e.g., Monarchs) required single individuals for analyses compared to smaller species such as the eastern tailed-blue (*Cupido comyntas*) that required ~12 individuals. As bumblebee queens are considerably larger, individual queens representing five species were analyzed individually in 2016 ($n = 16$), but composited in 2017 ($n = 8$). All tissue samples ($n = 90$) were analyzed for the presence of 168 pesticides and degradation products in both years (Hladik and Main, 2020). Any pollen that was located on the corbicula basket or leg/abdominal scopa of an individual bee was gently removed from the bee prior to sample preparation, frozen, and saved for future analysis. Bees or butterflies were placed in a clean, methanol-rinsed mortar and pestle. Liquid nitrogen was added to the mortar and the composite was formed by grinding the pollinators into a fine powder. Composites, therefore, are whole tissue samples that may include residues on both the external and internal parts of the bees or butterflies. The resultant powder was transferred to a Fisherbrand™ 20 mL HDPE scintillation vial and stored in the freezer for ~6 months before being shipped to the USGS California Water Science Center for chemical residue analysis. A minimum of 0.1 g of mass was required for pesticide analysis; sample mass ranged from 0.10 to 3.49 g with an average mean (± standard deviation) of 0.70 (± 0.61) g.

2.4. Sample extraction and quality control

The samples were extracted according to previously described methods (Hladik et al., 2016). The samples were homogenized with sodium sulfate (Na_2SO_4) and were spiked with $^{13}C_{12}$-*p,p′*-DDE, $^{13}C_4$ fipronil, d_4-imidacloprid, $^{13}C_6$-*cis* permethrin, and d_{10}-trifluralin (Cambridge Isotope, Cambridge MA) as recovery surrogates and extracted with 50:50 acetone: dichloromethane (DCM) using a Dionex 200 accelerated solvent extractor (ASE) at 1500 psi and 100 °C. Following extraction, co-extracted matrix interferences were removed with 1 g C18 (Bondesil C18 40 μm; Agilent Technologies, Santa Clara, CA) and 0.5 g *Z*-sep + (Sigma-Aldrich, St. Louis, MO) sorbents. The eluent was reduced to 1 mL and split into a gas chromatograph (GC) fraction and a liquid chromatograph (LC) fraction. The GC fraction was exchanged into ethyl acetate (final volume 100 μL; d_{10}-acenaphthene as internal standard) and the LC fraction was exchanged into acetonitrile (final volume 100 μL; $^{13}C_3$-caffeine as internal standard).

Samples were analyzed for a total of 168 pesticides and pesticide degradation products using either GC triple quadrupole mass spectrometry (GC–MS/MS; Agilent 7890 GC coupled to an Agilent 7000 MS/MS operating electron ionization (EI) mode) or LC-MS/MS (Agilent 1260 bio-inert LC coupled to an Agilent 6430 MS/MS) (Hladik and Main, 2020; Hladik et al., 2016). Data for all pesticides were collected in multiple reaction monitoring (MRM) mode with each compound having one quantifier MRM and at least one qualifier MRMs. The limits of detection (LOD), defined as the value greater than three times the signal-to-noise ratio, were 2 to 5 ng/g for a 0.5 g sample (Hladik and Main, 2020).

Mean (± standard deviation) recoveries of $^{13}C_{12}$-*p,p′*-DDE, $^{13}C_4$ _fipronil, d_4-imidacloprid, $^{13}C_6$-*cis*-permethrin, and d_{10}-trifluralin were 84 ± 8%, 103 ± 15%, 80 ± 6%, 95 ± 14%, and 99 ± 14%, respectively. No recovery corrections were made to any of the pesticide concentrations. Procedural blanks consisting of 1 g of baked Na_2SO_4 run with every batch of 18 samples did not contain detectable concentrations of pesticides. Due to the limited sample mass, environmental replicates and matrix spikes of tissue samples were not run; three matrix spikes of previously collected honeybee tissue (with no background pesticide levels) were analyzed and the recovery of the target compounds was in the acceptable range of 70% to 130%.

