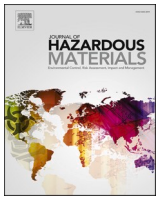
Journal of Hazardous Materials 480 (2024) 135910

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat



Pesticide exposure patterns in honey bees during migratory pollination

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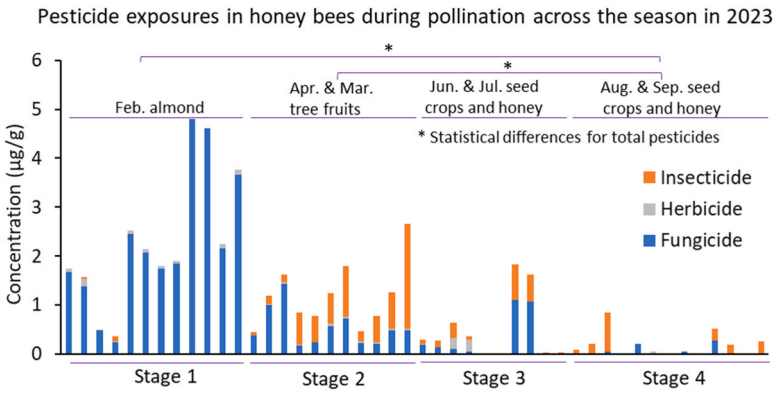
HIGHLIGHTS

- Pesticide exposures in honey bees exhibit a seasonal pattern in the northwestern U.S.
- The risk of pesticide exposures is most severe during spring fruit pollination.
- Insect growth regulators, a class of unregulated insecticides, threaten bee health.
- California policy and conservation efforts reduce insecticide exposures in pollinators.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Apis mellifera
Pollination
Pesticide
Toxicity
Exposure

ABSTRACT

Monitoring pesticide exposures in honey bees provides fundamental risk information that informs efforts to improve regulatory policy, pesticide use, and beekeeping management so pollinators are protected in realistic field conditions. We investigated pesticide exposures to bee colonies while colonies moved along commercial migratory routes in 2022 and 2023 to pollinate multiple pollinator-dependent, high-value U.S. specialty crops (e.g., almonds in California and apples and cherries in Washington). We found evident pesticide exposure patterns, including increasing exposures (both levels and number of pesticides) to fungicides during almond pollination, higher exposures to insecticides and persistent exposures to fungicides during springtime fruit pollination, and declining exposures in summer. Exposure risk assessment by risk quotient (RQ) model based on residues in bee bread indicates no concern of acute toxicity to adult honey bees during pollination, however, during colony inspections we found severe brood mortality in fields associated with high exposure to buprofezin, an insect growth regulator (IGR) thought to be safe for adult bees, which is permitted for use any time across the season. Our results suggest a need to improve compliance with insecticide label requirements during tree fruit pollination and a need for further research into the negative impacts of IGR on colony health especially on immature bees to inform potential policy changes.

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https://doi.org/10.1016/j.jhazmat.2024.135910
Received 12 July 2024; Received in revised form 17 September 2024; Accepted 18 September 2024
Available online 19 September 2024

1. Introduction

Pesticide exposure is one of the major causes of high honey bee colony loss in the U.S. and other countries which threatens the production of pollinator-dependent crops worldwide [1,2]. This comes despite long-term mandates that pesticidal products must be tested for LD_{50} toxicity (the chemical dosage that will kill 50 % of a laboratory population of adult honey bees in 96 h when applied to adult bee thoraces) before registration [3-5]. Pesticide exposure studies on stationary apiary locations or for short periods of time provide a valuable means to assess pesticide exposure risk placed on honey bees. However, honey bees are frequently moved between different crops for pollination across different regions and seasons [6–8] where pesticide exposures continuously occur based on the pesticides applied to each crop. A single colony can pollinate 4–6 different focal crops in a single season. In the U.S., 80% of colonies migrate between crops for pollination with a value over $15 billion every year [9]. A lack of pesticide exposure data collected during migratory pollination makes bee colonies vulnerable to potential pesticide risks and prevents beekeepers from adjusting their management practices accordingly. Large scale pesticide exposure surveys in honey bee colonies may cover different regions and reveal national or regional trends in pesticide exposures [10,11], however, the data are often low resolution and do not reveal detailed sampling time and locations. This prevents a greater understanding of pesticide exposures within specific crops which could allow for significant enhancements to pollinator protection.

In the U.S., the majority of migrating honey bee colonies (about two million colonies) start the season in California (CA) from January through March for almond pollination. Almonds are the single most valuable pollinator dependent crop in CA, and California-grown almonds account for 100% of U.S. almond production and 80% of worldwide almond production [4,12]. After almond bloom, these colonies are moved either throughout California or out to other states to pollinate tree fruits which bloom in late spring or early summer [6]. One destination includes Washington State (WA), a leading producer of tree fruits and small fruits (apple, sweet cherry, peach, blueberry, etc.). After spring fruit pollination, many of the tree fruit-pollinating colonies continue their migratory movement to pollinate seed crops in summer and fall within WA, the leading vegetable seed producer (e.g., carrot, onions, canola) in the U.S.; otherwise, they are moved to natural areas to produce honey. While research has examined pesticide exposures to honey bees during the pollination of large-scale nut and fruit crops in spring, there is limited information on pesticide exposures in seed-producing cropping systems. Of the studies that examined pesticide exposures in honey bees, one explicitly determined pesticide exposures in California almond orchards in March and found between 11 and 21 different pesticides in bee bread [13]. This study had two key limitations: a limited number of replication sites (i.e., one in each year of the study) were sampled, and pesticide exposures directly during the February almond bloom were not studied, but rather exposure data were collected after many heavy pesticide applications were completed. A pesticide survey from California, Maine, and Pennsylvania examined pesticide residues from freshly collected pollen (from pollen traps) in alfalfa, almonds, apples, blueberries, corn, and pumpkins and found 5, 15, 13, 4, 3, and 11 pesticides from each crop respectively [14]. This study had a key limitation that samples for residue analysis were collected when hives had spent only one day in each crop, potentially limiting the insecticides captured to those applied early in the crop's growing cycle. Another pesticide survey conducted in WA found that neonicotinoid exposures to honey bees would not cause lethal toxicity, since reported residues usually fell below the NOAEC (no observable adverse effect concentration) for agricultural landscapes and consistently fell below NOAEC for urban and rural landscapes (Lawrence et al., 2010), however, the risk from exposures to other pesticide classes were not examined.

The work presented here fills gaps in our knowledge of pesticide exposures and the associated risks to migratory honey bees in CA and WA, where several crop production systems of high economic importance rely on migratory beekeeping services. We tracked six groups of commercial colonies across two years (2022 and 2023). Each group of colonies moved between crop sites as a consistent unit. Bee bread (a product of stored pollen in comb) was collected for pesticide residue analysis using Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS) and Liquid chromatography–tandem mass spectrometry (LC–MS/MS) and for taxonomic identification using Internal Transcribed Spacer (ITS) amplicon sequencing. We discuss potential pesticide sources based on pollen taxonomy data as well as plant phenology and satellite-based land cover information. Monitoring pesticide exposures during hive migration provides fundamental information needed to assess exposure risk, which can enhance beekeeper and grower awareness of potential risk and preventative management activities, guide regulatory policy changes, and improve communication about the sustainability of the U.S. food system.

2. Methods and materials

2.1. Study sites, crops and pollination stages

A total of 32 study sites were identified by collaborating commercial beekeepers and our lab in California (CA) and Washington state (WA) based on the chronological pollination stages occurring in these two states in 2022 and 2023 (Table 1; refer to Table A.1 for more site information). Four pollination stages were defined according to a typical pollination schedule for commercial beekeepers, including almond pollination in CA in February (Stage 1 "S1"), tree and small fruit pollination in April and May in WA (Stage 2 "S2"), summer vegetable seed

Table 1
Experimental design with year, pollination stage, colony grouping and study sites.

Year	Group (colony number)	Crops (site number) pollinated by bee colonies in different stages[a]			
		Feb. (Stage 1)	Apr. & May (Stage 2)	Jun. & Jul. (Stage 3)	Aug. & Sep. (Stage 4)
2022	A (24)	Almond (1)	Blueberry (5)	Canola (9)	Buckwheat (13)
2022	B (24)	Almond (2)	Apple (6)	Onion (10)	Buckwheat (13)
2022	D (24)	Almond (3)	Apple (7)	Carrot (11)	Alfalfa (14)
2022	E (24)	Almond (4)	Apple-Cherry (8)	N/A	Alfalfa (15)
2022	F (24)	Almond (4)	Apple-Cherry (8)	N/A	Alfalfa (15)
2022	G (24)	Almond (3)	Apple (7)	Honey (pasture) (12)	Honey (pasture) (12)
2023	C (16)	Almond (16)	N/A	N/A	Honey (forest) (30)
2023	H (16)	Almond (17)	Cherry (21)	Canola (25)	Honey (forest) (31)
2023	K (16)	Almond (3)	Cherry (22)	Canola (26)	Sunflower (32)
2023	M (16)	Almond (18)	Apple-Cherry (23)	Honey (pasture & forest) (27)	Honey (pasture & forest) (27)
2023	N (16)	Almond (19)	Apple (24)	Onion (28)	Honey (pasture) (28)
2023	P (16)	Almond (20)	Apple-Cherry (23)	Honey (pasture & forest) (29)	Honey (pasture & forest) (29)

[a] At some sites two crops were grown at the same site and both pollinated by honey bees, such as apple and cherry at site 8. Sites used for honey production were either pasture land, forest or a combination of the two types of land together at a site. N/A indicates a failure in colony inspection and sample collection.

crop pollination in June and July in WA (Stage 3 "S3"), and fall seed crop pollination in August and September in WA (Stage 4 "S4"). Between January and March, beekeepers transported bee hives between CA and WA. In CA, a total of 9 almond orchards were used in two years, and in WA, the study used a total of eight tree- and small fruit locations in stage 2 (including apple, sweet cherry, and blueberry), six vegetable seed crop fields in stage 3 (including onion, carrot and canola), and four seed crop fields in stage 4 (including sunflower, buckwheat and alfalfa). With the exception of one almond orchard (site 3) used in both years, all other pollination locations were different between years (Table 1). In addition to crop pollination locations where honey bees fulfilled pollination contracts, six wild forage sites in pastures (grassland) and/or forests were also incorporated into our experimental design. This is because collaborating beekeepers chose these sites for honey production and colony health maintenance in stage 3 and/or stage 4 instead of crop pollination. In addition, two blueberry fields were also surveyed in the preliminary study in 2021 in order to provide additional exposure data for risk assessment during blueberry pollination.

