Environmental Toxicology and Chemistry, 2025, 1–13
https://doi.org/10.1093/etojnl/vgaf218
Advance access publication: September 2, 2025
Original Article

Hazard/Risk Assessment

Pesticides detected in two urban areas have implications for local butterfly conservation

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Abstract

Human-managed green spaces in urban landscapes have become important focal points for insect conservation, partly because of the desirable insect diversity that these areas support, and also because exposure to nature is important for human health and well-being. An important issue in insect conservation is the extent to which nonpest insects are impacted by pesticide applications, but this has been relatively less examined outside of agricultural landscapes. Here, we investigated green spaces, including parks and private yards, in two urban areas (Sacramento, California, and Albuquerque, New Mexico, United States), asking if larval host plants for butterflies in the two regions contained herbicides, insecticides, and fungicides. We assayed 336 individual plants in 19 genera, including woody and herbaceous plants. Pesticide presence was ubiquitous: only 22 samples had no detectable levels of pesticides; the median number of compounds detected in the other 314 individual plants was three; and the maximum detected in any one plant was 18. Within Sacramento, azoxystrobin was detected in 84% of all samples, whereas atrazine was detected in 70% of samples within Albuquerque. Two compounds (azoxystrobin and chlorantraniliprole) were found to exceed concentrations that are known to cause lethal and sublethal effects in 71 out of 336 plants. Our results suggest that the effects of pesticides on nontarget species should be further explored in urban areas, and that nontarget effects on desirable insects are possible in these areas without thoughtful management and elimination of nonessential pesticide applications.

Keywords: insecticide, herbicide, fungicide, nontarget insects, Lepidoptera

Introduction

In the midst of the ongoing insect biodiversity crisis (Crossley et al., 2021; Eisenhauer et al., 2019; Forister et al., 2021; Van Klink et al., 2020; Wepprich et al., 2019), exposure to pesticides has been identified as a threat to nontarget insect populations (Serrão et al., 2022). These findings are somewhat unsurprising, given the trend toward the use of compounds with increasing toxicity to invertebrates over time, even when controlling for reductions in application rate (Schulz et al., 2021). Although our understanding of the influence of pesticide use on insect population trajectories in and near agroecosystems continues to grow (Brereton et al., 2011; Forister et al., 2016; Gilburn et al., 2015; Kuechle et al., 2022; Shirey & Ries, 2023; Van Deynze et al., 2024), relatively little attention has been devoted to understanding pesticide concentrations in urban areas. These densely populated areas contain impervious infrastructure such as houses, roads, and commercial buildings, but also insect habitat such as gardens and public parks (Meftaul et al., 2020; Pickett et al., 2011). Adequate habitat quality within urban areas is integral in the preservation of both insects and the myriad ecosystem services they provide (e.g., pollination, nutrient cycling, and cultural value, among others; Schowalter et al., 2018). These managed green spaces also support biodiversity, and in some instances, have been shown to contain diverse insect pollinator communities (Hall et al., 2017).

Urban areas may house a more diverse array of pesticides than agricultural settings (Wittmer et al., 2010), as the compounds used in urban areas are applied to control a wider diversity of pests within a range of differing systems (e.g., structural, ornamental, and home garden), rather than management of a handful of known pests within a typical agricultural monoculture cropping system (Meftaul et al., 2020). Furthermore, application rates are known to far exceed those of conventional agriculture on a per-area basis (Haith & Duffany, 2007; Hoffman et al., 2000); considering all nonagricultural applications within the United States, a total of 42 million kg of conventional pesticides were used over the course of a single year (Atwood & Paisley-Jones, 2017). In addition, pesticides can be introduced to urban landscapes inadvertently because of agricultural field runoff or drift, particularly when these compounds are applied indiscriminately (Hoffman et al., 2000; Luo & Zhang, 2010). Pesticides can also be introduced to urban green spaces as a consequence of volatilization (Bedos et al., 2002). Given the limited habitat available to urban insects, the presence of pesticides in urban

Received: March 17, 2025. **Revised:** July 9, 2025. **Accepted:** August 7, 2025

green spaces could create an ecological trap, wherein an organism drawn to the space for food or other resources could experience lethal or sublethal effects following exposure to a given pesticide. Previous work has established patterns of pesticide contamination in urban surface waters (Nowell et al., 2021) and in pollen collected by bees near cities (Botías et al., 2017), but few studies have explored pesticides detected within known butterfly host plants in urban areas.

In this study, we assayed the presence and concentration of pesticides found within known or presumed butterfly host plants collected from urban spaces within Albuquerque, New Mexico, and Sacramento, California. We focused on butterfly host plants because both the life history of butterflies and their host plant associations are well established (Scott, 1986) relative to other insect taxa, facilitating an examination of pesticide residues contained within a set of known larval resources and potential effects on a major group of insects. Further, a variety of butterfly host plants can be present in urban spaces, often through intentional plantings of pollinator gardens, such as milkweed stands for monarch butterflies. In our study, host plants were opportunistically sampled from sites representative of urban areas, including parks, pollinator gardens, and private yards. Our primary objective was to describe the assemblage of pesticides that are present within urban landscapes and their field-realistic concentrations. To explore potential sources of detected pesticides, we gathered county-specific agricultural and nonagricultural application data available for Sacramento, CA, to examine whether some of the pesticides observed within the sampled urban areas were potentially attributable to drift or run-off from reported applications. Finally, we referenced the toxicological literature to provide context for our pesticide detection results and discuss instances wherein exposure to detected concentrations might result in sublethal or lethal effects for nonpest lepidopterans and other related species.

Materials and methods

Host plant sampling

Host plant samples were collected from Sacramento, California, and Albuquerque, New Mexico in the spring of 2022. To select the optimum timeframe in which to sample, we used iNaturalist to generate a list of commonly observed plants at both parks and natural areas within each city, as well as the approximate time periods in which those species were known to leaf out (iNaturalist, 2022). The plant list was then cross-referenced to establish which larval lepidopteran species were likely to use specific plants as larval hosts (Calflora, 2024; Cary & Toliver, 2024; Scott, 1986), and final candidate species were sampled (if present) within each city (more details below). Within Sacramento, we collected samples from 14 different sites, including public city parks ($n = 9$) and privately managed urban sites ($n = 5$), such as home yards or privately owned pollinator gardens, during the month of May (Figure 1A). Within Albuquerque, we collected samples from 10 different sites, chosen to represent a similar array of city parks ($n = 5$) and privately managed urban sites ($n = 5$) during the month of June (Figure 1B). A total of 19 host plant genera were sampled.

To sample privately managed urban sites, we recruited volunteers from the Native Plant Society and Master Gardener chapters within Sacramento and Albuquerque. Volunteers were asked to complete a survey that assessed the suitability of their property for sampling, including the number of target butterfly host plant genera within each yard and if they apply pesticides. Given the constraints of sampling at privately owned sites, we were unable to randomize the locations selected for sampling and instead chose sites in response to volunteer interest and access to locations. City parks were also selected opportunistically.