2.5. Summary statistics and potential risk to bees

As this was a pilot study, all pollinators were opportunistically collected in 2016 and 2017. Due to an unbalanced, small sample size between pollinator groups and years, data are presented only as summary statistics. Non-detections were set at ½ of the LOD.

Pesticide concentrations measured in pollinator tissues were also compared against published acute toxicity values, expressed as median lethal dose (LD_{50}), for native bees. Because our analytical methods could not differentiate between oral and contact exposure to pesticides, the most conservative (lowest) contact LD_{50} value was chosen. Concentrations for species nesting belowground were compared against *Nomia melanderi* LD_{50} values and species that nest aboveground were compared against acute values for *Megachile rotundata*. If native bee toxicity values did not exist, a contact toxicity value for the managed honeybee (*Apis mellifera*) was used as a surrogate. Despite the potential for additive or synergistic toxicity, pesticide concentrations were evaluated individually for each pesticide. In this study, we calculated the pesticide

concentration of ng/bee using the following calculation: $\frac{concentration\ (ng/g)\ x\ bee\ mass\ (g)}{numberofbees} = ng/bee.$

3. Results

3.1. Pesticide concentrations in wild bees

Due to the small individual and composite sample sizes analyzed in both years, data are presented as overall detections and concentrations for the entire pilot study (complete tables of the pesticides analyzed and pesticides detected can be accessed elsewhere; Hladik and Main, 2020). Among the combined 43 wild bee samples analyzed (excluding bumblebee queens), there were nine pesticides detected with at least one representative active ingredient from the evaluated insecticides, fungicides, herbicides and a plant growth regulator. All compounds were detected in >2% of samples (from greatest to least): metolachlor (19%), flumetralin (16%), atrazine (14%), tebuconazole (12%), tebupirimfos (9.3%), bifenthrin (5%), fludioxonil (5%), oxadiazon (2%), and imidacloprid (2%; Fig. 1). There were differences between years with fungicide concentrations in wild bees greater in 2016 and herbicide concentrations greater in 2017 (Fig. 2). Tebuconazole and imidacloprid were only detected in 2016 and tebupirimfos, fludioxonil, and oxadiazon were only detected in 2017. The greatest percentage of tissue samples analyzed contained herbicides (23%), followed by fungicides (16%) and insecticides (14%). Most bees tested contained only one pesticide (79%); however, 16% of bees contained two pesticides and 5% contained three different pesticides. Residues were most frequently detected in long-horned bees (73%; genus: *Melissodes*) and least frequently detected in sweat bees (13%, genus: *Halictus*; Fig. 1). No active ingredients were detected in composite bee samples from the genera

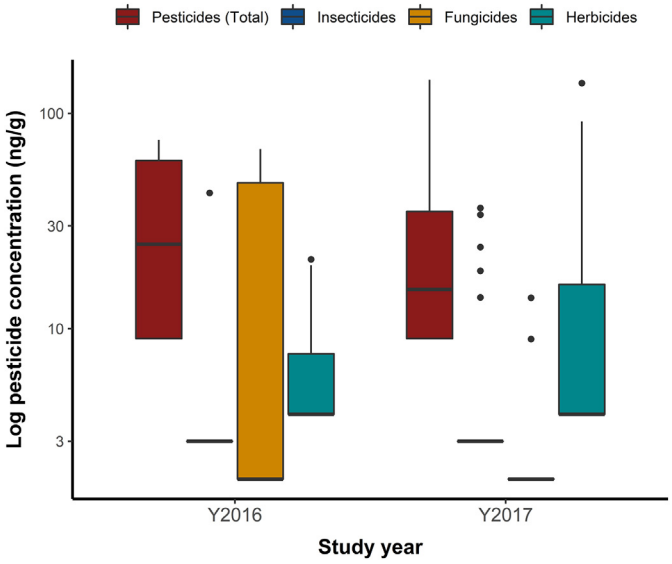

Fig. 2. Boxplot comparison of overall insecticide, fungicide, herbicide, and summed (total pesticide) concentrations in wild bee tissues collected from Conservation Areas in Missouri during 2016 ($n = 13$) and 2017 ($n = 30$). Dark horizontal lines are median values and filled circles are outliers.