2.2. Honey bee colony migratory management, health inspection, and sample collection

Six groups of 24 colonies were tracked across four pollination stages in 2022 (including A, B, D, E, F, and G), resulting in a total of 144 colonies studied (Table 1). While six groups of 16 colonies (a total of 96) were tracked across four pollination stages in 2023 (including group C, H, K, M, N, and P) (Table 1). Two groups (D and G) used in 2022 were owned and managed by Washington State University (WSU) apiary, and all others (10 groups) were from four collaborating commercial beekeepers. Each group was an experimental unit and continuously moved together from one site to another between various pollination stages, but at three wild forage sites for honey production (one in 2022 and two in 2023, i.e., Sites 12, 27, and 29) honey bees were kept at the same site for two stages for honey production (Table 1). Six sites in 2022 and one site in 2023 hosted two groups of colonies, while all other sites hosted only one group of colonies (Table 1). All colonies within a group were inspected once at each of four pollination stages (resulting in four inspections per group in a year). During inspection, colony health parameters were recorded, including colony mortality, pesticide poisoning symptoms (either massive death of individual brood or adult bees while the rest of whole colony remaining still alive), queen failure, and population size (e.g., frames of adult bees, a frame of bees being defined as 75% surface area of a frame covered with adult bees). During each inspection on a group of colonies (either 24 or 16), one wax comb (20.3 cm^2) containing fresh bee bread was cut from each of the four colonies with the largest adult bee populations and from each of the four colonies with the smallest adult bee populations based on the number of frames of bees. Since the population size range varied between each group, comparisons between the four largest and four smallest populations were relative (refer to Fig. A1 for a range of population size). A bee bread sample was also collected from each dead colony at each site. In 2022, additional bee bread was collected from dead colonies in groups A and B after they were transported from CA back to WA but before relocation to a tree fruit orchard (recorded as 2_A-D+ and 2_B-D+). These samples were collected to reflect the pesticide exposures in almond orchards that occurred after stage 1 inspections. Each comb was kept individually in a labelled bag and placed on ice during colony inspections. Comb was stored at −20 °C before bee bread was manually extracted using lab spatulas. Extracted bee bread was pooled across four colonies of either large population or small population size at each site and sampling date, leading to 24, 22, 18, and 24 pooled bee bread samples through stages 1–4, respectively, in two years. Bee bread from all the dead colonies (number varying from one to three) at each site and sampling date was also pooled, resulting in a total of 13 samples (Table 2). Due to a failure to perform some hive inspections, one group in stage 2 and three groups in stage 3 did not have bee bread collected in two years (Table 1).

Table 2
Bee bread sample collection from dead colonies during each pollination stage and potential factors contributing to colony death.

Year	Stage	Group	Sample ID[a]	Number of dead colonies[b]	Factors contributing to colony death[c]
2022	2	A	2_A-D+	2	Weak starting colonies from almond orchard and queen failure
	2	B	2_B-D+	3	Weak starting colonies from almond orchard and queen failure
	2	B	2_B-D	2	Weak starting colonies from almond orchard and queen failure
	2	D	2_D-D	1	High pesticide exposures with especially massive brood mortality
	2	G	2_G-D	1	High pesticide exposures with especially massive brood mortality
	3	D	3_D-D	2	High pesticide exposures with especially massive brood mortality in the previous stage 2
	3	B	3_B-D	1	Queen failure
	4	A	4_A-D	1	Queen failure
	4	B	4_B-D	2	Queen failure
	4	D	4_D-D	1	Continuously declining population after massive brood mortality in the previous stage (stage 2), and continuing exposure to high insecticides (buprofezin) in stage 4
	4	G	4_G-D	1	Continuously declining population after massive brood mortality in stage 2 with high pesticide exposures
2023	2	N	2_N-D	1	Queen failure
	4	K	4_K-D	1	Queen failure

[a] In stage 2 in 2022, samples from dead colonies within groups A and B were collected before migratory colonies were relocated to research sites (labeled as 2_A-D+ and 2_B-D+) or after being moved to a new site (i.e., during pollination, with samples labeled as 2_B-D).
[b] Bee bread from multiple colonies at a given site and a given sampling day were pooled and recorded with a unique sample identification (ID).
[c] Multiple contributing factors are included as they are not mutually exclusive.

2.3. Measurement of pesticide residues in bee bread and risk quotient (RQ) assessment

Pesticides in bee bread were extracted by QuEChERS method, and pesticide concentrations were analyzed by LC–MS/MS and GC-MS/MS. A total of 290 pesticides were measured, including 75 fungicides, 75 herbicides and 140 insecticides (Table A.2). A 2-gram pooled sample from each site and sampling date was added into a 50 mL centrifuge tube, followed by the addition of 10 mL deionized water and 5 mL acetonitrile. A volume of 20 µl of spike solution containing internal and external standards (issued with certificates of analysis) was added to each sample tube. We used a QuEChERS packet, containing 4 g MgSO4, 1 g NaCl, 1 g thermally stable carboxen (TCD), and 0.5 g dispersive hydrophobic sorbent (DHS) (EN 15662, Phenomenex, Torrance, California), according to manufacturer instructions, and shook the subsequent solution for 1 min and centrifuged for 5 min at 3000 rpm. The supernatant was transferred to a cleanup tube with graphitized carbon black in the dispersive solid-phase (GCB dSPE) (Phenomenex, Torrance, California), shook for 2 min, and then centrifuged for five minutes at 3500 rpm. The recovery rate was controlled between 70–130% and the drift rate from initial analysis was controlled below 30%. Ejected volumes of extracts for LC and GC were 4 µl and 1 µl, respectively.

Oral acute toxicity of pesticide exposures in adult honey bees through diets are determined by both residue levels and toxicity of certain pesticides. Risk quotient (RQ, a ratio of exposure/toxicity) was used to estimate acute toxicity according to the United States Environment Protection Agency (EPA) risk assessment methods [15], and the actual calculation method in our study is RQ = (0.0095 g/bee × insecticide residue level)/LD_{50}. In this equation, 0.0095 g indicates the estimated daily pollen consumption per adult honey bee [16]. The LD_{50} for insecticides included in this study is supported by the EPA (http://cfpub.epa.gov/ecotox/) and is also accessible through the previous publication [17]. Since multiple insecticides could be found in a sample, the RQ values we report per sample was calculated by summing the calculated RQ from each individual insecticide without incorporating potential synergism [18]. Three insecticides in the class of insect growth regulator (IGR), including buprofezin, methoxyfenozide, and pyriproxyfen, were not calculated for RQ since they do not cause acute toxicity to adult bees. Fungicides and herbicides usually do not cause acute toxicity and carry undefined LD_{50}, thus RQ was not given.

2.4. Identification of plant genera from bee bread

ITS amplicon sequencing was used to identify plant taxa from bee bread samples. A 0.25 g pooled sample was used for DNA extraction using the DNeasy 96 PowerSoil Pro Kit (Hilden, Germany). PCR was performed with PCR Master Mix from Promega (Madison, Wisconsin), with 1 μl of extracted bee bread DNA, and 0.4 μM of both the forward primer CGAAATCGGTAGACGCTACG and reverse primer CCATTGAGTCTCTGCACCTATC [19]. The sequential PCR conditions included 3 min of denaturation at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C, and 10 min of elongation at 72 °C. The amplicons were uploaded to 2 % agarose gel for a quality check and cleaned by shrimp alkaline phosphatase (SAP) and exonuclease I (EXO I) for 30 min at 37 °C, then inactivated at 95 °C for 5 min. The cleaned amplicons were further amplified by Master Mix from Promega with sequencing adapters, and PCR conditions were modified to include 8 cycles at 95 °C for 30 s instead of 40 cycles. Pooled, indexed PCR amplicons were sequenced by an Illumina NovaSeq 6000 (San Diego, California) using the NovaSeq Reagent Kit v1.5 (500 cycles). Sequence data was compared with GenBank [20] for taxonomic assignment. We aimed to identify plant sources to the level of genus that includes the common crops grown in the study area. When a pollen source was identified to a family that includes both the crops pollinated by our colonies and plausible wild flower species, the corrected genus name was assigned according to other information, including our field observations, known plant phenology, crop species present in the surrounding landscape based on crop cover data from the CropScape program established by the United States Department of Agriculture National Agricultural Statistics Service (USDA NASS), and plant distribution information from the USDA Plants Database as well as former studies regarding honey bee forage records on certain crops and wild flowers. If the family of a pollen source included several plant species from different genera which simultaneously bloomed in the study area, we rewrote the family name and included all potential genera together. When an identified genus included several geographically plausible crops with appropriate phenology, we listed the names of all plausible crops.