For each site, iNaturalist was referenced to establish the potential presence of plant species that are either known larval hosts or belong to genera containing known larval host species (iNaturalist, 2022). Plant sampling was conducted haphazardly by first taking a preliminary walk throughout the sampling area to establish the location of all focal plant genera. The website iNaturalist and other online resources were used to identify plants to the lowest taxonomic level possible (either genus or species; iNaturalist, 2022). A total of 10 plants per target species were sampled per site; for a site containing fewer than 10 plants of a given species, all plants were sampled (Tables S1 and S2, see online supplementary material). Plant samples were chosen to represent their spatial extent within a site. For example, for sites with an elevational gradient extending from upslope down to a riverbank, we sampled individuals from across the gradient. When unconstrained by site-level variables (such as the location of plants, plant availability, and plant size), plants were sampled from the edges to the center of a site. Examples of plant locations within a site are provided in Figure 1C and D.

We sampled between 5–10 g (wet wt) of leaf tissue per plant. Smaller forbs were often close to the 5 g minimum sample size, and thus, we sampled the majority, if not all, leaves. Tall trees were sampled by removing accessible leaves toward the base of the plant, whereas smaller trees or shrubs were sampled by removing leaves from the center of the plant (neither apical nor basal). Clippers were cleaned with 90% isopropyl alcohol between samples. Sampled leaves were placed into squares of aluminum foil, wrapped, and transported in plastic bags with ice packs. After collection, samples were stored in the freezer at −20 °C and then overnight shipped to the Cornell Chemical Ecology Core Facility for analysis.

Chemistry

A modified version of the EN 15662 QuEChERS procedure (European Committee for Standardization, 2008) was used to extract host plant leaves, and the extracts were screened for 94 pesticides (including pesticide metabolites and pesticide breakdown products) by liquid chromatography mass spectrometry (LC-MS/MS; Table S3, see online supplementary material). Our list of analytes was compiled according to three main parameters: (1) the pesticide is used extensively in United States agriculture or by homeowners, (2) the pesticide is relevant to insects (e.g., studies have shown that it is either toxic to insects at environmentally realistic concentrations or in combination with other pesticides), and (3) the pesticide can be quantified using ultraperformance LC-MS/MS with a limit of quantification (LOQ) that is of a relevant toxicity to insects. Host plant leaves were extracted as follows. Frozen leaves (5–10 g) were cut into small pieces and mixed with 5 mL of water and 10 mL of acetonitrile. The resulting mixtures were homogenized for 1 min with ceramic beads (∼10 g, 2.8 mm diameter) using a Bead Ruptor 24 (OMNI, International, United States). A mixture of salts (6.5 g, EN 15662) comprised of 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate tribasic dihydrate, and 0.5 g sodium citrate dibasic sesquihydrate was added. Samples were shaken vigorously for 1 min on the Bead Ruptor, centrifuged at $7,300 \times g$ for 5 min, and 1 mL of the resulting supernatant was placed into a dispersive solid phase extraction tube containing 900 mg $MgSO_4$ and 150 mg primary secondary amine (PSA). The samples were vortex-mixed for 1 min and centrifuged at $7.3 \times g$ for 5 min. Two hundred ninety-four microliters of supernatant were collected and 6 μL of a

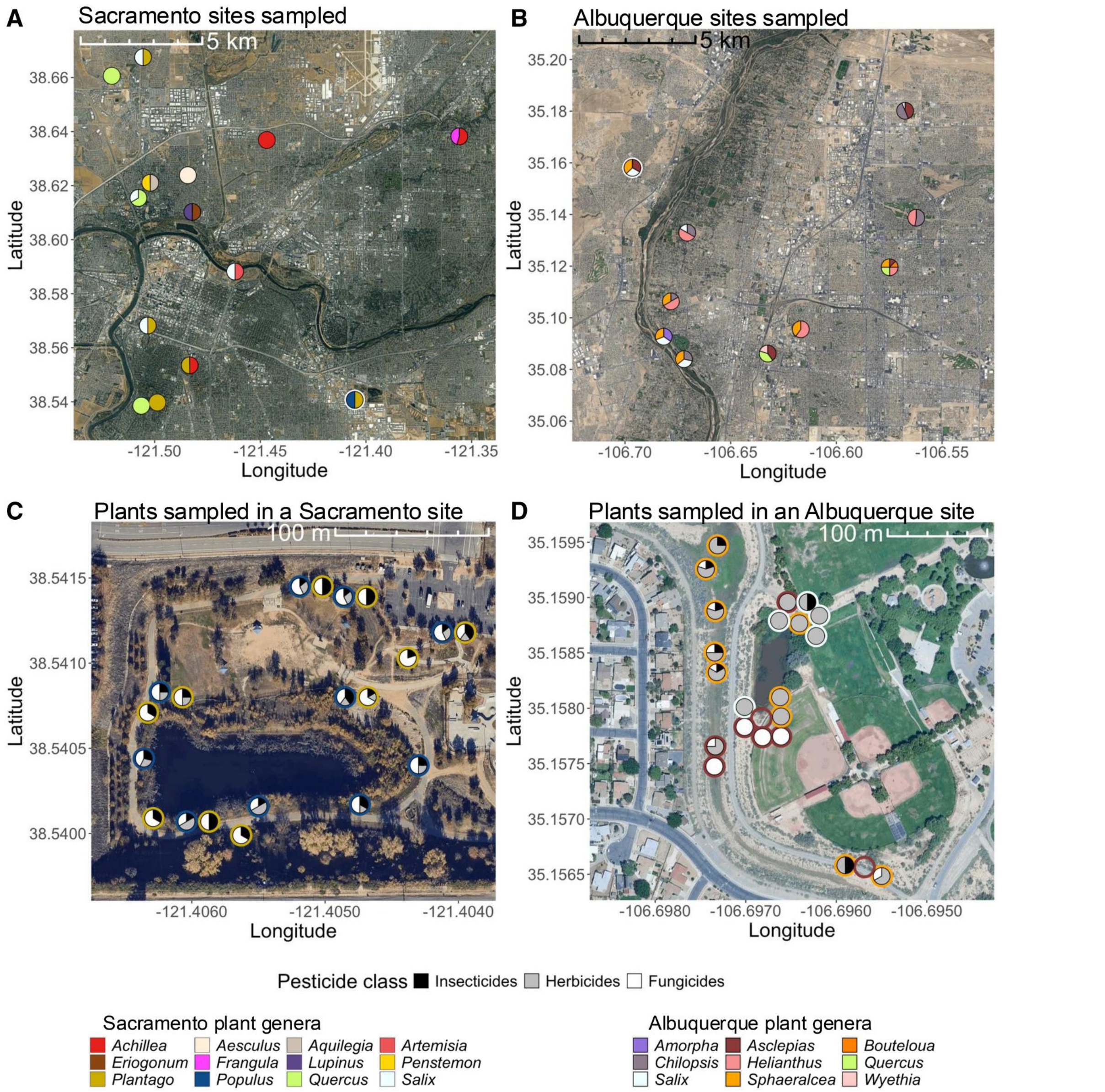

Figure 1. (A–B) Maps depicting the sampling locations for Sacramento, CA, USA (A; $n = 14$) and Albuquerque, NM, USA (B; $n = 10$). Pie charts are color coded by the plant genera sampled within each site. Pie charts outlined in white indicate the focal site that is featured in the bottom panels, C and D. (C–D) Maps of representative parks from within Sacramento, CA (C) and Albuquerque, NM (D). Pie charts show the proportion of pesticide detections belonging to each pesticide class in individual plants sampled within the park, with plant locations jittered to avoid visual overlap. The outline color of each pie chart indicates the genera of the plant sampled.

solution containing three internal standards (0.3 μg/mL 13C3-metalaxyl; 0.3 μg/mL 2H3-pyraclostrobin; 0.15 μg/mL 2H4-fluopyram) was added. Resulting samples were filtered (0.22 μm, polytetrafluoroethylene) and stored at −20 °C before analysis.

