Lethal and sub-lethal concentrations of pesticides detected in milkweeds purchased from nurseries across the United States

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Abstract

The decline of monarch butterflies in both the eastern and western United States has garnered widespread public interest. Planting milkweed has been promoted as one action that individuals can take, but very little is known with respect to potential pesticide contamination of store-bought milkweeds during the process of production and transport to market. In this study, we collected 235 milkweed leaf samples from 33 retail nurseries across the US to screen for pesticides. Across all samples, we detected 61 different pesticides with an average of 12.2 compounds per leaf. While most of the compounds have unknown effects on caterpillars, 48 samples contained a pesticide at a concentration that exceeds a published lepidopteran LD_{50} and 89 contained a pesticide above a concentration shown to have a sub-lethal effect for a monarch. We detected only modest effects of store size and milkweed species; however, plants with labels advertising their value for wildlife were more likely to contain pesticides at concentrations known to have negative effects on monarchs. These results demonstrate the extensiveness of pesticide exposure within nursery milkweeds and the potential impacts on monarchs and other insects consuming store-bought plants.

Introduction

Insect populations are facing an expansive and interacting set of stressors [1–3]. Among native insects declining in the US, the monarch (*Danaus plexippus*) is a widely recognized butterfly whose declines have been substantial in both the eastern and western populations [4–6]. The causes of these declines are complex and region-specific, but proposed hypotheses implicate climate change, the loss of overwintering habitat, natural enemies, and pesticides, especially herbicides affecting the abundance of native milkweeds (*Asclepias sp*.). [7–10]. Many monarch conservation strategies have been suggested, from changes in agricultural practices to the planting of milkweeds by individuals in gardens and yards [11]. For people wanting to help imperiled insects, planting beneficial plants is a seemingly simple action, however the practices used to bring nursery plants to shelf often involve pesticide treatments [12,13]. A recent study found pesticides in milkweeds growing across diverse landscapes in the Central Valley of California, including up to 31 compounds in individual retail plants [14]. Milkweeds in that study were purchased from only two nurseries in one metropolitan area, thus one of the conclusions was that retail locations required further investigation.

In this study, we address the need to better understand contamination of retail plants by quantifying the concentrations of pesticides (insecticides, fungicides, and herbicides) found in the leaves of milkweed plants sold in nurseries across the United States. Stores ranged from local nurseries to large national chains and were all in areas where monarchs breed. First, we present an exploratory analysis of the detected compounds, including a search of published toxicological data for lethal effects of those compounds on lepidopterans. Second, we examine associations between observed pesticide concentrations and factors relevant to a milkweed buyer. Finally, we offer suggestions for buyers interested in planting milkweeds for monarch conservation.

Methods

Sample collection

Five species of milkweed plants were purchased from 33 retail stores across 15 states from May 15 to June 29, 2021 (Table S1). Whenever possible, five plants of each type (species x supplier) were purchased (fewer plants were available in two places). These collections were intended to represent a snapshot of milkweed plants available to the public across the monarch's migratory range. After purchase, collectors clipped at least 5 grams of leaves from an individual plant, and wrapped samples in tin foil stored in sealed plastic bags. Clippers were cleaned with soap and water or rubbing alcohol between samples. Samples were shipped with ice packs to a central location for temporary storage in a freezer, and ultimately shipped on dry ice to the lab for chemical analysis. In one instance, 10 leaf samples were collected immediately after purchase, and another 10 were collected after growing the plants outdoors in pots with daily watering for two weeks (these additional samples were excluded from the primary analysis but discussed below).

Chemical analysis

Using methods similar to previously mentioned milkweed study [14], frozen milkweed leaves were extracted by a modified version of the EN 15662 QuEChERS procedure [15] and screened for 92 pesticides by liquid chromatography mass spectrometry (LC-MS/MS). Sample analysis was carried out with a UHPLC system coupled with a TSQ Quantis mass spectrometer. MS/MS detection was carried out using the selected reaction monitoring (SRM) mode. Additional details including a full table of retention times and optimized SRM acquisition parameters for pesticides and internal standards can be found in the supplement (Table S2).