2.5. Statistical analysis

Concentration and frequency of total fungicides, herbicides and insecticides in each sample as well as RQ were compared using Kruskal-Wallis rank sum test in R, Version 2023.03.1 (Posit PBC, Boston). Dun Kruskal-Wallis was used to perform multiple comparisons, and the reported *P* value was adjusted by the Hom method.

3. Results

A total of 49 pesticides comprised of 23 fungicides, 6 herbicides and 20 insecticides were found in 2022 and 2023 out of 75 fungicides, 75 herbicides and 140 insecticides tested for (Table A.3, Figs. 1–3). Though each research site was different between the two years (except for site 3 used in both years), 23 out of 51 pesticides were found in both years. The number of fungicides, herbicides and insecticides found in bee bread samples were 18, 5 and 16 in 2022, and 19, 3 and 12 in 2023, respectively (refer to Supplemental Data 1 for detailed pesticide residues in each pollination stage). The highest detection frequency of fungicides, herbicides and insecticides were trifloxystrobin (32 %), pendimethalin (49 %), and buprofezin (39 %), respectively. The majority of detected pesticides (48 out of 50) in our study are historically reported for use in studied crops except spiromesifen and diphenylamine (Table A.4).

3.1. Fungicide residue patterns across the season

In 2022, the highest fungicide residue levels and number of pesticides per sample were observed in stage 2 (data from stage 2 statistically higher than stages 1 and 4); while in 2023, the most severe residue scenario occurred in stage 1 (with residues in both stages 1 and 2 being statistically higher than stage 4) (Tables 3 and 4, Fig. 1). Residues in stage 2 in 2023 were consistent with those found in 2022 with the same detection frequency (100 % detection rate) and comparable range (< 2 μg/g). Number of fungicides per sample, overall detection frequency across samples (i.e., sample with detection of fungicides), and residue levels declined in stages 3 and 4 in both years, but with elevated exposures in 2022 compared to 2023 (Fig. 1). In stage 2 in 2022, five pooled samples collected from dead colonies at each site had higher levels of fungicide residues than other samples collected from live colonies due to multiple potential reasons such as bee bread desiccation and residues from unconsumed bee bread collected at almond fields where high fungicide contamination occurred. The only two samples collected at blueberry fields in the 2021 preliminary study also contained very high levels of fungicide residues, reaching 12.99 μg/g, even higher than that found in 2022 and 2023 (Table A.5).

Although the overall fungicide residues had evident seasonal patterns, there was no single residue pattern that explained the presence of each specific type of fungicide. Fungicide types were very diverse across the season, and many frequently detected types were consistently found at multiple stages and/or years, such as boscalid, fluopyram, tebuconzole and trifloxystrobin. There was also yearly variation for frequently found fungicide types. For example, tebuconzole was consistently found across stages 1–3 in 2022, but was found only in stage 1 in 2023.

3.2. Herbicide residue patterns across the season

Herbicides were frequently found in bee bread in both stages 1 and 2 in both years with most samples having low residual levels at < 0.1 μg/g, except for two samples with residues between 0.1–0.2 μg/g (Tables 3 and 4, Fig. 2). Detection frequency declined in stage 4 in 2022 and in stages 3 and 4 in 2023, but two samples had elevated total residues levels at 0.2–0.3 μg/g in stage 3 in 2023. In both years, residue levels of total herbicides in stages 1–3 were statistically higher than these from stage 4 (Tables 3 and 4, Fig. 2). Pendimethalin was the dominant herbicide among all detected herbicides that were consistently found across stages 1–3. In 2022, other herbicides were mainly found at either stage 2 or 3, three or four times each, and atrazine was only found once in stage 4. In 2023, only three herbicides were detected one time each in either stage 1 or 3. Herbicides were found in bee bread at low levels (< 0.3 μg/g), however, since our pesticide analysis methods could not measure glyphosate, the top pesticide used in the U.S. by quantity [21,22], we cannot conclude that herbicide exposures are not a threat to honey bee health.

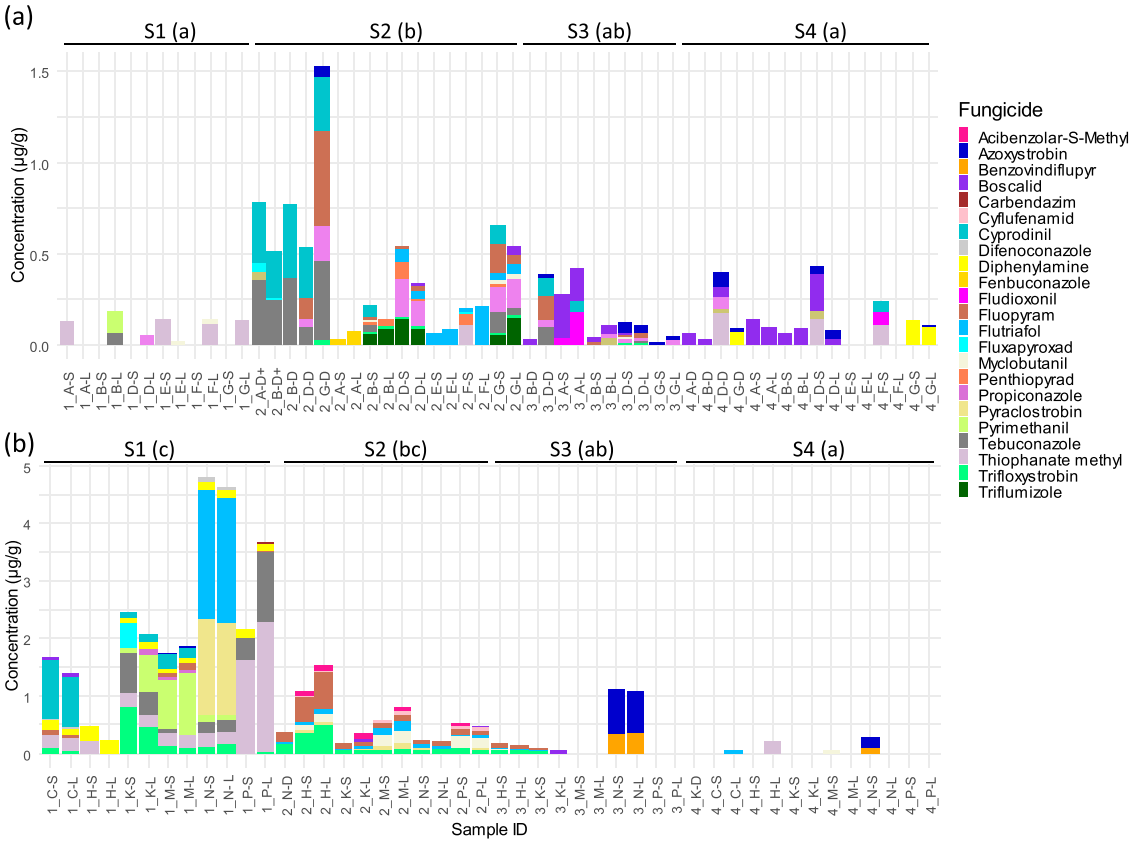

Fig. 1. Fungicide concentrations (μg/g) in bee bread across seasons in two years, 2022 (a) and 2023 (b). Sample identification (ID) include one digit and two letters, informing the pollination stage (including four stages S1–4), group of bee colonies, and adult bee population size of live colonies (L "large" and S "small") and dead colonies "D" (refer to Table 1 and Table A.1 for more information). Different letters following stage name (S1 to S4) indicates statistical differences in concentrations of all detected pesticides between stages (Tables A3 and A4).

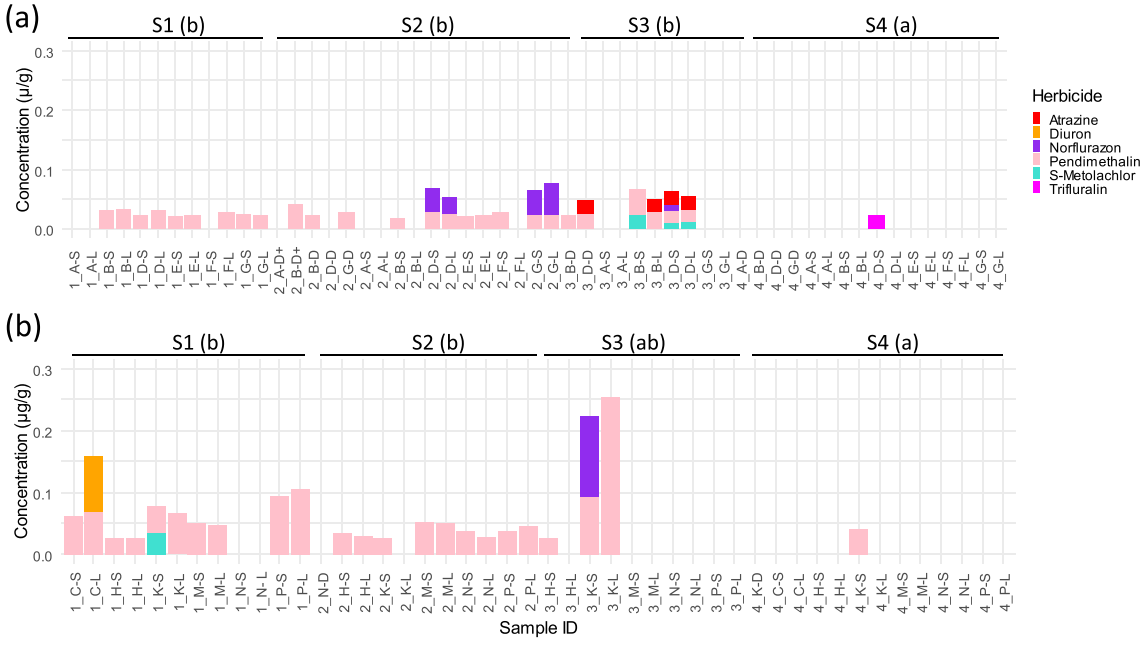

Fig. 2. Herbicide concentrations (μg/g) in bee bread collected in 2022 (a) and 2023 (b).