Samples were analyzed by LC-MS/MS using a Vanquish Flex UHPLC system (Dionex Softron GmbH, Germering, Germany) equipped with a Waters Acquity UPLC BEH C18 column (2.1 mm x 100 mm, 1.7 μm particle size), in conjunction with a TSQ Quantis mass spectrometer (Thermo Scientific, San Jose, CA, United States). A complete description of the parameters used for this analysis is contained in the Supplementary Methods section (see online supplementary material). The range of limit of detection (LOD) and LOQ for each compound (assuming 1 g of sample) varied between runs and are summarized for compounds included in the pesticide screening in Table S4 (see online supplementary material). Level of detection refers to minimum concentration at which a compound can be distinguished from a blank sample (but the concentration of the sample cannot be reliably quantified), whereas LOQ is the concentration at which a compound can be quantified. In our study, LOQ is equal to three times the LOD. When a pesticide was detected in a sample at a concentration that fell below the LOQ value, we used the mass-adjusted LOD (i.e., LOD multiplied by the sample mass) value for that sample. This approach could underestimate the concentration for these samples but provides a conservative value for samples with trace amounts.

Finally, it is also important to note the limits of the assay used to determine the presence and concentration of pesticides within our samples. Multi-residue detection in complex sample matrices can be difficult, potentially resulting in signal suppression or enhancement ("matrix effects"; Veiga-del-Baño et al., 2024). Although the analytes selected for this analysis were carefully chosen to reflect pesticides that are commonly used within the

United States, we also acknowledge the potential error associated with the method employed for pesticide detection.

Analysis

The sampling design for this study, described above, was largely opportunistic and guided by the presence and location of target plants, and availability of volunteer yards. Because of the nature of that sampling, we focused our analyses on descriptive, summary statistics, and visualizations of data, rather than the development of analyses for which assumptions (e.g., independence and random samples) might not be met. Thus, our presentation of results is aimed at documenting a snapshot of the pesticide landscape rather than evaluating specific hypotheses. Summary statistics and figures were all generated in R version 4.2.2 (R Core Team, 2024). Maps were generated using the ggmap (Kahle & Wickham, 2013), ggspatial (Dunnington, 2023), and PieGlyph (Vishwakarma et al., 2024) packages.

Exceedance data literature search

To examine the potential biological relevance of our findings, we compared the detected pesticide concentrations to established oral lethal concentrations found within the literature (where available) and interpreted exceedances of oral lethal concentrations in the context of the published residual level values (RL50) for a given compound, which contain information about rate of decay in the environment. We emphasize that this study is not a traditional risk assessment, and the studies and their reported thresholds referenced herein are to provide context for the concentrations that we detected. One of those thresholds is lethal concentration (LC), which refers to the concentration of a chemical that is expected to cause death in a certain percentage of the study animals after a specified period of exposure (Heath, 1972). Using ISI Web of Science and the ECOTOXicology Knowledgebase (Olker et al., 2022), we used the search terms (lepidop* OR butterfl* OR moth*) AND (name of compound) for each of the detected compounds to identify studies that experimentally exposed caterpillars to a given compound and reported LC endpoints. Lethal concentration values are directly comparable to our study units, which are in ppb (quantified as ng of compound/g of plant). In contrast, lethal dose values (e.g., LD50) are in units of compound per units of body weight (Trevan, 1927) but were included in our literature search in instances where LC endpoints were lacking. When information on nonpest lepidopterans was unavailable, we referenced the pest lepidopteran literature. If neither butterfly nor pest lepidopteran data were available, we referenced the bee literature. Although both pest lepidopterans and bees have limited relevance to butterfly physiology (e.g., Hoang et al., 2011; Voelckel & Jander, 2014), information derived from these groups is still better than a complete lack of information and were thus used to inform our understanding of relative toxicity of detected compounds for butterflies.

To assess environmental fate and whether chronic or acute exposure would be possible at a given detected concentration, we referenced the University of Hertforshire's Pesticide Properties Database for RL50s (Lewis et al., 2016). Residual level is the range of days for which a given compound's concentration declines by 50% when the compound is contained within or on the surface of a particular plant matrix, wherein "plant matrix" refers to the plant tissue type (e.g., leaf or flower) and species in which a concentration for a given compound was measured. We use established RL50 values as a proxy for how detected compounds may behave in sampled host plants.

Pesticide use data

To explore how patterns of pesticide detection relate to pesticide use patterns, we obtained data for pesticide application methods and the amount of active compounds applied for Sacramento County, California and Yolo County, California from the California Pesticide Use Report for the months before plant sampling occurred in Sacramento: January–May of 2022 (California Department of Pesticide Regulation, 2024a). Using these data, we fit a generalized linear model with a binomial distribution that examined the relationship between log-transformed pesticide application data (in total pounds of chemical applied) and the proportion of plant samples for which a given compound was detected. Although all sampling was conducted within the boundaries of Sacramento County, we elected to include Yolo County data to account for movement of pesticides applied within this county, as this region borders the sampled area. Pesticide application data were filtered to only include pesticides that host plant leaves were assayed for in the pesticide screen, yielding 87 compounds of the 94 that were tested. Degradants were included under the parent compounds. See Table S4 (see online supplementary material) for a complete breakdown of application data, including references for synonyms and degradants. These data include all pesticide applications made to support agricultural production except for seed treatments. They also include nonagricultural pesticide applications made to maintain roadside and railroad rights-of-way, parks, golf courses, cemeteries, and any application of a restricted material and/or made by a licensed pest control operator (e.g., landscaping or pest management professional). Consumer home-and-garden uses are not included. To the best of our knowledge, data on pesticide use are not available for Albuquerque, New Mexico.

Results and discussion

A total of 336 individual plants from 19 different genera were sampled (Figure 1; Tables S1 and S2, see online supplementary material), of which 314 samples were found to contain at least one pesticide. We detected 47 of the 94 pesticides included in the screening, of which 14 were insecticides, 10 were herbicides, 20 were fungicides, one was an antibiotic, one was the breakdown product of an insecticide, and one was an adjuvant (Figure 2). Adjuvants are commonly used in pesticide mixtures to enhance the potency of the active ingredients. For samples in which pesticides were detected, the median number of compounds per plant was three, and the maximum number of compounds per plant was 18. Sampled host plants contained a range of 0–10 different fungicides, 0–7 different herbicides, and 0–7 different insecticides. Below, we detail compounds that were detected in high prevalence, reference potential sources of pesticides that were detected, and explore the potential consequences of the compounds that were detected given their concentrations.