Literature search

To gain insight into potential biological impacts of observed concentrations, we collected published oral and contact LD_{50} values for honeybees from EPA records in the ECOTOX and PubChem databases as well as from the University of Hertfordshire's Pesticide Properties Database (Table S3). Honeybee LD_{50} values are widely used to estimate risk to terrestrial invertebrates, however, adult bee and caterpillar physiology are of course different, and we only use these values in a heuristic sense. To find the most directly relevant data, we also conducted a literature search for bioassay studies that quantified LD_{50} values on caterpillars. For studies that specifically looked at monarchs, we also made note of any reported sub-lethal effects. This was done in January of 2022 for all compounds identified in our samples using ISI Web of Science and the terms (lepidopt* OR butterfl* OR moth*) (Table S4).

Statistical analysis

To examine relationships between pesticides and explanatory variables, we modeled pesticide richness, diversity, and the number of exceedances of a published lethal concentration in lepidoptera (including monarchs), or sub-lethal concentration in monarchs. Diversity was represented as the effective number of compounds by taking the exponential of the Shannon diversity index [16]. Exceedance was treated as a binary category: either a sample did or did not contain a pesticide at or above a concentration known to exhibit lethal or sub-lethal effects. The predictor variables we explored were store size, milkweed species, region (eastern or western US), and whether the plant contained a label at the point of purchase with information or a claim about its value for wildlife (e.g. "attracts butterflies"). Pesticide richness, diversity, and the number of exceedances were each modeled using a generalized mixed effects model with a Poisson error structure (log link), a Gaussian error structure, and a binomial error structure (logit link) respectively. The individual store the plants were purchased from was included as a random

intercept to account for store-specific effects not encompassed by other explanatory variables. We could not separately evaluate the identity of suppliers because in most cases wholesalers provided plants to a single store.

Random effects models were built in R using the lme4 package [17] and variance partitioning was performed using the partR2 and modEvA packages [18,19]. We also explored compositional differences using principal coordinates analysis, but the results added little to the overall picture of contamination and are not discussed further but are included in supplementary material (Fig S1). Finally, to investigate the importance of wildlife labels, we used a random forest classifier with the presence of a label as the response and the presence of each compound as predictor variables (using the randomForest package [20]).

Results

We detected 61 unique compounds out of a possible 92 in the test panel: 24 insecticides, 26 fungicides, 10 herbicides, and 1 synergist (Fig. 1). The fungicides azoxystrobin, metalaxyl, and difenoconazole were the most common, found in over 75 percent of samples. We obtained honeybee contact and oral LD_{50} values for 59 of the 61 compounds, and lepidoptera LD_{50} values for 15 of the 61 compounds (from 69 studies). We found that 72 of the 235 samples contained a pesticide at a concentration that exceeded a honeybee contact or oral LD_{50}. These were associated with ten compounds and with 13 of the 25 locations (towns or cities) (Table S5). We found that 48 of the 235 samples contained a pesticide at a concentration that exceeded a published lepidopteran LD_{50}, coming from 7 locations and involving five compounds (Fig. 1, Table S6). We also detected exceedances of published monarch sub-lethal effects in 89 samples, driven by azoxystrobin, clothianidin, and trifloxystrobin, occurring in 18 of 25 locations (Table S7).

Milkweed species and store size explained the greatest variation in pesticide richness, and store size and the presence of wildlife-friendly labels explained the largest amount of variation in pesticide diversity (Table 1, Fig. S2). Specifically, larger stores were associated with higher richness (z = 2.644, p = 0.01, Table 2, Table S8, Fig. S3) and higher diversity ($t_{23.1}$ = 2.764, p = 0.01, Table 2, Table S9, Fig. S4). The presence of a wildlife friendly label was associated with lower diversity, likely due to lower evenness across compounds ($t_{30.8}$ = -2.707, p = 0.01, Table 2, Table S9, Fig. S4). While pesticide richness and diversity are one aspect of contamination, these metrics are not sufficient for understanding the realized effects of these pesticides on caterpillars. When considering the number of exceedances of a lethal or sub-lethal concentration, we found that milkweed species and the presence of wildlife labels explained the most variation (Table 1, Fig. S2). Most notably, plants with a label claiming the plant's value for wildlife had an increased chance of exceeding a lethal or sub-lethal concentration of at least one pesticide (z = 2.262, p = 0.02, Fig. 2, Table S10). Random forest analysis identified azoxystrobin and trifloxystrobin among the most predictive of whether a sample was labeled, both of which were often detected above the sub-lethal threshold in labeled plants (Fig. 2).