Agapostemon, *Augochlora*, *Ceratina*, *Lasioglossum*, *Osmia*, or *Svastra*, but each was represented by <2 samples. The greatest maximum insecticide concentration was the organophosphate tebupirimfos detected in tissues of leaf-cutter bees (genus: *Megachile*) at 34.4 ng/g (Table 1). The fungicide tebuconazole was detected in three different bee genera,

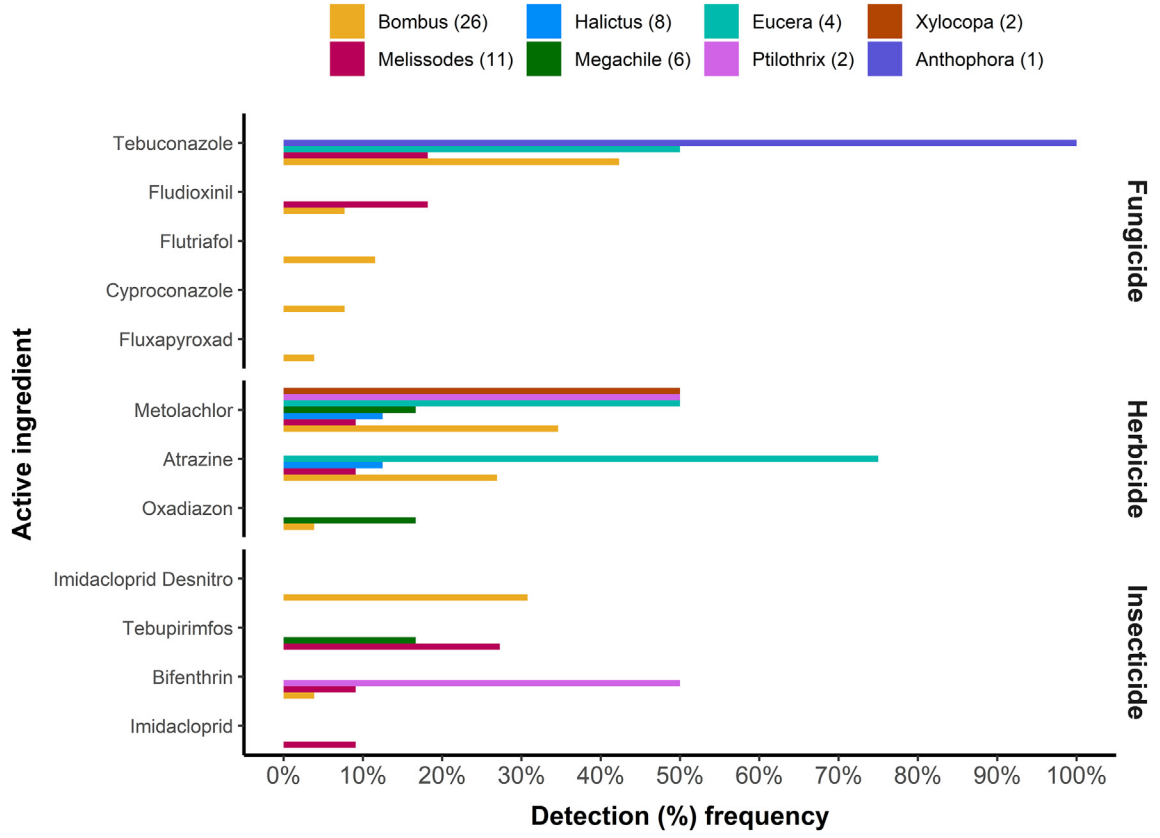

Fig. 1. Detection frequency of fungicides, herbicides, and insecticides in tissues from 8 genera of wild bees (*Bombus, Halicutus, Eucera, Xylocopa, Melissodes, Megachile, Ptilothrix, Anthophora*) collected from Conservation Areas in Missouri during 2016 and 2017 (combined). All concentrations presented were detected in >2% of wild bees. Numbers in parentheses represent sample sizes by individual genera.