Pesticide detection

Pesticide detection across cities and plant genera

Although pesticides were ubiquitous within both cities, several differences can be noted in the prevalence and assemblage of detected pesticides in each city (Figure 2). In Sacramento, we sampled more individual plants (181 plants from 12 genera) and detected a slightly higher number of pesticides (37 compounds) than in Albuquerque (31 compounds detected in 155 plants representing nine genera; Figure 2). The difference in the number of compounds was primarily due to a difference in the number of fungicides, with more fungicide compounds detected in

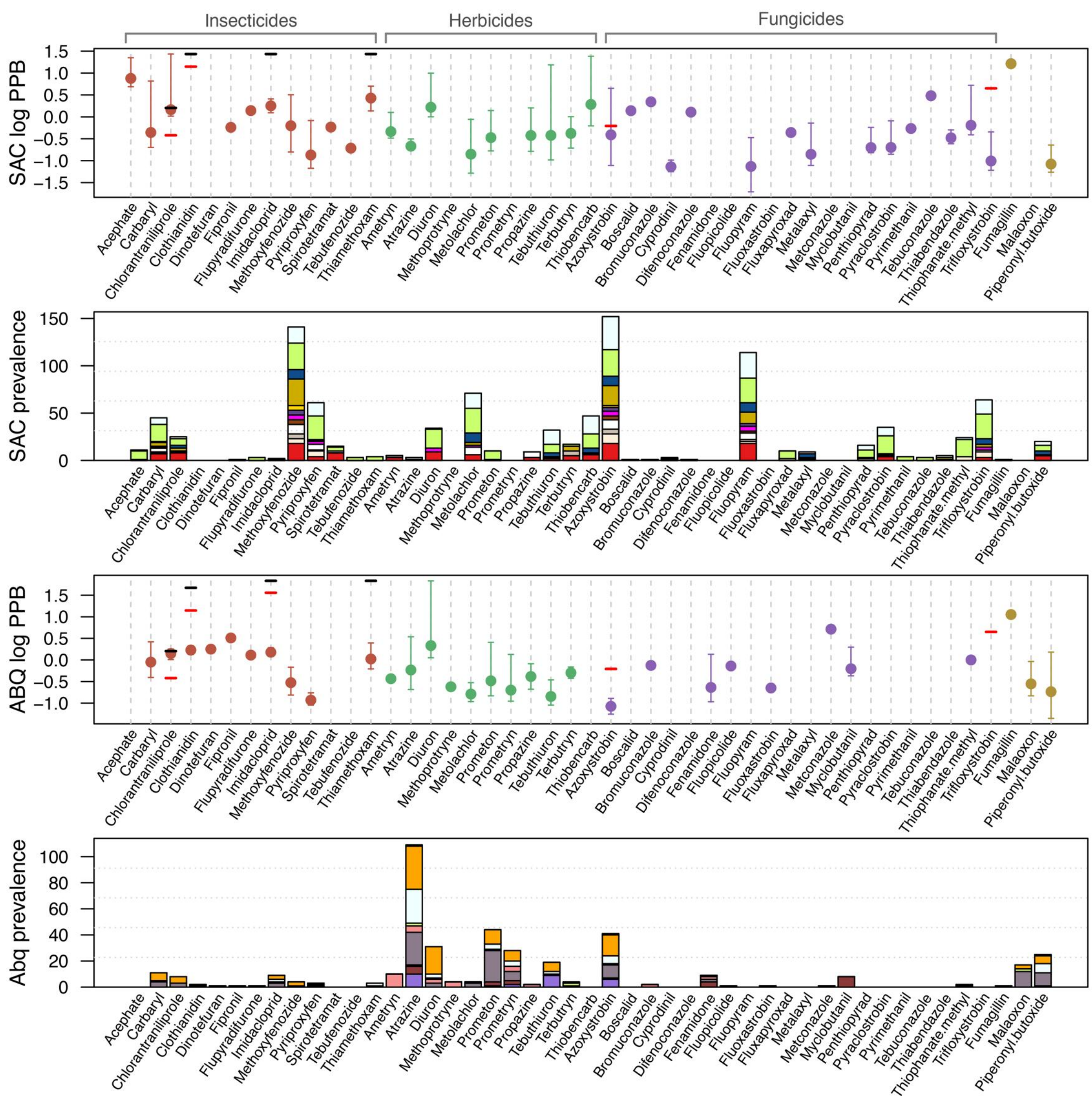

Figure 2. Panels A and C: Mean and range concentration of pesticide detected (in parts per billion [PPB] on a log scale) by compound and compound class in Sacramento (SAC; A) and Albuquerque (ABQ; C). Averages were calculated for each compound after excluding samples in which that respective compound was not detected. Compounds without a datapoint were not detected in that city. Red horizontal lines, where present, indicate an established 10% lethal concentration (LC10) value or sublethal effect for nonpest lepidopterans. Black horizontal lines, where present, indicate established LC50 values for nonpest lepidopterans. Colors of the mean and range indicate the compound class to which the pesticide belongs, wherein insecticides are dark red, herbicides are green, fungicides are purple, and others are dark yellow. Panels B and D: Compound prevalence in Sacramento (B) and Albuquerque (D). Bars show the total number of plant samples in which compounds were detected (out of 181 and 155 total sampled plants for Sacramento and Albuquerque, respectively). Colors within the bars correspond to plant genus (as depicted in the legend) and indicate the number of respective samples for each plant genus.

Sacramento (Figure 2). The average number of compounds contained within a sample was 5.5 and 2.6 for Sacramento and Albuquerque, respectively. Within Sacramento, three compounds were detected in the majority of samples, wherein azoxystrobin (fungicide) was the most prevalent compound, contained in 84% of all samples; methoxyfenozide (insecticide) was in 78% of samples; and fluopyram (fungicide) was in 63% of samples (Figure 2B). This result is consistent with previous work that established methoxyfenozide, azoxystrobin, and fluopyram as being amongst the most prevalent compounds detected within milkweed plants, an important host plant for the monarch butterfly, across various habitat types (including urban areas) in the Central Valley of California (Halsch et al., 2020). Azoxystrobin was also detected within more than 75% of milkweed nursery plants sampled from 15 states within the United States (Halsch et al., 2022). Conversely, atrazine (herbicide) was the only prevalent compound within Albuquerque, contained in 70% of all samples (Figure 2D). This variation between cities is not surprising given that Sacramento is within the most productive agricultural region in the United States (the Central Valley), and therefore likely exposed to drift from neighboring agricultural areas (see Potential sources of pesticides in Sacramento).

Overall, woody plants contained more compounds, and the highest numbers of pesticides were detected in *Salix* and *Quercus*, which contained 28 and 29 compounds, respectively (Figure 3). However, this pattern might arise because of sampling limitations for herbaceous species, as representation of woody genera was higher across sites. Within the herbaceous genera, *Achillea* contained the highest number of compounds detected (22); however, compound accumulation curves for this and other