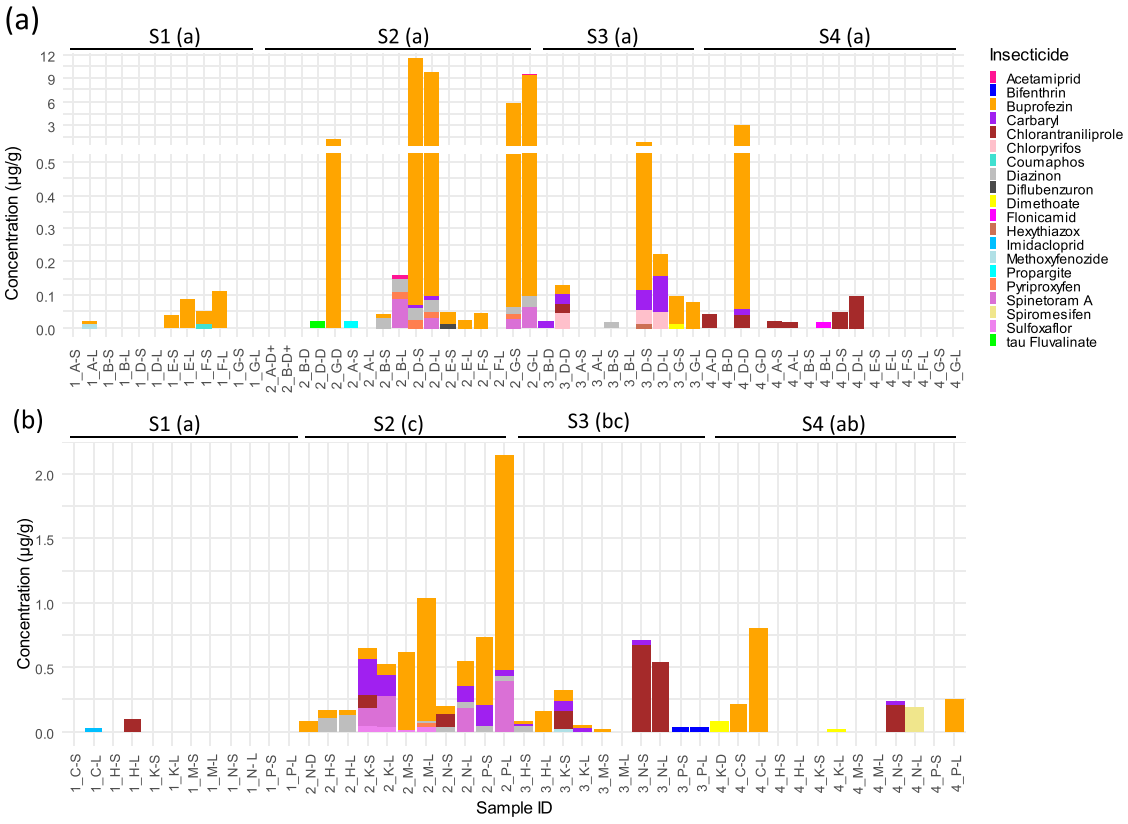

Fig. 3. Insecticide concentrations (μg/g) in bee bread collected in 2022 (a) and 2023 (b).

Table 3
Comparisons of pesticide concentrations in bee bread across sampling stages.

Year	Stage	Pesticide concentration in μg/g, mean (median, min-max)[a]			
		Fungicides	Herbicides	Insecticides	All pesticides
2022	1	0.066 (0.033, 0 −0.187) a	0.02 (0.0235, 0 −0.034) b	0.025 (0, 0 −0.112) a	0.111 (0.1025, 0.02 −0.281) a
2022	2	0.258 (0.205, 0.032 −0.661) b	0.029 (0.023, 0 −0.076) b	3.087 (0.0465, 0 −11.5879) a	3.375 (0.2725, 0.052 −12.1999) b
2022	3	0.142 (0.1073, 0.12 −0.42) ab	0.029 (0.025, 0 −0.067) b	0.155 (0.0475, 0 −0.8287) a	0.326 (0.2155, 0.107 −1.0156) b
2022	4	0.114 (0.0925, 0 −0.426) a $\chi^2 = 2.26$, $P = 0.001534$	0.001 (0, 0 −0.023) a $\chi^2 = 15.48$, $P = 0.001449$	0.016 (0, 0 −0.097) a $\chi^2 = 6.3302$, $P = 0.0966$	0.132 (0.1125, 0 −0.497) a $\chi^2 = 19.595$, $P = 2.06 \times 10^{-4}$
2023	1	2.26 (1.963, 0.233 −4.794) c	0.059 (0.0375, 0 −0.159) b	0.009 (0, 0 −0.095) a	2.329 (1.652, 0.355 −4.794) c
2023	2	0.59 (0.501, 0.165 −1.522) bc	0.034 (0.036, 0 −0.053) b	0.678 (0.5835, 0.165 −2.144) c	1.305 (1.2135, 0.464 −2.665) bc
2023	3	0.264 (0.072, 0 −1.112) ab	0.05 (0, 0 −0.254) ab	0.193 (0.0665, 0 −0.711) bc	0.508 (0.2845, 0 −1.823) ab
2023	4	0.048 (0, 0 −0.277) a $\chi^2 = 31.974$, $P = 5.29 \times 10^{-7}$	0.003 (0, 0 −0.042) a $\chi^2 = 17.232$, $P = 6.331 \times 10^{-4}$	0.142 (0.0085, 0 −0.8) ab $\chi^2 = 23.021$, $P = 3.999 \times 10^{-7}$	0.194 (0.1185, 0 −0.845) a $\chi^2 = 27.083$, $P = 5.656 \times 10^{-6}$

[a] Different letter(s) after mean concentration valuesindicate(s) statistical differences between four pollination stages within eachyear ($P < 0.05$). Kruskal-Wallis rank sum test was used to generatemodel summaries, while Kruskal-Wallis multiple comparison was used to comparemean values between stages.

Table 4
Comparisons of number of pesticides detected in bee bread across sampling stages.

Year	Stage	Number of pesticides, mean (median, min-max)			
		Fungicides	Herbicides	Insecticides	All pesticides
2022	1	0.7 (1, 0 −2) a	0.7 (1, 0 −1) b	0.5 (0, 0 −2) a	2 (2, 1 −4) a
2022	2	4 (3.5, 1 −9) c	1 (1, 0 −2) b	2.5 (2, 0 −6) a	7.6 (6.5, 1 −16) b
2022	3	3 (2.5, 3 −6) bc	1.3 (1, 0 −4) b	1.5 (1, 0 −5) a	5.8 (4, 2 −15) b
2022	4	1.3 (1, 0 −4) ab $\chi^2 = 19.55$, $P = 2.1 \times 10^{-4}$	0 (0, 0 −1) a $\chi^2 = 15.794$, $P = 0.00125$	0.4 (0, 0 −1) a $\chi^2 = 7.3641$, $P = 0.06116$	1.8 (2, 0 −6) a $\chi^2 = 19.595$, $P = 2.06 \times 10^{-4}$
2023	1	6.1 (7, 1 −9) c	1 (1, 0 −2) c	0.1 (0, 0 −1) a	7.3 (8, 3 −10) bc
2023	2	5.6 (6, 3 −7) c	0.9 (1, 0 −1) c	3.3 (3.5, 2 −5) c	9.8 (10, 8 −12) c
2023	3	1.6 (1.5, 0 −5) b	0.4 (0, 0 −2) bc	1.6 (1, 0 −4) bc	3.6 (3.5, 0 −9) ab
2023	4	0.4 (0, 0 −2) ab $\chi^2 = 31.166$, $P = 7.845 \times 10^{-7}$	0 (0, 0 −1) a $\chi^2 = 18.577$, $P = 3.344 \times 10^{-4}$	0.5 (0.5, 0 −2) ab $\chi^2 = 29.002$, $P = 2.237 \times 10^{-7}$	1 (1, 0 −4) a $\chi^2 = 29.66$, $P = 1.627 \times 10^{-6}$

3.3. Insecticide residue patterns across the season and exposure risk assessment by RQ model and field colony inspection

In both years, for level and number of insecticides per sample and detection frequency, insecticide residues were consistently low in stage 1 and highest in stage 2 compared with other stages (Fig. 3). However, the differences were not statistically different between stages in 2022, while residues from stage 2 were statistically higher than stages 1 and 4 in 2023 (Tables 3 and 4, Fig. 3). Residue levels decreased in stages 3 and 4, but stage 4 had more samples without insecticides and fewer types of insecticides detected than in stage 3 (Fig. 3). Buprofezin was consistently found across all stages, reaching its highest concentrations in stage 2. Pyriproxylfen, spinetoram and sulfoxaflor were frequently found in stage 2, and chlorpyrifos frequently in stage 3. Chlorantraniliprole, carbaryl, and diazinon were frequently detected across multiple stages, including stages 3 and 4, stages 2–4, and stages 2 and 3, respectively. Coumaphos and tau-fluvalinate were miticides commonly used to control the parasitic Varroa mite (*Varroa destructor*). It was applied by beekeepers, and the presence of those two insecticides in our samples is likely derived from past Varroa control treatment.