Table 1
Pesticides detected in tissue composites of whole wild bees from 5 genera: *Bombus, Melissodes, Halictus, Megachile, Eucera*. Data presented include the mean (± SE) and maximum concentration of detections above the limit of detection (LOD). Only pesticides with >2% detections are presented. ND = not detected and "–" is not calculated.

Pesticide type	Pesticide concentrations (ng/g)									
	Bombus (n = 26)*		Melissodes (n = 11)		Halictus (n = 8)		Megachile (n = 6)		Eucera (n = 4)	
Active ingredient	Mean (± SE)	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
Insecticide										
Bifenthrin	–	3.91	–	19.2	ND	ND	ND	ND	ND	ND
Imidacloprid	ND	ND	–	22.5	ND	ND	ND	ND	ND	ND
Imidacloprid Desnitro	4.94 ± 2.72	72.1	ND	ND	ND	ND	ND	ND	ND	ND
Tebupirimfos	ND	ND	7.12 ± 3.33	31.8	ND	ND	–	34.4	ND	ND
Fungicide										
Cyproconazole	1.63 ± 0.46	12.1	ND	ND	ND	ND	ND	ND	ND	ND
Fludioxonil	1.55 ± 0.42	11.5	2.71 ± 1.20	12.9	ND	ND	ND	ND	ND	ND
Flutriafol	6.40 ± 3.06	58.3	ND	ND	ND	ND	ND	ND	ND	ND
Fluxapyroxad	–	8.37	ND	ND	ND	ND	ND	ND	ND	ND
Tebuconazole	14.5 ± 4.05	69.3	11.7 ± 7.23	67.3	ND	ND	ND	ND	21.9 ± 12.4	49.4
Herbicide										
Atrazine	2.55 ± 0.90	23.2	–	4.63	–	12.5	ND	ND	3.57 ± 1.23	6.80
Metolachlor	4.42 ± 1.60	39.2	–	4.35	–	5.13	–	11.4	5.84 ± 2.86	12.2
Oxadiazon	–	2.75	ND	ND	ND	ND	–	11.6	ND	ND
Plant growth regulator										
Flumetralin	–	1.27	–	1.65	30.1 ± 16.9	135.2	ND	ND	ND	ND

* includes bumblebee queens.

Bombus, *Melissodes*, and *Eucera* at maximum concentrations of 69.3, 67.3, and 49.4 ng/g, respectively. Flumetralin, a plant growth regulator, was measured at 135.2 ng/g in one composite sample of Halictus (Table 1). All concentrations in wild bees were <LD_{50} values for individual pesticides.

3.2. Pesticide concentrations in butterflies, moths, and caterpillars

We analyzed 23 samples for the presence of pesticides: butterfly (16), moth (2), and caterpillar (5). The majority of samples contained herbicides (35%), followed by insecticides (30%) and fungicides (17%). There were eight pesticides detected with at least one active ingredient from the insecticides, fungicides, and herbicides evaluated. Metolachlor was the most frequently detected pesticide (26%) followed by bifenthrin (22%), p,p′-DDD (22%), imidacloprid desnitro (17%), atrazine (17%), tebuconazole (13%), azoxystrobin (4%) andp,p′-DDT (4%). Although the majority of collected butterfly, moth, and caterpillar samples tested contained no pesticides (44%), other samples contained between one (17%) and five (4%) pesticides. Maximum concentrations were greatest for metolachlor in the genus *Colias* (174.1 ng/g), tebuconazole in monarchs (genus: *Danaus*; 49.7 ng/g), and bifenthrin in the genus *Hylephilia* (32.8 ng/g; Table 2). The organochlorine insecticide p,p′-DDT and its metabolite p,p′-DDD were detected in tissues of small butterflies such as skippers (genus: *Hylephila*; p,p′-DDT = 17.9 ng/g) and large monarchs (p,p′-DDD = 5.4 ng/g). The total pesticide concentration or sum of all detected active ingredients was greater in butterfly/moth tissues compared to caterpillars (Fig. 3).