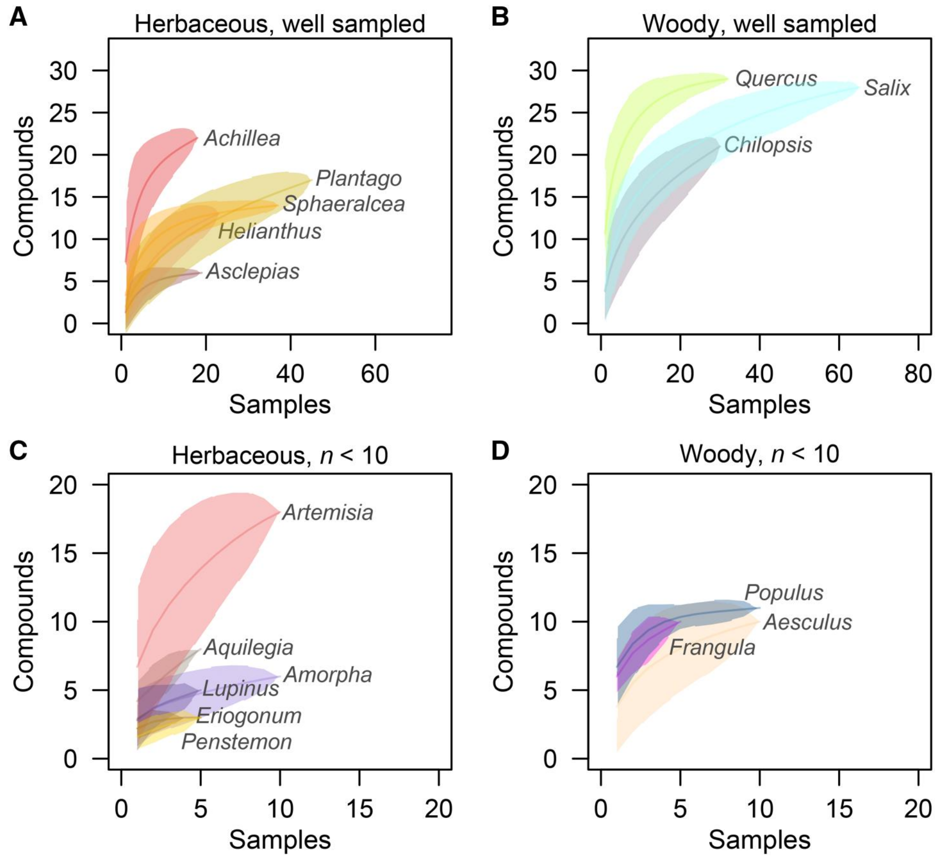

Figure 3. Compound accumulation curves by woody and herbaceous genera. *Salix* and *Quercus* were sampled in both Sacramento, CA, USA, and Albuquerque, NM, USA, and thus compound numbers for these genera are pooled across cities. All other genera were sampled within one city only. This graph excludes *Bouteloua*, which contained too few samples to rarefy. See Tables S1 and S2 (see online supplementary material) for a complete list of plant genera that were sampled.

herbaceous genera suggest these numbers are conservative and compound richness would likely increase with additional sampling (Figure 3A and C). Across genera, 79% of plant samples contained residues of multiple compounds. Only 10 (out of 181) and 12 (out of 155) plants were devoid of pesticides within Sacramento and Albuquerque, respectively. Both *Salix* and *Quercus* samples collected from Sacramento frequently contained a combination of insecticides, herbicides, fungicides (Figure S1, see online supplementary material), and the plateaus evident in compound accumulation curves for these genera suggest that they were both sufficiently sampled to estimate compound richness (Figure 3). Beyond arising as an artifact of sampling, woody genera may accumulate more pesticides because they are exposed to pesticide application for the entirety of the year, rather than dying back during the winter months. Further, if agricultural runoff is reaching urban sites, deeper roots may facilitate the uptake of pesticides contained within groundwater. In contrast, the herbaceous genera *Plantago* and *Helianthus* less frequently contained mixes of compounds (Figure S2, see online supplementary material).

Pesticide detection within sites and limitations of pesticide detection

Accumulation curves visualized at the level of individual sites were generally more indicative of rising compound richness with additional samples (Figure 4). However, it is interesting to note that the patterns of richness detected, even for curves that have not reached a plateau, revealed comparable ranges of pesticide richness across public and private areas for each city (Albuquerque private range: 5–14 compounds; Albuquerque public range: 9–15; Sacramento private range: 8–20; Sacramento public range: 4–23). We acknowledge that our estimates of the number of compounds in those spaces are certainly conservative and would increase with additional study. Private sites seemed to be slightly more prone to under-sampling (Figure 4A and C), but it is important to remember that this study was dependent on landowners volunteering access to their property. In the intake survey that was provided to screen for potential volunteers, most volunteers who allowed us to sample from their backyards indicated that they rarely (if ever) applied pesticides to their gardens and backyards, and we would thus anticipate that a higher number of compounds would be found within the backyard of an average or randomly sampled homeowner.

In addition to sampling limitations arising from nonrandom site access, we expect our estimation of pesticide residues in urban areas to be conservative because the pesticide screening did not exhaustively assay all possible pesticides. Notably, we were unable to test for the presence of pyrethroid insecticides, which represent a potentially large source of contamination within the California Central Valley: within the last decade, pyrethroids have increased in surface water detections within the California Central Coast (DeMars et al., 2021), and they are commonly used to control pests within the Central Valley (California Department of Pesticide Regulation, 2024a). We were also unable to test for glyphosate, an herbicide commonly used across urban and agricultural landscapes (Duke, 2018). As we measured pesticide concentrations of host plants at a single time point in early spring, we were unable to capture variability of compounds that might

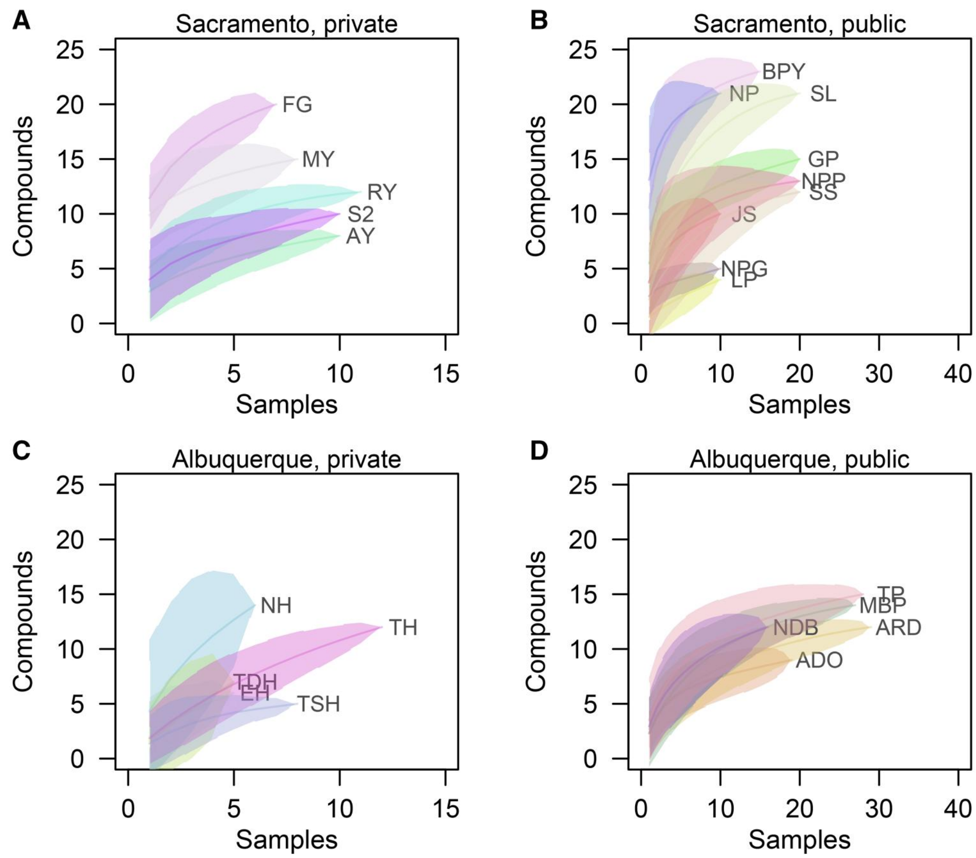

Figure 4. Compound accumulation curves by city and site type (public vs. private). Panels A and B depict Sacramento, CA, USA, private and public sites, respectively, and Panels C and D depict Albuquerque, NM, USA, private and public sites, respectively. Sites are denoted with different colors. Site codes are provided for reference within Tables S1 and S2 (see online supplementary material), where a breakdown of plants sampled at each site is provided.

have been detected throughout the year or across years (Olaya-Arenas & Kaplan, 2019). We might expect to see more or different compounds in plant tissues at different sampling times given that certain compounds are applied and detected earlier or later in the growing season (Brühl et al., 2021; Hladik et al., 2023; Olaya-Arenas & Kaplan, 2019). The sampling timeframe that we selected, however, overlaps with when a variety of developing larvae are known to emerge and begin to feed (Scott, 1986; Shirey et al., 2022). Even if not actively feeding, larvae could still experience cuticular exposure to pesticides being applied on vegetation or soil. Ultimately, the findings from host plants in Sacramento, California, and Albuquerque, New Mexico suggest nonpest Lepidoptera using host plants within urban green spaces are likely to be exposed to a variety of pesticides.