Stages 1 and 4 had extremely low RQ (too low to be visible in 2022 and only one observable low RQ value, <0.005 in 2023), with stages 1 and 4 being statistically lower than stage 2 in 2023 ($df = 3$, $\chi^2 = 26.005$, $P = 9.517 \times 10^{-7}$, Fig. 4) and stage 1 being statistically lower than stage 2 in 2022 ($df = 3$, $\chi^2 = 9.529$, $P = 0.023$, Fig. 4). Furthermore, RQ from stage 1 was statistically lower than stage 3 in 2023. Higher RQ in stages 2 and 3 was mainly driven by bifenthrin, carbaryl, chlorpyrifos, imidacloprid, diazinon and sulfoxaflor. Among the above five insecticides, diazinon and carbaryl were consistent drivers of RQ across multiple stages in both years; while the other three insecticides occurred in one stage in one year. Despite higher insecticide exposure risk in tree fruit and small fruit pollination in WA, RQ is far below the concern value of 0.4, the level at which acute toxicity occurs in honey bees as defined by the U.S. EPA [23,24]. RQ reflects the exposure risk on a day, however, honey bees' exposure to a pesticide in field can occur in continuously days potentially causing increased risk [25]. The accumulative RQ calculated in a week (duration for a nursing bee as the major bee bread consumer) increased to a level at 0.2, approaching the concern level of 0.4 (Fig. A.2).

In contrast to the low exposure risk to adult bees suggested by RQ, extreme levels of dead brood were observed inside and in front of bee hives in groups G and D (a total of 48 colonies) right after pollination to fruit trees in stages 2 and 3 in 2022. Bee bread collected from two groups contained the highest concentrations of buprofezin across the season. We did not observe massive brood or adult bees in other groups. Exposures to high levels of buprofezin caused long-term effect on honey bee health that bee colony populations could not be sustained without ability to reproduce sufficient new generation of adult bees, leading to colony mortality late in the season (Table 2). Not all colony death was caused by exposures to pesticides, and weak starting colonies and queen failure were other reasons for colony mortality (Table 2). By collecting bee bread from colonies with the largest and smallest honey bee populations at each site and pollination stage, we attempted to reveal the variation in pesticide exposures in honey bees within the same site; however, pesticide exposures were very similar between colonies with varying population size (Table A.6, Fig. A.3).

3.4. Plant taxonomy of pollen from bee bread

Except for blueberry, pollen from all focal crops (almond *Prunus*, apple *Malus*, cherry *Prunus*, canola *Brassica,* carrot *Dacus,* onion *Allium*, sunflower *Helianthus*, alfalfa *Medicago*, European buckwheat *Fagopyrum*) was intensively collected by forager honey bees (Fig. 5). For example, apple and cherry pollen was consistently found in all samples of stage 2 in both years, and canola pollen was present in all samples collected in stage 3 in 2023. Pollen from non-focal crops grown within foraging

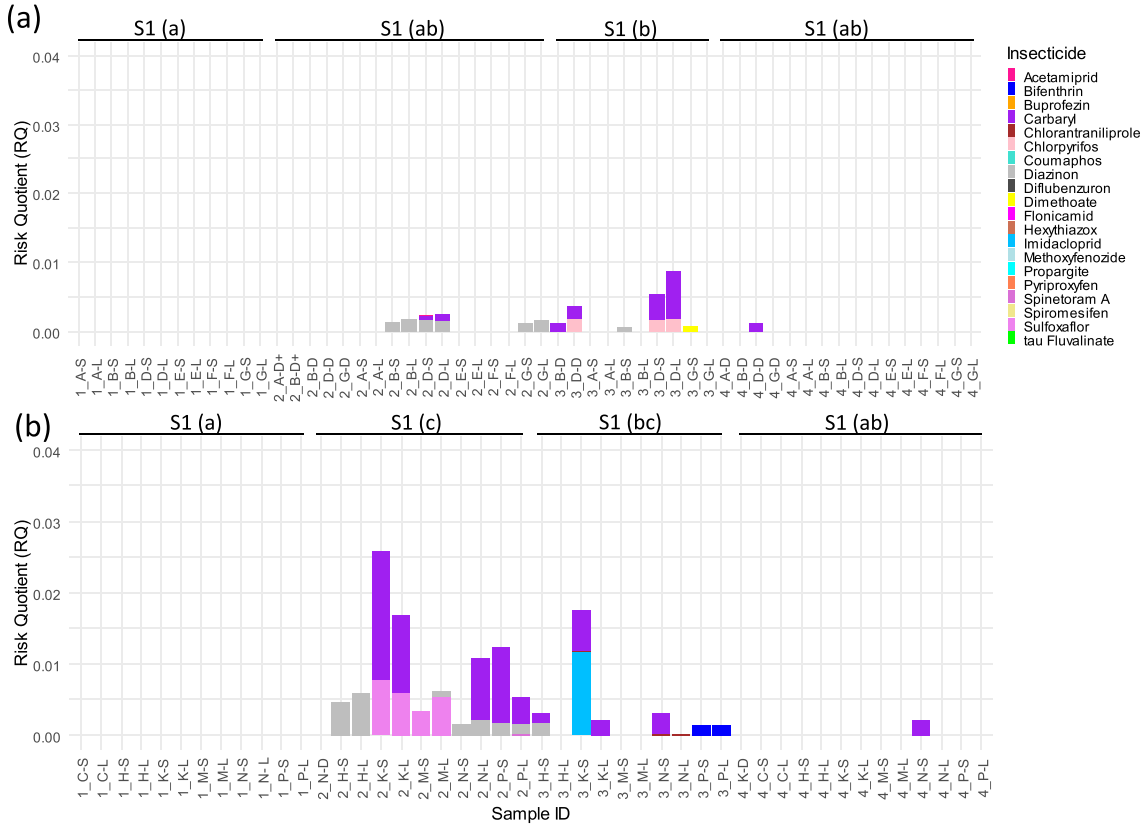

Fig. 4. Risk assessment of acute toxicity of insecticide exposures to adult bees consuming bee bread using risk quotients (RQ) in 2022 (a) and 2023 (b).

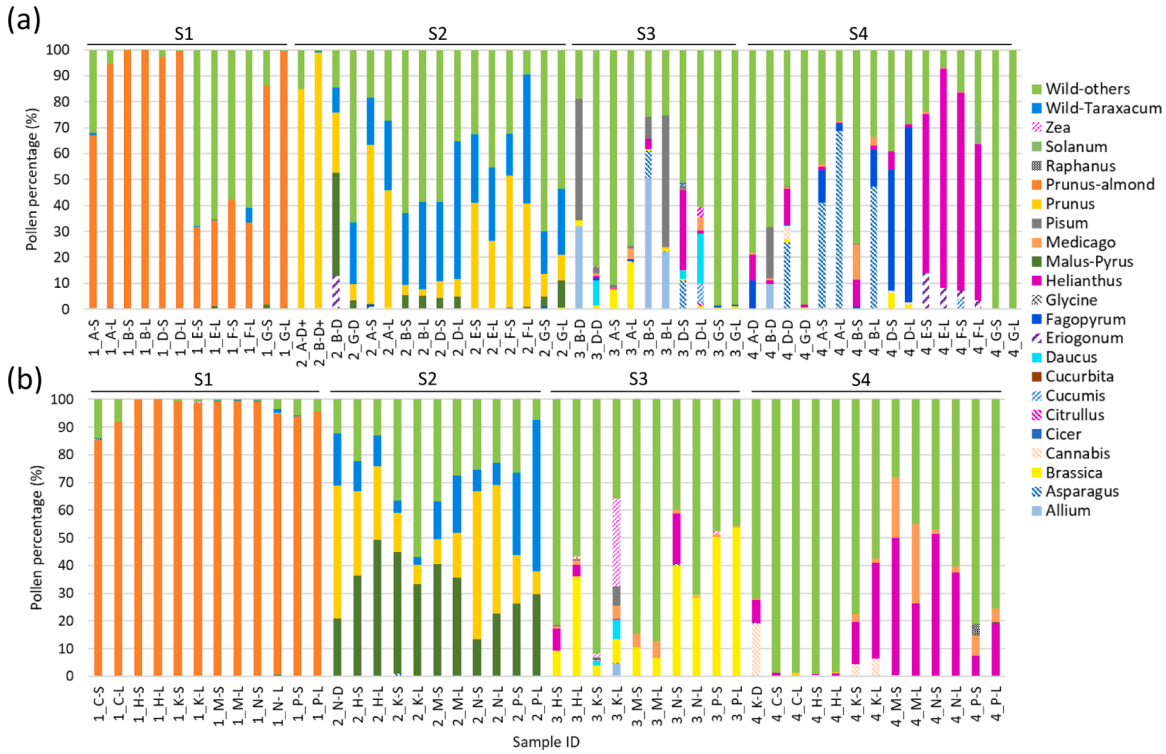

Fig. 5. Percentage of bee bread from various crops and wild plants labelled by genus in 2022 (a) and 2023 (b). Wild dandelion blooming in crop fields (Wild-*Taraxacum*) is illustrated, and pollen from all other wild plants is combined into one category (Wild-others).