3.3. Exposure of bumblebee queens to pesticides

Approximately 71% of the 24 bumblebee queen samples collected during our study contained at least one pesticide active ingredient with most bee tissues containing a fungicide (54%) compared to herbicides (42%) or insecticides (33%). The most frequently detected pesticides were tebuconazole (46%), imidacloprid desnitro (33%), metalochlor (33%), atrazine (25%), flutriafol (13%), cyproconazole (8%), fludioxonil (8%), oxadiazon (4%) and bifenthrin (4%). Tebuconazole, imidacloprid desnitro, flutriafol, and cyproconazole

Table 2
Pesticides detected in composite tissues of whole wild butterfly (genera: *Danaus, Papilio, Hylephila, Colias*) and caterpillars. Data presented include the mean (± SE) and maximum concentration of detections above the limit of detection (LOD). Only pesticides with >2% detections are presented. ND = not detected and "–" is not calculated.

Pesticide type	Pesticide concentrations (ng/g)									
	Caterpillars (n = 5)		Danaus (n = 4)		Papilio (n = 4)		Hylephila (n = 3)		Colias (n = 3)	
Active ingredient	Mean (± SE)	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
Insecticide										
Bifenthrin	–	2.18	5.22 ± 2.65	11.98	ND	ND	16.4 ± 9.20	32.8	ND	ND
Imidacloprid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Imidacloprid desnitro	ND	ND	2.57 ± 0.93	4.61	ND	ND	–	24.4	–	16.0
p.p.′-DDD	ND	ND	–	5.42	–	3.12	19.7 ± 3.31	25.1	ND	ND
p.p.′-DDT	ND	ND	ND	ND	ND	ND	16.4 ± 9.20	17.9	ND	ND
Fungicide										
Azoxystrobin	ND	19.1	ND	ND	ND	ND	ND	ND	ND	ND
Tebuconazole	–	47.9	–	49.72	–	49.67	ND	ND	ND	ND
Herbicide										
Atrazine	–	12.9	ND	ND	ND	ND	–	19.8	12.3 ± 5.96	21.3
Metolachlor	ND	ND	ND	ND	ND	ND	15.5 ± 3.00	21.4	–	174

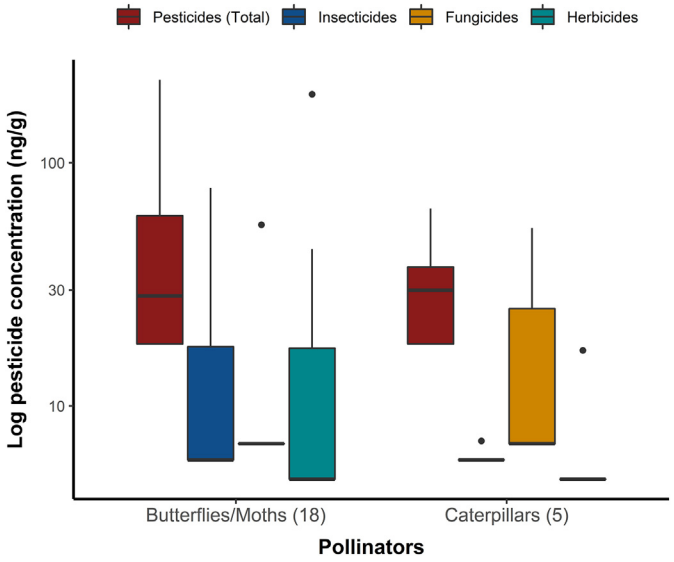

Fig. 3. Boxplot comparison of overall insecticide, fungicide, herbicide, and summed (total pesticide) concentrations in butterfly/moth and caterpillar tissues collected from Conservation Areas in Missouri during 2016. Numbers in parentheses represent sample sizes. Dark horizontal lines are median values and filled circles are outliers.

were only found in bumblebee queens collected in 2016 whereas bifenthrin, fludioxonil, and oxadiazon were only detected in 2017. Total pesticide concentrations were greatest in queens of the species *Bombus auricomus* and lowest in queens of the species *B. bimaculatus* (Fig. 4). Approximately 33% of bumblebee queens contained at least two active ingredients with 25% of samples containing >3 pesticides. Only two insecticides were measured in tissues of queens: the pyrethroid bifenthrin and a neonicotinoid metabolite, imidacloprid desnitro. Imidacloprid desnitro concentrations ranged from not-detected (ND) to a maximum of 72.1 ng/g in *B. griseocollis* (Table 1). Fungicide concentrations were greatest for tebuconazole (69.3 ng/g), flutriafol (58.3 ng/g) and cyproconazole (12.1 ng/g) whereas measured herbicide concentrations were greatest for metolachlor (17.9 ng/g) and atrazine (8.8 ng/g). All concentrations in bumblebee queens were $<LD_{50}$ values for individual pesticides.