Potential sources of pesticides in Sacramento

Broadly, pesticide application amounts reported within Yolo County and Sacramento County during the spring of 2022 were weakly, but positively, associated with the frequency at which we detected compounds within sampled host plants (ß = 0.9476 ± 0.0371 on the log scale, $df = 1$, $p < 0.0001$, $R^2 = 0.27$; Figure 5). This finding suggests that detected compounds may have been partly attributable to movement from application sites (sampled sites were 577–5,429 m away from the nearest agricultural field), within the range of distances at which pesticide movement has been previously observed (Baio et al., 2019; Lenard et al., 2025; Pimentel & Levitan, 1986). Conversely, the low variance explained is likely attributable at least in part to the complexity of the sources of detected pesticides beyond reported applications, as well as the course spatial grain of the analysis.

Frequently detected pesticides may have originated from various application types. The prevalent insecticide methoxyfenozide was reported only in agricultural settings (California Department of Pesticide Regulation, 2024a), where it was sprayed from ground equipment onto fruit and nut crops (Figure S3, see online supplementary material). The prevalent fungicides azoxystrobin and fluopyram were predominantly applied via ground equipment (with some aerial application) in an agricultural setting, with some applications in nonagricultural settings (California Department of Pesticide Regulation, 2024a; Figure S3, see online supplementary material). Although these results suggest that the presence of these compounds in host plant samples collected in the city of Sacramento may be partly attributable to agricultural drift, these pesticides may have also originated from nonreported uses. Given the high prevalence of azoxystrobin detected within sampled nursery plants (among other active ingredients; Halsch et al., 2022), nurseries may serve as a potential source of compounds. Homeowner applications are also a potential source because each of these active compounds can be found in both restricted-use and general-use pesticide mixes (Britten, 2024; California Department of Pesticide Regulation, 2024b). Similarly, milkweed plants sampled from agricultural, urban, and wildlife refuge areas had comparable ranges of methoxyfenozide concentrations, making it difficult to infer the sources of this compound (Halsch et al., 2020). Methoxyfenozide was also detected ambiently within five wildlife refuges dispersed across the Sacramento Valley (Lenard et al., 2025).

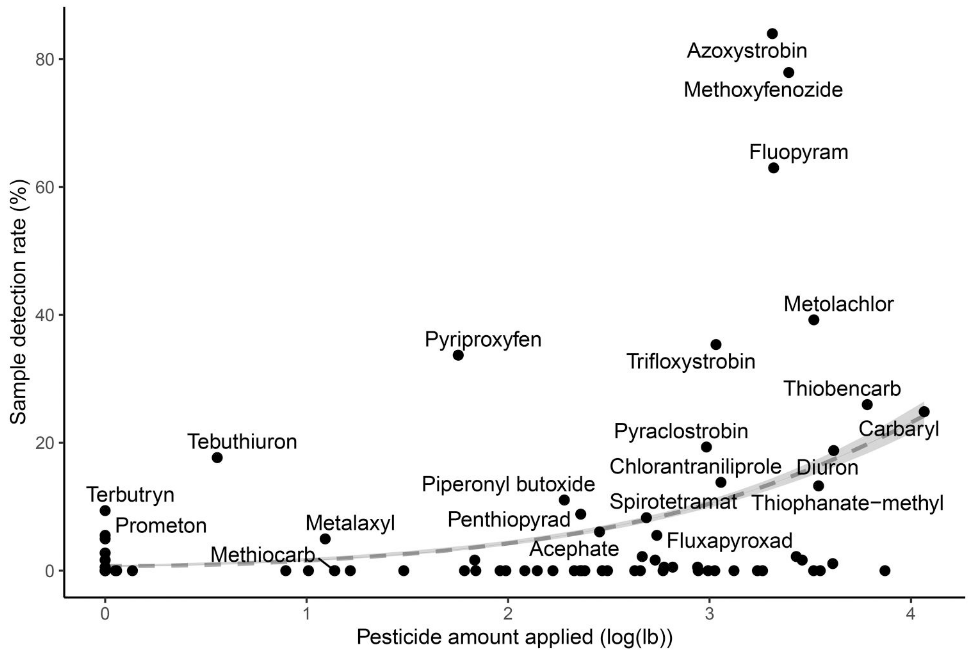

Figure 5. Log-transformed pesticide application data from the months of January through May of 2022, as compiled from both Sacramento and Yolo, CA, USA counties (California Department of Pesticide Regulation, 2024a) versus the percentage at which pesticides were detected within host plants sampled from urban areas in the city of Sacramento. The dark gray dashed line shows the fit of the generalized linear model with a binomial distribution predicting percent sample detection by log-transformed pesticide amount applied in pounds, with the associated 95% confidence interval in light gray (ß = 0.9476 ± 0.0371 on the log scale, $\chi^2 = 1,040$, $df = 1$, $p < 0.0001$, $R^2 = 0.27$). The following compounds were detected in plant samples but did not show records of use during the focal period: ametyryn, atrazine, prometon, propazine, terbutryn, thiabendazole, fumagillin.

In contrast to methoxyfenozide, we can infer that detections of thiobencarb in Sacramento stemmed from exclusively agricultural applications. Thiobencarb is a restricted-use pesticide that is applied solely in rice within the state of California (California Department of Pesticide Regulation, 2024a), which is grown to the north and west of Sacramento (United States Department of Agriculture, 2024). This compound was reported as having been applied aerially during the spring months before sampling (California Department of Pesticide Regulation, 2024a; Figure 5; Figure S3, see online supplementary material). Spray drift from aerial application poses a risk to nontarget terrestrial plants (U.S. Environmental Protection Agency, 1997), indicating that the detection of thiobencarb within both public and private urban green spaces resulted from movement out of agricultural landscapes. Atrazine is another restricted-use compound that was detected, but no applications of this compound were reported in the 5 months preceding our sampling within the focal counties. Applications were thus either not reported or the residues we detected originated from movement outside of the focal counties. Such movement is possible, as this compound can persist in soil (half-life of 130 days), leaches easily through soil substrate, and is known to contaminate groundwater (U.S. Environmental Protection Agency, 2016). Atrazine can also reach the atmosphere via volatilization or particle transport, after which it can be deposited hundreds of kilometers away (Thurman & Cromwell, 2000). In addition to its detection in Sacramento, this compound was the most frequently detected in Albuquerque. Although pesticide application data are not available for Albuquerque, atrazine was detected in New Mexico drinking water during the year of sampling and years preceding sampling (Environmental Working Group, 2023). Within this state, atrazine is commonly used to control broadleaf and grassy weeds in rangeland and agricultural ecosystems (Environmental Working Group, 2023; Young & Spackman, 2021).