range in surrounding landscapes was also identified. For example, we identified asparagus *Asparagus*, chickpea *Cicer*, cannabis *Cannabis*, watermelon *Citrullus*, cucumber, cantaloupe and honeydew *Cucumis*, squash and pumpkin *Cucubita*, wild buckwheat *Eriogonum*, soybean *Glycine*, pea *Pisum*, radish *Raphanus*, and potato *Solanum*, and corn *Zea*. Honey bees also consistently collected wild forage outside crop fields across the season, but in almond orchards the percentage of wild forage was significantly reduced, especially in 2023. The predominance of almond pollen was very consistent in stage 1 of 2023 and all bee bread samples contained 85–100 % almond pollen that year. Four samples containing almond pollen in quantities lower than 50 % were collected from a site near a city (Vasilia, CA) in 2022, where urban ornamental flowers could compete for honey bee visitations. Because the ITS amplicon sequencing protocol used in this study could not separate apple (*Malus*) and pear (*Pyrus*) pollen and because these two crops were either in proximity to the studied colonies or present in the landscape within honey bee foraging range, we integrated two crops into one category (*Malus-Pyrus*). Focal fruit crops as well as wild flowers (i.e., dandelion *Taraxacum*) growing under tree fruit canopies were major pollen sources in stage 2; however, honey bees collected considerable amounts of pollen from other wild flowers as well (refer to Supplemental Data 2 and 3 for a full list of wild flowers foraged by honey bees in 2022 and 2023, respectively).

4. Discussion

4.1. Peak fungicide and low insecticide exposures during almond pollination

In 2023, fungicide exposures in almond orchards were highest compared with other pollination stages, however, we detected major differences in fungicide residues between almond pollination in 2022 and 2023 as a result of a mismatch between the window for major fungicide applications in California (CA) almonds and our sampling time. In 2022, we collected our samples on February 16–18 when almonds were in early bloom, and fungicide spray frequency was still low across CA [4]. Although our sampling time in 2023 was only one week later (i.e., February 21–23), fungicide use in the state had rapidly increased during peak bloom, and our sampling perfectly caught the impact of the rapid change in fungicide use [4]. We took additional samples (e.g., 2_A-D+, 2_B-D+) from dead colonies immediately after colonies returned from CA almonds but before they were relocated to tree fruit locations in 2022. Those additional samples can demonstrate fungicide exposure conditions later in February of 2022 and support our assumption regarding an increase in fungicide applications. Fungicide application is a routine practice for most almond growers as it is used widely across the state each year for disease prevention and accounts for a majority of pesticide use in almond orchards based on the application data from the California Department of Pesticide Regulation (https://www.cdpr.ca.gov/) (refer to Table A.4 for a summary of public pesticide use data) [26]. Due to their wide use, we predicted fungicide exposures in stage 1 in 2022 would be as severe as that in 2023, however, our results did not directly demonstrate this because we collected samples earlier in 2022 relative to 2023, missing the potential peak in application.

Our findings that insecticide exposures were low in both number of pesticides per sample and overall detection frequency, and residual level in stage 1 is in agreement with the previous statement of low pesticide use frequeny during during almond bloom [4]. These low levels of insecticide exposures are likely a testament to efforts of the almond industry to invest in and promote research, outreach, and communication related to pollinator protection which reduced the inappropriate use of insecticides of high and medium toxicity which are not permitted to be used during almond bloom.

4.2. Persistent fungicide and peak insecticide exposures during spring fruit pollination

The elevated number of insecticide detections and residue levels we identified in bee bread from tree and small fruit locations in Washington

(WA) indicates honey bees face greater risk during this pollination stage compared to the risks associated with other pollination stages throughout the season. The higher insecticide residues detected in our samples overlap with historically reported, heavily used pesticide types in fields, for example, carbaryl, chlorpyrifos, and diazinon contributing to acute toxicity, and buprofezin contributing to brood mortality (Table A.4). In contrast, some intensively used insecticides in CA such as bifenthrin, chlorantraniliprole, methoxyfenozide, and spinetoram did not increase insecticide exposures in our experimental colonies. It is not reported whether pesticide applications in pollinated crop fields in WA were well managed or not. However, pesticide exposures during the pollination of tree fruits and small fruits in WA can be complicated by the complex patchwork of crops with differing bloom periods, narrow pollination timing, and spray application requirements. For example, it is common to have colonies contracted for apple pollination within flight range of pear/cherry blocks that have begun to receive post-bloom applications. Alternatively, it is possible that pollination contracts in cherries may place bees near apples that are receiving pre-bloom applications of these higher risk pesticides while herbaceous flowering plants bloom in the understory.

In 2023, exposures to fungicides in stage 2 decreased relative to the high exposures identified in stage 1 (without statistical difference), but exposures remained higher than in stages 3 and 4. This numerical difference may have arisen because in stage 2, tree fruits constituted a lower percentage of landscape coverage relative to the ubiquitous coverage of almond in stage 1 as well as lower total use of most fungicides that were detected in our samples (Fig. A.2). This is supported by findings from our taxonomic analysis that in stage 2, a lower proportion of pollen arose from the focal tree fruit crop and a higher proportion of pollen arose from wild flowers, where the likelihood of contamination by pesticides is lower.

Unexpectedly, we found evident differences in insecticide residues in stage 2 in between 2022 and 2023. In 2022 we found low levels of contamination in several samples (e.g., 2_A-S, 2_A-L), while in 2023, detected residues remained fairly consistent. We hypothesize this may also be caused by differences in the timing of both pesticide application and sample collection between years, since samples with low or no insecticide residues were collected in the middle of April when temperatures were still low, carrying low insect pressure and low insecticide usage. The insecticide, buprofezin, was widely found at all stages and was a dominant residue in stage 2. The exact buprofezin volume used annually is only reported in pear (7900 pound or 3583 Kg), but the actual buprofezin used in all fruit crops is predicted to be much higher since apple, peach, and blueberry cultivation occupied significantly greater area than pear in WA (USDA-NASS, 2023).

It is challenging to make an ideal, complete comparison of spring pesticide exposures between this study and previous studies since analytical methods vary across studies. However, from our study and across the literature, fungicides are consistently found to be dominant constituents of pesticidal residues while insecticides are found to be main contributors to acute toxicity in orchards in spring [27-30]. Only one neonicotinoid (imidacloprid) was found in one sample in our study although neonicotinoids are widely used insecticides across the U.S. Concentrations of specific pesticides can be significantly different between studies due to differences in cropping systems or landscapes between regions. For example, neonicotinoids were frequently detected in a previously conducted study where hives were surrounded by soybean and corn monocultures in spring [28]. Since, soybean and corn production require some of the highest neonicotinoid applications of all crops grown in the U.S. [31], neonicotinoid detections trend higher near plots of soybean and corn. In WA where there is very limited soybean and corn cultivation, we suspect our single detection of imidacloprid is less likely from a corn field, instead arose from its use in summer crop(s) that cannot be confirmed due to limitations in our research methods.

4.3. Decreasing pesticide exposures during seed crop pollination and honey production

Public pesticide use data in seed crops is lacking in WA (Table A.4), however, several factors may contribute to the low levels of fungicides we identified from bee bread in stages 3 and 4. Climate and weather variation as well as fungal disease phenology in certain crops may play a role. Unlike the widespread use of fungicides to protect against fungal infections in the flowers of almond, tree fruits and small fruits in early spring, dry and/or hot weather in the summer, especially in central WA decreases fungal disease occurrence rates [32-34]. Unlike in springtime tree fruits, fungicides are less frequently used during bloom for seed crops (e.g., onion, carrot, sunflower), because few devastating fungal diseases affect the flowers of seed crops. The only exception is fungicide use during canola bloom. There, fungicides are not applied to protect canola flowers from diseases, but instead are applied to prevent infections of the stem (sclerotinia stem rot) from fungal spores that can be spread by drifting pollen or fallen flowers. Despite the potentially reduced use of fungicides in stages 3 and 4, forager bees have the capacity to leave the target crop area and forage from other blooming crops nearby (Fig. 5) which may be sprayed with fungicides. Wildflowers adjacent to other crops which receive drift from fungicide applications can also be a source of exposure regardless of if those non-focal crops bloom or not. The source of insecticide exposures in stage 3 could be similarly diverse and potentially more unpredictable than those occurring during almond and fruit pollination in spring.

We uncovered overall variations in fungicide residues in stages 3 and 4 between sampling years with generally lower levels and number of detections in 2023 relative to 2022. This is potentially because more samples were collected from sites in pastures and/or forests in 2023 (Table 1, Fig. A.4), where pollen forage is less likely subject to pesticidal contamination. This yearly variance was also observed for insecticides in stages 3 and 4, however, this variation was not as severe as it was for fungicides. In both years, samples collected from sites with more natural habitat in the surrounding landscapes had no or significantly decreased pesticide residues (e.g., no pesticides detected in a total of three samples from groups E and F in stage 4 in 2022, and in one sample from group C in stage 3 and one sample from each of groups H, M and P in stage 4 in 2023). In other large-scale survey studies, which included sites in landscapes with very high percentages of natural habit coverage, an overall trend of decreasing pesticide exposures was observed in honey bees, indicating the value of natural habit to mitigate pesticide exposures [28,35,36].