Discussion

Monarch butterflies are in decline in both the eastern and western United States, leading to an upswell in conservation efforts, including planting milkweeds in gardens and yards. We detected pesticides in milkweeds bought from nurseries in regions of the US where monarchs breed. The number of compounds and their concentrations were highly variable, however many plants contained pesticides at levels known to have deleterious effects on insects. Plants from larger stores contained higher numbers of pesticides (but not necessarily more lethal or sub-lethal concentrations), and most staggeringly, plants with a label referring to its value for wildlife were

more than twice as likely to contain at least one pesticide at a level potentially dangerous to monarchs.

Pesticides are also known to have sub-lethal effects that can affect survival and reproduction [21]. In monarchs, sub-lethal effects are under-studied, but a few have been identified [22,23]. Pecenka and Lundgren found that clothianidin as low as 1ppb, a concentration we observed in 5 samples from 2 states [22], can reduce larval size. Olaya Arenas et al. investigated the impacts of six compounds, all of which we detected, and found that azoxystrobin and trifloxystrobin both reduce adult monarch wing size [23]. The concentrations that caused these effects in their study were exceeded 73 times by azoxystrobin and 22 times by trifloxystrobin in our samples. Azoxystrobin and trifloxystrobin were more common in samples labeled as valuable for wildlife and were the primary drivers of higher exceedances in those samples compared to those that were unlabeled. This pattern of use might be explained by the fact that these compounds are fungicides and are not used to target caterpillars, but fungicides do have direct impacts on monarchs and other insects [23–27]. Critically, most of the compounds we detected (including some of the most prevalent) have not been directly tested on monarchs, so harmful levels of contamination are likely underestimated and may be widespread in nursery plants.

In addition to exceedances of lethal and sub-lethal concentrations, there are other findings in these results worth discussing. The herbicide atrazine was found in 62% of samples. While one study did not find it to exhibit effects on monarchs [23], its detection frequency poses the question of how these plants became exposed in the first place. In one case, plants that were certified organic still contained atrazine, raising the possibility of environmental conditions in addition to nursery practices being a source of contamination. Another noteworthy result is the potential for additive and synergistic effects from mixtures of compounds. On average, plants

contained 12.2 different pesticides and some of these pesticides have been shown to interact to increase lethality [28–30]. Interestingly, Olaya Arenas et al. also examined mixtures and did not identify lethal or sub-lethal effects [23], but it is unclear how these findings, pertaining to a mixture treatment containing 6 compounds (with 1 insecticide), might apply to our samples which contained, on average over 12 compounds including multiple insecticides. Finally, in one instance leaves from the same plants were screened at two different time points, immediately after purchase and two weeks later, to gain some insight into pesticide degradation over time. Although sample sizes were too small for statistical analyses, we observed reductions in the concentrations of some pesticides over this time. Compounds like spinosad and acephate which have short half-lives saw the greatest reduction, while other compounds like azoxystrobin (which has a longer half-life) did not change (Table S11, Fig. S5).

What should the average person do if they want to support monarch conservation by planting milkweed? We recommend that plants be purchased from nurseries that implement a robust approach to minimizing their reliance on pesticides. This extends beyond insecticides, as fungicides can also have negative effects on monarch caterpillars and were ubiquitous in our samples. Consumers should ask retailers to source plants grown using ecologically sound pest management strategies [31,32]. On the regulatory front, the U.S. Environmental Protection Agency could take steps to address risk, including reducing permissible nursery application rates to prevent residues toxic to pollinators. Finally, further work could investigate sources of contamination in nursery plants, which may become contaminated at multiple points from production to sale. The monarch is the most famous declining butterfly in the US, but it is one of many, and may not even be the most dire case [33]. The planting of beneficial plants can help

imperiled insects, yet, for small scale insect conservation efforts like native plant gardens to be effective, it is critical that nurseries provide plants free from harmful pesticide residues.