4. Discussion

At present, much of the current literature evaluating pesticide impacts on wild pollinators has focused on neonicotinoid insecticides. Neonicotinoid insecticides are shown to negatively impact wild bees via changes in foraging behavior, reductions in reproductive success, and losses in wild bee density or species richness (Blacquière et al., 2012; Main et al., 2018; Rundlöf et al., 2015). However, although we hypothesized that wild pollinators collected on Conservation Areas would be buffered against most pesticides, our results demonstrate that wild bee communities may be exposed to a wide range of fungicides, herbicides, and insecticides beyond neonicotinoids.

Based on available LD_{50} values for wild bee species, only two of 16 pesticides measured in this study are considered "highly toxic" (e.g., LD_{50} < 2 μg/bee) to bees (e.g., bifenthrin, imidacloprid; Hooven et al., 2013). Generally, both fungicides and herbicides are considered relatively less toxic to bees than insecticides. Across both years, no individual pesticide concentrations surpassed the most conservative LD_{50} values for the native ground-nesting bee species *Nomia melanderi* (alkali bee) or the cavity-nesting species, *Megachile rotundata*. (alfalfa leaf-cutter bee). As all pollinators were collected alive in 2016 and 2017, it is unlikely that the concentrations measured within pollinator tissues would cause acute mortality. However, our data provide evidence of pesticide exposure (and potentially accumulation) in wild pollinators at concentrations that may result in sublethal effects or infer chronic exposure. In areas where crops may be rotated more frequently, it is important to note that pollinator exposure to a range of pesticides may vary between years depending on the commodity or annual pest pressure.

Insecticides, though less frequently detected in our samples, are likely to be the most concerning for wild bees and butterflies. Previous research has demonstrated that legacy insecticides, such as *p,p′*-DDT, are toxic to newly emergent bees, leaf-cutter bees (family: Megachilidae), and social bee genera such as *Apis* (Johansen, 1977). Only one long-horned bee sample (*Melissodes* spp.) contained the neonicotinoid imidacloprid at a concentration of 22.5 ng/g. Although the genus *Melissodes* is made up of solitary species, eusocial species such as *Bombus impatiens* are shown to have reduced queen survival and worker movement when chronically exposed to imidacloprid at 16 ng/g (Scholer and Krischik, 2014). By comparison, little to no data are available for both tebupirimfos (organophosphate) and the