4.4. Complementary RQ assessment method with field monitoring

The low acute risk to adult bees resulting from our RQ assessment ($<$ 0.03) falls below the EPA recommend concern value of 0.4. This aligns with our field observations, since we found no acute poisoning symptoms in adult bees. RQ as well as other commonly used evaluation methods such as hazardous quotient (HQ) [37] usually use toxicity information collected from adult bees in acute poisoning tests and do not take into account the potential for chronic toxicity or acute toxicity to brood. This can lead to underestimated impacts of pesticides on overall colony health. Despite evidence that IGR do not pose a threat via acute toxicity in adult bees [38,39], the traditional field monitoring used in this study found severe brood mortality in two groups of colonies (groups D and G including 48 colonies) right after colonies moved out from free fruit focal fields, which was exposed to high levels of buprofezin in stage 2. No field risk assessment data exists for buprofezin, but analyses of other IGRs have demonstrated decreased egg eclosion, decreased brood capping, and increased pupal death [40,41]. This instance of brood poisoning by buprofezin presents an alarming scenario that highlights a need to monitor pesticide exposures in honey bees under natural conditions in the field as well as a need to implement regulatory policy changes surrounding pesticide safety. The inability to

evaluate chronic, and larval impacts from certain classes of insecticides (e.g., the IGR, buprofezin) carried by the RQ method points to a need to further investigate the impact of high exposures to insecticides and fungicides that were previously determined to be safe for use around honey bees.

RQ usually calculates the consequential toxicity of one pesticide application or one day of pesticide exposure, however, honey bees can be exposed to the same pesticide across multiple days by recurrently visiting focal fields sprayed with pesticides bearing long-lasting residues or visiting nearby fields treated with the same pesticides across multiple days. Contaminated bee bread stored in bee hives can be consumed across a longer term (ranging from several days to weeks depending on overall food storage and colony needs), which extends potential negative impact across a longer period of time. Furthermore, severe impacts of pesticide exposures during one pollination stage can have long lasting consequences, for example, we noted continuously declining bee populations in our experimental colonies that were exposed to high levels of buprofezin early in the season, which scenario resulted in colony death at the end of the season.

When comparing RQ results across studies, research conclusions or statements have to be carefully examined because different equations are used for RQ calculations (Fig. A5). Studies using pesticide concentration in bee bread (pollen) or honey (nectar) as pesticide exposure without considering food consumption rate will overestimate oral exposure risk [42,43]. The toxicity of pesticides can be adjusted based on honey bee body weight, however, this method of risk assessment can also overestimate risk because the body weight of a honey bee (around 0.1 g) is higher than bee bread consumption (around 0.01 g) [29].

Since fungicides lack acute toxicity to adult bees, RQ used in our study did not assess exposure risk from high levels of fungicides. However, fungicides can cause sublethal toxicity by affecting many aspects of honey bee health, including but not limited to, interrupting gut physiology, decreasing brood production, and increasing queen loss [5, 44-49]. The concentrations of fungicides that cause negative impact on honey bee health in the field are much lower than concentrations that cause negative impacts measured in lab scenarios. Further, under realistic, field-based scenarios, honey bees are exposed to widely variable combinations of fungicides. Concurrent exposure to multiple pesticides can worsen health impacts via synergisms between fungicides, insecticides or various environmental stressors [26].

4.5. Sources of pollen and pesticide exposures

By measuring pesticide residues in bee bread or pollen samples, the quantity and diversity of pollen in the landscape that is contaminated with pesticide residues usually cannot be identified, because honey bees are generalists that forage pollen from many plant species [25]. However, the analysis of pesticide residues from collected pollen, backed by supporting information of pollen sources can still indicate likely sources of pesticide exposure in the field. Under some circumstances, we can be fairly confident identifying the origin of the residuals. For example, in our study, we found multiple samples collected from almond orchards consisting of over 99 % (up to and including 100 %) almond pollen, demonstrating with confidence that high fungicide exposures were likely due to fungicidal applications in almond orchards. In addition, almond is the first crop to bloom annually in CA and the first crop to require pesticide applications, while, other major nuts and fruits in the study area (e.g., grape) just break the dormancy and bloom later than almond and are usually not treated with pesticides in February.

As in almond orchards, the source of pesticide exposures in honey bees in WA tree fruits can also be predicted using knowledge of the flowering phenology for crops and wild plants in the landscape. The analysis of pesticides carried out in our study in combination with reported pesticide use supplied by the USDA NASS can increase the predictability of pesticide poisonings in honey bees. Our study suggests fungicide exposures arose from contaminated pollen produced by blooming fruit trees as well as dandelions growing in the understory. Dandelions were consistently present in bloom at all study fields during stage 2. Dandelion presence carries both pros and cons because they serve as a major forage resource for honey bees but also trap fungicides and compete with target crops for pollination [27]. Flowering dandelions can be mowed to reduce pollination competition with the target crop [50]; however, this practice requires additional labor from growers. Further, it is unknown if honey bees compensate for the loss of dandelion pollen by foraging other wild flowers in surrounding natural or urban habitats that are less likely to be contaminated with pesticides in early spring.

In summer and fall when pesticide use is common in many crops across the foraging landscape of honey bees, pollen taxonomic information tends to be less powerful to predict the source of pesticide residues in bee bread. This is because a honey bee colony can simultaneously collect pollen from several crop species, obfuscating the ability to identify which plant is the source of pesticide exposure. For example, spiromesifen is an insecticide used in both corn and melons which were not focal crops, and pollen from both crops was found in our bee bread samples. However, pollen taxonomy data can still provide directions for further investigation to identify potential sources of pesticide exposure.

In conclusion, pesticide exposures to pollinating honey bees have various patterns crosslinked with season and geographical regions. For example, high fungicide exposures tend to occur in almond orchards in CA and high fungicide and insecticide exposures tend to occur in tree fruit orchards in WA. Our identification of exposure patterns provides an opportunity to mitigate exposure risk for pollinators by informing pesticide use education and/or policy changes aimed at reducing insecticide use during crop bloom. This could be especially impactful for insecticide use in tree fruit orchards in WA. Although pesticides are required to pass lab-based risk assessments or semi-field-based risk assessments before approval for use in the field, the case of extreme buprofezin exposure identified here and subsequent brood mortality indicates a need to expand risk assessment for pesticide exposures under realistic field conditions in different cropping systems. Although fungicide use is a necessary practice to prevent fungal infections in the flowers of nut and fruit trees, conflicts between fungicide use and pollinator protection should be mitigated through the analysis of sublethal fungicide effects and through appropriate management from beekeepers. In contrast with high almond crop cover in CA which causes repeated fungicide exposures in a large area, many tree fruit fields are less densely distributed in WA, resulting in situations where pesticide exposures are less frequent and potentially mitigable at the focal field level. For example, beekeepers may close hive entrances for short periods of time during and right after application in the focal crop. Alternatively, they may remove contaminated pollen from forager bees using pollen traps. There are other potential techniques such as detoxification agents designed to cure pesticide poisonings in honey bees, but such products are still in development [51]. In summer and fall when many crops are simultaneously treated with pesticides, existing farming practices like avoiding pesticide use during crop bloom and preventing pesticide drift remain a key method to protect bees and other pollinators, even when focal fields have no honey bee colonies installed.

Environmental implications

Our study collected biological samples (bee pollen) in 240 honey bee hives at 32 fields across California and Washington State in the U.S. in two years' period. Pesticide contamination occurs to bee pollen as pesticides are applied at crop fields. Pollen (namely bee bread) samples were analyzed for pesticide residues for risk assessment on honey bees using RQ assessment model. A total of 290 pesticides causing either acute and chronic toxicity were tested. Simultaneously, bee health was measured in fields in addition to analysis of pesticides in bee pollen, which can be used to explore the negative impact of pesticide exposures.

CRediT authorship contribution statement

Ge Zhang: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Brandon Hopkins:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Rae Olsson:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Ryan William Kuesel:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Xia Liu:** Writing – review & editing, Visualization, Formal analysis. **Riley Reed:** Writing – review & editing, Investigation.

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.

Data availability

Data have been attached as supplementary material and supplemental data.

Acknowledgement

This study was supported by the Technical Assistance for Specialty Crops Program of the United States Department of Agriculture, Foreign Service Agency (grant number: TASC-2021-17) and Specialty Crop Block Grant Program of Washington State Department of Agriculture (grant number: AWD005725).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.135910.