Beyond atrazine, six other compounds were detected in our samples but not listed as having been applied in either county relevant to our Sacramento samples, including ametryn, prometon, propazine, terbutryn, thiabendazole, and fumagillin, none of which are restricted use. The detection of the systemic fungicide thiabendazole could be explained by movement from other counties, carryover from previous applications, or possibly by their use as seed treatments. Similarly, thiophanate-methyl was applied in fruit and nut crops (Figure S3, see online supplementary material), but detections may have also originated from its unreported use as a seed treatment, because the planting of pesticide-coated seed is currently not tracked by California's pesticide use reporting system. A full list of frequently detected compounds and their status as both systemic and/or seed use treatments can be found within Table S6 (see online supplementary material). Other compounds that were detected but not reported may have originated from noncommercial applications.

Although our findings suggest that agricultural applications of pesticides are likely important sources of pesticides within urban environments, it is also imperative to consider what is applied within urban spaces. For example, carbaryl was one of the more frequently detected insecticides (25%) in our samples from Sacramento and is frequently used in home and garden settings (Atwood & Paisley-Jones, 2017). However, these applications are more difficult to track, and we were unable to account for them in our examination of pesticide use in Sacramento because private applications of unrestricted pesticides are not reported to the California Department of Pesticide Regulation. This emphasizes the role of the homeowner in privately owned spaces. Outside of constraints on consumer availability, the only regulation of homeowner pesticide applications is what is available in stores and through the instructions provided on the label. Sampling of urban waterways shows that runoff and leaching from urban applications occur (Nowell et al., 2021). The risk of this increases with home applications, because training is not required for private pesticide applications of unrestricted compounds, and untrained applicators might fail to follow application instructions and spread pesticides beyond the intended application area. Although the modern agricultural and nonagricultural spheres have become dependent on pesticides, we can be more selective about what is applied to our landscapes to achieve multiple outcomes. Pests can be managed through numerous nonchemical means (Sawyer & Casagrande, 1983), while still allowing urban spaces to support and bolster biodiversity, and consequently, human health.

Exceedances of lethal and sublethal values for butterflies

Of the 47 compounds that were detected, oral LC10, oral LC50, oral LC90, or other records of established sublethal effects for nonpest lepidopterans were available for atrazine, azoxystrobin, chlorantraniliprole, clothianidin, imidacloprid, pyraclostrobin, s-metolachlor, thiamethoxam, and trifloxystrobin (Table S5, see online supplementary material). These studies were conducted primarily on the monarch (Nymphalidae: *Danaus plexippus*), and secondarily on two butterflies in the family Lycaenidae, *Lycaeides melissa* and *Polyommatus icarus*. We considered chronic toxicity, acute toxicity, and sublethal effects to ascertain a more robust picture of the potential threat posed to developing larvae. Although some studies were unable to distinguish between oral versus contact toxicity, these were still included.

The fungicide azoxystrobin (detected in 84% samples in Sacramento; 26% of samples in Albuquerque) and the insecticide chlorantraniliprole (detected in 14% samples in Sacramento; 0.05% samples in Albuquerque) were found in exceedance of at least one of these established values, considering chronic toxicity, acute toxicity, and sublethal effects (Table S7, see online supplementary material). Azoxystrobin concentrations in 51 plants sampled from within Sacramento either met or exceeded 0.67 ppb—the mean field-realistic concentration of milkweed plants bordering agriculture in the midwestern United States and the concentration at which reductions in wing size were illustrated to occur for monarchs (*Danaus plexippus*) in response to chronic exposure (Olaya-Arenas et al., 2020). Azoxystrobin has a residual level (RL50) ranging from 0.4 to 17.5 days on and in plant matrices (Lewis et al., 2016). On average, developing monarch larvae feed for 10–14 days (Oberhauser & Solensky, 2004). Only 15 of the 51 samples contained azoxystrobin concentrations that would still exceed the concentration at which sublethal effect are known to occur after degrading to one-half of their original value. Consequently, azoxystrobin is likely to degrade in plant tissues fast enough to limit exposure, assuming that repeated applications do not occur. However, even with a single application, under the right conditions (i.e., RL50 $\geq$ 10 days), it is possible that monarch larvae feeding on plants containing this compound could experience chronic exposure.

Of the 33 plants that contained chlorantraniliprole, the detected concentration of this compound either met or exceeded the chronic oral LC50 value for all monarch larval instars in 7 plants, and all 33 plants containing chlorantraniliprole exceeded the chronic oral LC10 value for all monarch larval instars and acute oral LC10 value for second instars (Krishnan et al., 2021). Conversely, detected concentrations of chlorantraniliprole did not exceed the concentration that was established to kill 62% of the test population for the lycaenid, *Lycaeides melissa* (Halsch et al., 2023). Within and on a variety of plant matrices, chlorantraniliprole has an RL50 ranging from 2.2 to 12.6 days (Lewis et al., 2016). A feeding larva being exposed to a concentration of chlorantraniliprole exceeding the chronic LC50 value would have been unlikely, as the six observed exceedances would be under the threshold of toxicity following the reduction of the concentration of this compound to half of its original value. For all 33 plants containing chlorantraniliprole, concentrations would have remained above the acute oral LC10 and chronic oral LC10 values, even if the substance were to degrade to half of its original concentration. Thus, if monarch larvae were feeding within several days following this study, acute oral toxicity would have been possible, and chronic oral toxicity would have been less likely, as it would hinge on the degradation process exceeding 9 days. However, these RL50 values are not representative of all methods of application. Chlorantraniliprole is also injected into trees to manage foliar pests; following injection, the concentration of this compound gradually increases within the leaves and peaks 2 months after application (Coslor et al., 2019). If chlorantraniliprole was applied via an injection in the *Populus* and *Quercus* samples that were found to contain the compound, it may have persisted longer in these plants than suggested by the RL50 range, thereby increasing the probability of chronic oral exposure. Although most chlorantraniliprole applications were reported as having been applied via spraying, some "unspecified" listings could indicate their application via injection (California Department of Pesticide Regulation, 2024a).

Our results suggest a range of nonpest lepidopteran species could be exposed to these pesticides at potentially harmful concentrations, as azoxystrobin was found across a wide array of both herbaceous and woody plants in Sacramento, and chlorantraniliprole was found in a wide array of woody and herbaceous genera in Sacramento, and one herbaceous genus (*Sphaeralcea*) and one woody genus (*Chilopsis*) in Albuquerque (Figure 2). Notably, interpretation of these exceedances using RL50 values hinges on the assumption that the compound would behave similarly in sampled plants as compared to how the compound behaved in and on tested crop plant tissues. This study also does not account for routine spraying, which could well have occurred after we sampled. Furthermore, although it was the best that could be done given the available information, using data for one species (e.g., monarch) as a proxy for other nonpest lepidopterans is a limited approach. Beyond differing developmental times, and thus differing lengths of exposure to pesticides, there is also considerable variability in response to the same compound even within a taxonomic family—monarchs have been noted to be 70 times more sensitive to clothianidin, as compared to the painted lady (Nymphalidae: *Vanessa cardui*; Krishnan et al., 2021; Peterson et al., 2019). Similarly, the monarch is much more sensitive to chlorantraniliprole as compared to the Melissa blue (*Lycaeides melissa melissa*; Halsch et al., 2023; Krishnan et al., 2021). Therefore, more information is needed to understand how the diverse butterfly species using the host plants in our focal urban areas would be affected.