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Contribution of authors

C.H.: data curation, formal analysis, methodology, visualization, writing – original draft, writing – review and editing; S.H.: data curation, project administration, funding acquisition, writing – review and editing; A.C.: funding acquisition, writing – review and editing; J.F.: methodology, writing – review and editing; M.F.: methodology, supervision, visualization, writing – review and editing.

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Competing interests

The authors declare no competing interests.

Data availability

Data will be made available on dryad when the paper is accepted for publication.

Supporting Information

Full description of chemical analysis.

Table S1. Summary of purchased milkweeds.

Table S2. Retention times and optimized SRM acquisition parameters for pesticides and internal standards (RT: Retention time, CE: Collision Energy)

Table S3. Contact and oral LD_{50} data for honey bees.

Table S4. Studies from Lepidoptera literature review.

Table S5. Exceedances of honeybee LD_{50} concentrations by compound.

Table S6. Number of exceedances of lepidopteran LD_{50} concentrations by compound.

Table S7. Number of exceedances of monarch sub-lethal threshold concentrations by compound.

Table S8. Summary statistics for model predicting pesticide richness.

Table S9. Summary statistics for model predicting pesticide diversity.

Table S10. Summary statistics for model predicting pesticide exceedances.

Table S11. Changes in pesticide concentrations in 10 samples over two weeks.

Figure S1. Semi partial variance explained among important predictor variables.

Figure S2. Summary of pesticide richness found in milkweed samples purchased in stores across the United States.

Figure S3. Summary of pesticide diversity found in milkweed samples purchased in stores across the United States.

Figure S4. Summary of lethal and sub-lethal exceedances found in milkweed samples purchased in stores across the United States.

Figure S5. Changes in pesticide concentrations two weeks after purchase.

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Table 1. Variance explained by covariates predicting pesticide richness, pesticide diversity, and the presence of a lethal or sub-lethal exceedance. The total explained variance is the explained variance of the model when including all terms and the covariate specific rows are the semi-partial variance explained by each term.

	Pesticide richness	Pesticide diversity	Exceedance
Total explained variance	20.5%	30.0 %	33.5 %
Store size	9.8%	3.9%	1.0 %
Milkweed species	9.5 %	7.9%	13.2%
Region	0.5%	0%	0.0%
Label	0%	17.2%	25.4%

Table 2. Means and standard errors of pesticide richness and pesticide diversity for each covariate level.

Covariate		Pesticide richness	Pesticide diversity
Store size	Single store	11.25 (± 0.39)	2.98 (± 0.13)
	2-100 stores	11.85 (± 0.58)	2.94 (± 0.18)
	> 100 stores	16.43 (± 0.83)	4.02 (± 0.32)
Milkweed species	*Asc. curassavica*	12.18 (± 0.59)	2.69 (± 0.17)
	Asc. fascicularis	13.50 (± 0.67)	4.53 (± 0.73)
	Asc. incarnata	12.03 (± 0.59)	3.58 (± 0.22)
	Asc. speciosa	9.12 (± 0.68)	2.70 (± 0.37)
	Asc. tuberosa	12.92 (± 0.65)	3.13 (± 0.17)
Region	East	12.32 (± 0.40)	3.04 (± 0.12)
	West	12.04 (± 0.52)	3.23 (± 0.18)
Label	Yes	13.33 (± 0.49)	2.77 (± 0.14)
	No	10.64 (± 0.84)	3.79 (± 0.20)