References

[1] 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 (6229), 1255957.
[2] Kulhanek, K., Steinhauer, N., Rennich, K., Caron, D.M., Sagili, R.R., Pettis, J.S., et al., 2017. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. J Apic Res 56 (4), 328–340.
[3] eCFR (2007). Data Requirements for Pesticides, 40 CFR 158. ⟨https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-158⟩ (accessed on 16 May 2024).
[4] Durant, J.L., Goodrich, B.K., Chang, K.T., Yoshimoto, E., 2021. Growers follow the label: an analysis of bee-toxic pesticide use in almond orchards during bloom. Calif Agric 75 (2), 91–99.
[5] European Food Safety Authority (E.F.S.A.), Adriaanse, P., Arce, A., Focks, A., Ingels, B., Jölli, D., et al., 2023. Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA J 21 (5), e07989.
[6] Marcelino, J., Braese, C., Christmon, K., Evans, J.D., Gilligan, T., Giray, T., et al., 2022. The movement of western honey bees (*Apis mellifera* L.) among US States and territories: history, benefits, risks, and mitigation strategies. Front Ecol Evol 10, 850600.
[7] Martínez-López, V., Ruiz, C., De la Rúa, P., 2022. Migratory beekeeping and its influence on the prevalence and dispersal of pathogens to managed and wild bees. Int J Parasitol: Parasites Wildl 18, 184–193.
[8] Mavrofridis, G., Tataris, G., Petanidou, T., 2023. Traditional migratory beekeeping in Greece, 18th–mid 20th century. J Apic Res 63 (3), 1–14.
[9] Reilly, J.R., Artz, D.R., Biddinger, D., Bobiwash, K., Boyle, N.K., Brittain, C., et al., 2020. Crop production in the USA is frequently limited by a lack of pollinators. Proc R Soc B 287 (1931), 20200922.
[10] Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., VanEngelsdorp, D., et al., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PloS One 5 (3), e9754.
[11] Traynor, K.S., Tosi, S., Rennich, K., Steinhauer, N., Forsgren, E., Rose, R., et al., 2021. Pesticides in honey bee colonies: Establishing a baseline for real world exposure over seven years in the USA. Environ Pollut 279, 116566.
[12] Crump, A., Farrar, J., Fournier, A.J., Ellsworth, P.C., 2018. Employing California pesticide use data for evaluating integrated pest management programs and informing pesticide policy and regulation. In: Zhang, M., Jackson, S.H., Robertson, M.A., Zeiss, M.R. (Eds.), Managing and Analyzing Pesticide Use Data for Pest Management, Environmental Monitoring, Public Health, and Public Policy. American Chemical Society, Washington, D.C., pp. 225–237
[13] Meikle, W.G., Weiss, M., Beren, E., 2020. Landscape factors influencing honey bee colony behavior in Southern California commercial apiaries. Sci Rep 10 (1), 5013.
[14] Frazier, M.T., Mullin, C.A., Frazier, J.L., Ashcraft, S.A., Leslie, T.W., Mussen, E.C., et al., 2015. Assessing honey bee (Hymenoptera: Apidae) foraging populations and the potential impact of pesticides on eight US crops. J Econ Entomol 108 (5), 2141–2152.
[15] USEPA (2014). Guidance for assessing pesticide risks to bees. ⟨https://www.epa.gov/sites/default/files/2014–06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf⟩ (accessed on 28 Augst 2024).
[16] Stewart, S.D., Lorenz, G.M., Catchot, A.L., Gore, J., Cook, D., Skinner, J., et al., 2014. Potential exposure of pollinators to neonicotinoid insecticides from the use of insecticide seed treatments in the mid-southern United States. Environ Sci Technol 48 (16), 9762–9769.
[17] Sanchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees – a risk assessment. PLoS One 9 (4), e94482.
[18] Graham, K.K., McArt, S., Isaacs, R., 2024. High pesticide exposure and risk to bees in pollinator plantings adjacent to conventionally managed blueberry fields. Sci. Total Environ. 922, 171248.
[19] Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., et al., 2007. Power and limitations of the chloroplast trn L (UAA) intron for plant DNA barcoding. Nucleic Acids Res 35 (3) e14-e14.
[20] D.A. Benson I. Karsch-Mizrachi D.J. Lipman J. Ostell D.L. Wheeler. GenBank Nucleic Acids Res 1 2 2005 33.
[21] Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur 28, 1–15.
[22] Zhan, Y., Zhang, M., 2014. Spatial and temporal patterns of pesticide use on California almonds and associated risks to the surrounding environment. Sci Total Environ 472, 517–529.
[23] Thompson, H.M., Pamminger, T., 2019. Are honeybees suitable surrogates for use in pesticide risk assessment for non-*Apis* bees?. Pest Manag Sci 75 (10), 2549–2557.
[24] Vanegas, M., 2017. The silent beehive: how the decline of honey bee populations shifted the environmental protection agency's pesticide policy towards pollinators. Ecol Law Q 44 (2), 311–342.
[25] Long, E.Y., Krupke, C.H., 2016. Non-cultivated plants present a season-long route of pesticide exposure for honey bees. Nat Commun 7 (1), 11629.
[26] Wade, A., Lin, C.H., Kurkul, C., Regan, E.R., Johnson, R.M., 2019. Combined toxicity of insecticides and fungicides applied to California almond orchards to honey bee larvae and adults. Insects 10 (1), 20.
[27] Favaro, R., Bauer, L.M., Rossi, M., D'Ambrosio, L., Bucher, E., Angeli, S., 2019. Botanical origin of pesticide residues in pollen loads collected by honeybees during and after apple bloom. Front Physiol 10, 1069.
[28] Graham, K.K., Milbrath, M.O., Zhang, Y., Soehnlen, A., Baert, N., McArt, S., et al., 2021. Identities, concentrations, and sources of pesticide exposure in pollen collected by managed bees during blueberry pollination. Sci Rep 11 (1), 16857.
[29] 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 (1), 7189.
[30] McArt, S.H., Fersch, A.A., Milano, N.J., Truitt, L.L., Böröczky, K., 2017. High pesticide risk to honey bees despite low focal crop pollen collection during pollination of a mass blooming crop. Sci Rep 7 (1), 46554.
[31] Tooker, J.F., Douglas, M.R., Krupke, C.H., 2017. Neonicotinoid seed treatments: limitations and compatibility with integrated pest management. Agric Environ Lett 2 (1) ael2017-08.
[32] Al-Sadi, A.M., 2016. Epidemiology and management of fungal diseases in dry environments. In: Farooq, M., Siddique, K.H. (Eds.), Innovations in Dryland Agriculture. Springer, New York, pp. 187–209.
[33] Launay, M., Caubel, J., Bourgeois, G., Huard, F., de Cortazar-Atauri, I.G., Bancal, M.O., et al., 2014. Climatic indicators for crop infection risk: application to climate change impacts on five major foliar fungal diseases in Northern France. Agric, Ecosyst Environ 197, 147–158.
[34] Sardella, D., Muscat, A., Brincat, J.P., Gatt, R., Decelis, S., Valdramidis, V., 2016. A comprehensive review of the pear fungal diseases. Int J Fruit Sci 16 (4), 351–377.
[36] Wang, X., Goulson, D., Chen, L., Zhang, J., Zhao, W., Jin, Y., et al., 2020. Occurrence of neonicotinoids in Chinese apiculture and a corresponding risk exposure assessment. Environ Sci Technol 54 (8), 5021–5030.
[35] Drummond, F.A., Ballman, E.S., Eitzer, B.D., Du Clos, B., Dill, J., 2018. Exposure of honey bee (*Apis mellifera* L.) colonies to pesticides in pollen, a statewide assessment in Maine. Environ Entomol 47 (2), 378–387.
[37] Wen, X., Ma, C., Sun, M., Wang, Y., Xue, X., Chen, J., et al., 2021. Pesticide residues in the pollen and nectar of oilseed rape (*Brassica napus* L.) and their potential risks to honey bees. Sci Total Environ 786, 147443.
[39] Fine, J.D., Foster, L.J., McAfee, A., 2023. Indirect exposure to insect growth disruptors affects honey bee (*Apis mellifera*) reproductive behaviors and ovarian protein expression. Plos One 18 (10), e0292176.
[38] Devillers, J., Devillers, H., 2020. Lethal and sublethal effects of pyriproxyfen on *Apis* and non-*Apis* bees. Toxics 8 (4), 104.
[41] Fine, J.D., 2020. Evaluation and comparison of the effects of three insect growth regulators on honey bee queen oviposition and egg eclosion. Ecotoxicol Environ Saf 205, 111142.

[40] Chen, Y.W., Wu, P.S., Yang, E.C., Nai, Y.S., Huang, Z.Y., 2016. The impact of pyriproxyfen on the development of honey bee (*Apis mellifera* L.) colony in field. J Asia Pac Entomol 19 (3), 589–594.
[42] Knapp, J.L., Nicholson, C.C., Jonsson, O., de Miranda, J.R., Rundlöf, M., 2023. Ecological traits interact with landscape context to determine bees' pesticide risk. Nat. Ecol. Evol. 7 (4), 547–556.
[43] Nicholson, C.C., Knapp, J., Kiljanek, T., Albrecht, M., Chauzat, M.P., et al., 2024. Pesticide use negatively affects bumble bees across European landscapes. Nature 628 (8007), 355–358.
[45] Fisher, I.I., A., DeGrandi-Hoffman, G., Smith, B.H., Johnson, M., Kaftanoglu, O., Cogley, T., et al., 2021. Colony field test reveals dramatically higher toxicity of a widely-used mito-toxic fungicide on honey bees (*Apis mellifera*). Environ Pollut 269, 115964.
[46] 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 (2), 520–528.
[44] Belsky, J., Joshi, N.K., 2020. Effects of fungicide and herbicide chemical exposure on *Apis* and Non-*Apis* bees in agricultural landscape. Front Environ Sci 8, 81.
[47] Serra, R.S., Martínez, L.C., Cossolin, J.F.S., Resende, M.T.C.S.D., Carneiro, L.S., Fiaz, M., et al., 2023. The fungicide azoxystrobin causes histopathological and cytotoxic changes in the midgut of the honey bee *Apis mellifera* (Hymenoptera: Apidae). Ecotoxicology 32 (2), 234–242.
[48] Traynor, K.S., Lamas, Z.S., 2021. Social disruption: Sublethal pesticides in pollen lead to *Apis mellifera* queen events and brood loss. Ecotoxicol Environ Saf 214, 112105.
[49] USDA-NASS (2022). ⟨https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=WASHINGTON⟩ (accessed on 5 September 2024).
[50] Free, J.B., 1968. Dandelion as a competitor to fruit trees for bee visits. J Appl Ecol 5 (1), 169–178.
[51] Zhang, G., Dilday, S., Kuesel, R.W., Hopkins, B., 2023. Phytochemicals, probiotics, recombinant proteins: enzymatic remedies to pesticide poisonings in bees. Environ Sci Technol 58 (1), 54–62.