Although we found exceedances of sublethal and lethal concentrations in two of the nine compounds for which there were available data, we emphasize the incomplete understanding of how the remaining 38 detected compounds may impact nonpest lepidopterans. These compounds with limited data include two of the most prevalent compounds we detected within Sacramento—fluopyram and methoxyfenozide. Although studies were lacking on the impact of fluopyram on lepidopterans, the acute oral LD50 value established for honeybee exposure to fluopyram (100,000 ppb) likely implies limited toxicity for butterflies for the range of concentrations we detected (0.16–3.2 ppb). Studies on the effects of methoxyfenozide on all lepidopterans were limited to application rates at which reductions in crop injury were found to occur—not on lethal or sublethal concentrations (e.g., Abd-Ella, 2015); consequently we cannot make inferences as to the potential impacts of this compound at the concentrations we detected, nor can we use the LC50 value that was established for bee species because this compound was formulated as an ecdysone mimic that induces a lethal premature molt in lepidopteran larvae specifically (LaLone et al., 2014). Investigation into methoxyfenozide effects on nontarget lepidoptera could be especially productive, as butterflies are listed as one of the target pests for some registered pesticide mixes that contain methoxyfenozide as an active compound (California Department of Pesticide Regulation, 2024a), and the U. S. Environmental Protection Agency pesticide screening tool (SeqAPASS) suggests that monarchs may be susceptible to this compound (LaLone et al., 2014).

For remaining detected insecticides, herbicides, and fungicides, LC50 values and other toxicity endpoints were unavailable for pest lepidopterans. We instead reference the thresholds established for acute contact LD50 values established for honeybees: highly toxic (<2 μg of active ingredient per bee), moderately toxic (2–11 μg active ingredient per bee), and practically nontoxic (>11 μg of active ingredient per bee) as a proxy for relative toxicity (U.S. Environmental Protection Agency, 2012). The insecticide acephate and the herbicide terbutryn may be highly toxic for butterflies, and the insecticide fipronil may also be moderately toxic (Lewis et al., 2016). All remaining pesticides were considered practically nontoxic to honeybees (Lewis et al., 2016), demonstrating the potential for limited toxicity to butterflies. However, these results should be interpreted with caution given the differences in physiology between hymenopterans and lepidopterans. Furthermore, the U.S. Environmental Protection Agency's toxicity ratings are based on lethality, not sublethal effects. As we discuss above, azoxystrobin, considered a "practically nontoxic" fungicide, has been found to reduce monarch wing size (Olaya-Arenas et al., 2020). Such nonlethal effects can still cause population-level impacts.

Implications for urban butterflies

Only one of the compounds that was detected in exceedance of sublethal thresholds (where established) had documentation regarding the effects of exposure on butterfly physiology or behavior. The sublethal effect documented for azoxystrobin in monarchs at 0.67 ppb was a 12.5% reduction in wing size (Olaya-Arenas et al., 2020). Even when effects are occurring at the sublethal level, an otherwise marginal reduction in wing length still has the potential to negatively impact energetic expenditure and fitness outcomes (Kingsolver, 1999). For the monarch, a critical component of survival is the ability to migrate for up to thousands of kilometers (Reppert & de Roode, 2018); therefore, over long distances, a marginal reduction in wing size may be a critical determinant of the survivorship of a monarch. Extending these effects to consider other species, wing length has been established as a proxy for dispersal capacity (Sekar, 2012), which can be especially critical in navigating a patchwork of variable habitat quality in urban areas to opportunistically exploit resources.

However, interpretability of sublethal and lethal concentration exceedances in a field-realistic context is more complex than considering the effects of each compound in isolation. Experiencing a single instance of acute toxicity (a single, short period of exposure to a given compound) is relatively unlikely for a feeding caterpillar. Furthermore, the establishment of lethal concentration and lethal dose values is always conducted in a set of controlled conditions (Morris-Schaffer & McCoy, 2021), and although necessary to solidify a clean relationship between dose and response, pesticides in urban centers represent only one axis of stress in a multi-dimensional landscape of stressors that may interact additively or synergistically. This is well-exemplified by a study examining the survivorship of *Lycaeides melissa* in response to multiple stressors—exposure to both chlorantraniliprole and environmental warming additively decreased survival rates compared to isolated pesticide exposure (Halsch et al., 2023). Pesticides can also have synergistic effects that are often overlooked in assessments of lethal limits. Some fungicides have the potential to enhance the efficacy of other compounds (Sanchez-Bayo & Goka, 2014). In addition to being one of the more prevalent compounds that we detected, the fungicide fluopyram was frequently found in concert with other pesticides, possibly affecting their toxicity. Consequently, although these studies provide a baseline from which to assess the potential effects of the compounds we detected, they do not fully encapsulate a field-realistic representation of what a larva may experience in an urban green space and may underestimate the risk pesticide exposure poses to pollinator populations.

Conclusion

We documented widespread presence of pesticides within multiple butterfly host plants in green spaces within two urban centers: Sacramento, California, and Albuquerque, New Mexico, United States. Host plants contained a mixture of insecticides, herbicides, and fungicides, which highlights the potential for additive or synergistic effects. Within each city, pesticides were ubiquitous across host plant genera collected from both public and private lands. However, we found notable differences in the composition of compounds found in the two cities: more compounds were commonly detected in Sacramento than in Albuquerque, a pattern that may relate to the presence of intensive agriculture in the immediate vicinity of Sacramento. In comparison to some other insect taxa that have been studied, lepidopteran biodiversity is particularly sensitive to urbanization (Fenoglio et al., 2021; Theodorou et al., 2020), and declines have been documented in urban butterflies across a range of study systems (Ramírez-Restrepo & MacGregor-Fors, 2017). Our results suggest that one potential contributor to this pattern could be the pervasiveness of pesticides contained in butterfly host plants in urban green spaces. We acknowledge that future studies could more directly document host use in areas assayed for pesticides, rather than inferring host use, as we have done, from the literature or from congeneric host relationships. It is also essential to remember that we lack an understanding of the nontarget effects of most of these compounds in field-realistic settings, therefore, our study highlights a suite of pesticides whose effects on nonpest lepidopterans should be considered in future studies.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

The data underlying this article are available in a repository in Zenodo, at https://doi.org/10.5281/zenodo.16929572.

Author contributions

Clare Dittemore (Data curation, Formal analysis, Visualization, Writing—original draft, Writing—review & editing), Aaron Anderson (Conceptualization, Data curation, Funding acquisition, Methodology, Writing—review & editing), Aimee Code (Conceptualization, Data curation, Funding acquisition, Methodology, Writing—review & editing), Angie Lenard (Data curation, Visualization, Writing—review & editing), Maggie Douglas (Visualization, Writing—review & editing), Chris Halsch (Visualization, Writing—review & editing), and Matthew Forister (Conceptualization, Visualization, Writing—review & editing)

Funding

C.M.D. and M.L.F. acknowledge support from the National Science Foundation (grant number DEB-2114793). A.A. and A.C. thank the Carroll Petrie Foundation (grant numbers 2022-113 and 2023-141).

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank W.A. and the Cornell Chemical Ecology Core Facility for performing the chemical analysis, and the volunteers in Sacramento and Albuquerque who allowed us to sample in their yards.

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