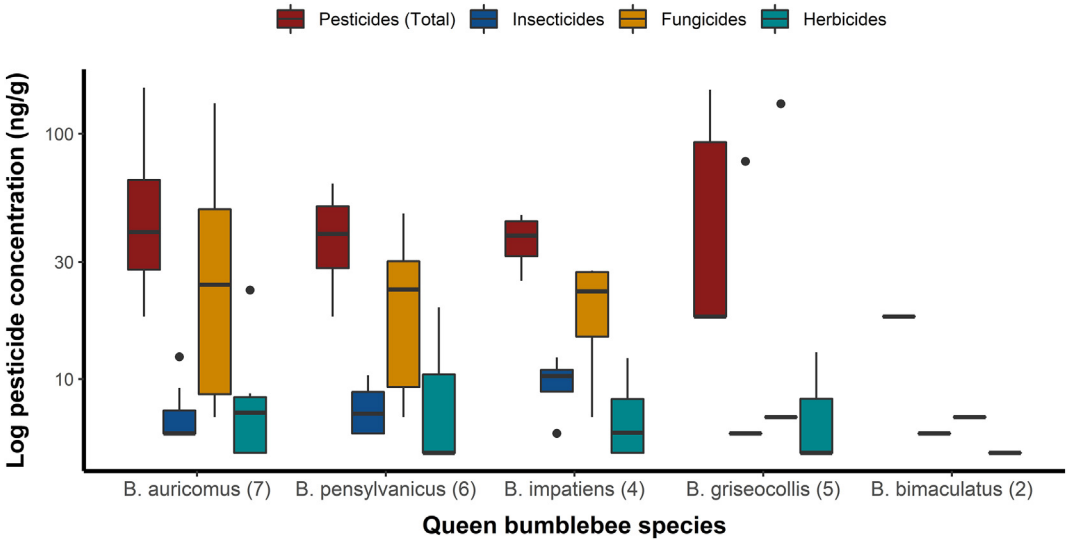

Fig. 4. Boxplot comparison of overall insecticide, fungicide, herbicide, and summed (total pesticide) concentrations in tissues of Queen bumblebees (genus: *Bombus*) collected from Conservation Areas in Missouri during 2016 and 2017 (combined). Numbers in parentheses represent sample sizes by individual species. Dark horizontal lines are median values and filled circles are outliers.

imidacloprid metabolite, desnitro (neonicotinoid) regarding toxic effects on bees or butterflies.

The synthetic pyrethroid bifenthrin, which was frequently detected in both bees and butterflies, is highly toxic to pollinators; the contact LD_{50} for wild bees ranges from 6 to 140 ng/bee (Mineau, 2014). In studies evaluating various pyrethroids, bifenthrin is shown to significantly reduce fecundity and development of honeybees in laboratory studies (Dai et al., 2010) and contact exposure for the bumblebee, *Bombus terrestris*, resulted in 100% mortality of bumblebee workers (Besard et al., 2010). Data on bifenthrin toxicity toward butterflies are absent in the literature. Experiments evaluating toxicity of the synthetic pyrethroid deltamethrin toward cabbage white butterflies (*Pieris brassicae*) reported sublethal effects (feeding inhibition and smaller pupae and adults) because of exposure (Çilgi and Jepson, 1995). Other pyrethroids, such as permethrin, can delay larval development in Painted Lady butterflies (*Vanessa cardui*; Peterson et al., 2019). It is important to note that deltamethrin is similarly toxic to *Megachile rotundata* while permethrin is more toxic than bifenthrin (contact LD_{50} 5 ng/bee and 18 ng/bee, respectively; Mineau, 2014). In our study, concentrations of bifenthrin in pollinator tissues ranged between 2.2 and 32.8 ng/g, but as we analyzed composited whole tissue samples, it is unclear as to whether pollinators were exposed via contact, ingestion, or both.

The majority of pollinators collected contained fungicides within their tissues, but fungicides are typically considered non-toxic to pollinators. Tebuconazole, for example, was widely detected in bees collected from corn and soybean fields in 2016, but not in 2017. The soybean seed planted in 2017 was protected with a different suite of fungicides (prothiconazole, penflufen, metalaxyl, and oxathiapiprolin). However, according to the University of California's "bee precaution" pesticide ratings, both tebuconazole and flutriafol are considered to potentially increase toxicity toward pollinators; tebuconazole may specifically be detrimental if it is mixed with pyrethroids, neonicotinoids, or diamides (e.g., chlorantraniliprole; University of California, 2020). In a study of acute toxicity toward parasitoid wasps (including mortality and loss of motor skills), a low dose of the neonicotinoid thiacloprid was found to be synergized by the addition of tebuconazole, with the effect increasing at higher doses (Willow et al., 2019). Similarly, oral toxicity testing of *Osmia cornuta* indicated that exposure to thiacloprid and the fungicide tebuconazole reduced normal food consumption by adult females (Vanderhaegen, 2016). As tebuconazole was mainly detected in tandem with imidacloprid desnitro, it is unclear as to whether any potential synergism was likely.

An important consideration is that many of the pollinators collected in our pilot study were of varying sizes or have differing life history traits that may make them potentially more (or less) susceptible to current-use pesticides. Indeed, some of the larger species collected in this study, such as bumblebee queens or carpenter bees (*Xylocopa virginica*), may fly several kilometers from their nest to forage. It is possible that due to CAs often being situated in locations surrounded by conventional cropland, larger bee or butterfly species may have been exposed to pesticides from privately-owned agricultural fields. Wild pollinators can be exposed to pesticides through a range of environmental media including pollen, contact with leaves/flowers, or soil (Kopit and Pitts-Singer, 2018). In our study, 75% of bees evaluated over both years were ground-nesting species. If pesticides are retained in soils, they may provide a route of chronic exposure to ground-nesting bees (Kopit and Pitts-Singer, 2018; Main et al., 2020); however, this remains relatively unexplored in the literature. Tebupirimfos, for example, was detected in >9% of all wild bee tissues collected and is considered to be persistent due to a soil half-life of ~300 days (PPDB, 2020). Seventy-five percent of bees containing tebupirimfos were ground-nesting species such as *Melissodes*. Tebupirimfos is used on corn crops and since residues were only measured in bees collected in 2017, there may have been a delayed response where bees were exposed the previous year. Similarly, imidacloprid desnitro is a major soil metabolite of imidacloprid and the majority of bumblebee queens containing this pesticide were species that nest in soil (e.g., *Bombus impatiens*, *B. auricomus*; Colla et al., 2011). As both tebupirimfos and imidacloprid are most-often applied as seed treatments or in-furrow applications (University of Nebraska, 2019), it is likely that ground-nesting species were more likely to interact with these pesticides. Interestingly, genera such as *Ceratina*, *Megachile*, and *Xylocopa* that nest above-ground in pith, stems, and wood mainly contained herbicides and fungicides which may reflect bees foraging on plants post-spray application (e.g., atrazine).

Pollinator exposure to pesticides may also be influenced by flight distance as smaller butterflies such as skippers (e.g., *Hylephila* spp.) and larger butterflies (e.g., Monarchs, Swallowtails) both contained pesticides. For smaller species, this could be a result of interacting with crop plants, different host preferences, or limited movement between fields; conversely, the long-distance migration of Monarchs may simply increase their exposure to pesticides. This was likely the case for the presence of p,p′-DDT and p,p′-DDD in Monarchs and Swallowtails that migrate through many states and countries. Although small bees may face a more concentrated exposure due to limited foraging ranges (Greenleaf et al., 2007), most of the wild bees containing pesticides in our study were medium to large species, which is consistent with results from Colorado, Texas, and the UK (Botías et al., 2017; Hladik et al., 2016; Longing et al., 2020). Due to a greater foraging range, it is likely that larger species may simply be exposed to a wider range of agrochemicals. Indeed, a number of bumblebee queens contained representative active ingredients from all groups of insecticides, fungicides, and herbicides.

5. Conclusion

Here, we evaluated exposure of different wild pollinator species to a suite of 168 pesticides and degradation products. Although limited by a small sample size and infrequent detections, we provide evidence that wild bee and butterfly genera may face exposure to a wide range of insecticides, fungicides, and herbicides despite being collected from areas managed for conservation. We must caution that we cannot say definitively that all bees were exposed to pesticides on Conservation Areas alone as many bees may forage beyond the extent of these managed landscapes to surrounding private fields. However, as solitary bees are shown to limit their foraging activity to within 600 m of established nests (Gathmann and Tscharntke, 2002), a high number of pollinators were likely exposed to pesticides on Conservation Areas. Much of the current research has focused on neonicotinoid insecticides; conversely, pollinator exposure to a range of other pesticides remains poorly understood. Importantly, these data indicate that exposure not only varies between bees and butterflies, but also among genera as well. It is possible that pesticide interactions may also increase concerns for regional pollinators. Future studies could consider evaluating a wider range of agrochemical impacts to pollinators rather than focusing exclusively on one chemical class.

CRediT authorship contribution statement

Anson R. Main: Conceptualization, Investigation, Writing - original draft, Visualization. **Michelle L. Hladik:** Conceptualization, Validation, Formal analysis, Resources, Writing - review & editing. **Elisabeth B. Webb:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Keith W. Goyne:** Writing - review & editing, Supervision, Funding acquisition. **Doreen Mengel:** Conceptualization, Writing - review & editing, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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