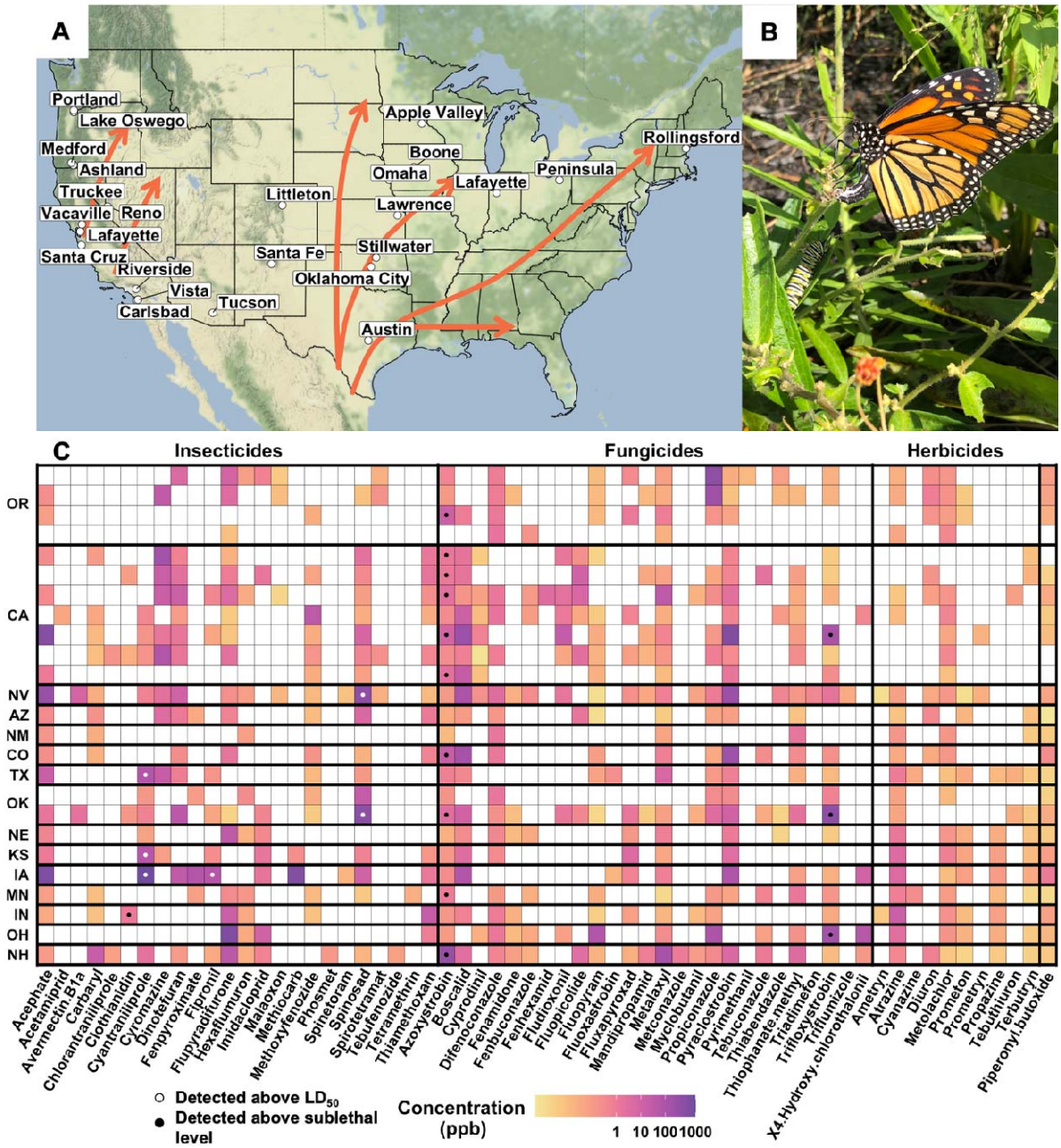


Figure 1. Pesticides found in milkweed samples. A) Locations where milkweeds were purchased. Orange arrows denote major monarch migration routes. B) Adult monarch butterfly and caterpillar on *Asclepias tuberosa*. C) Mean concentrations of compounds by location. Values are shown in parts per billion on a log scale. White circles indicate compounds found above a lepidopteran LD_{50}. Black circles indicate compounds above a monarch sub-lethal concentration.

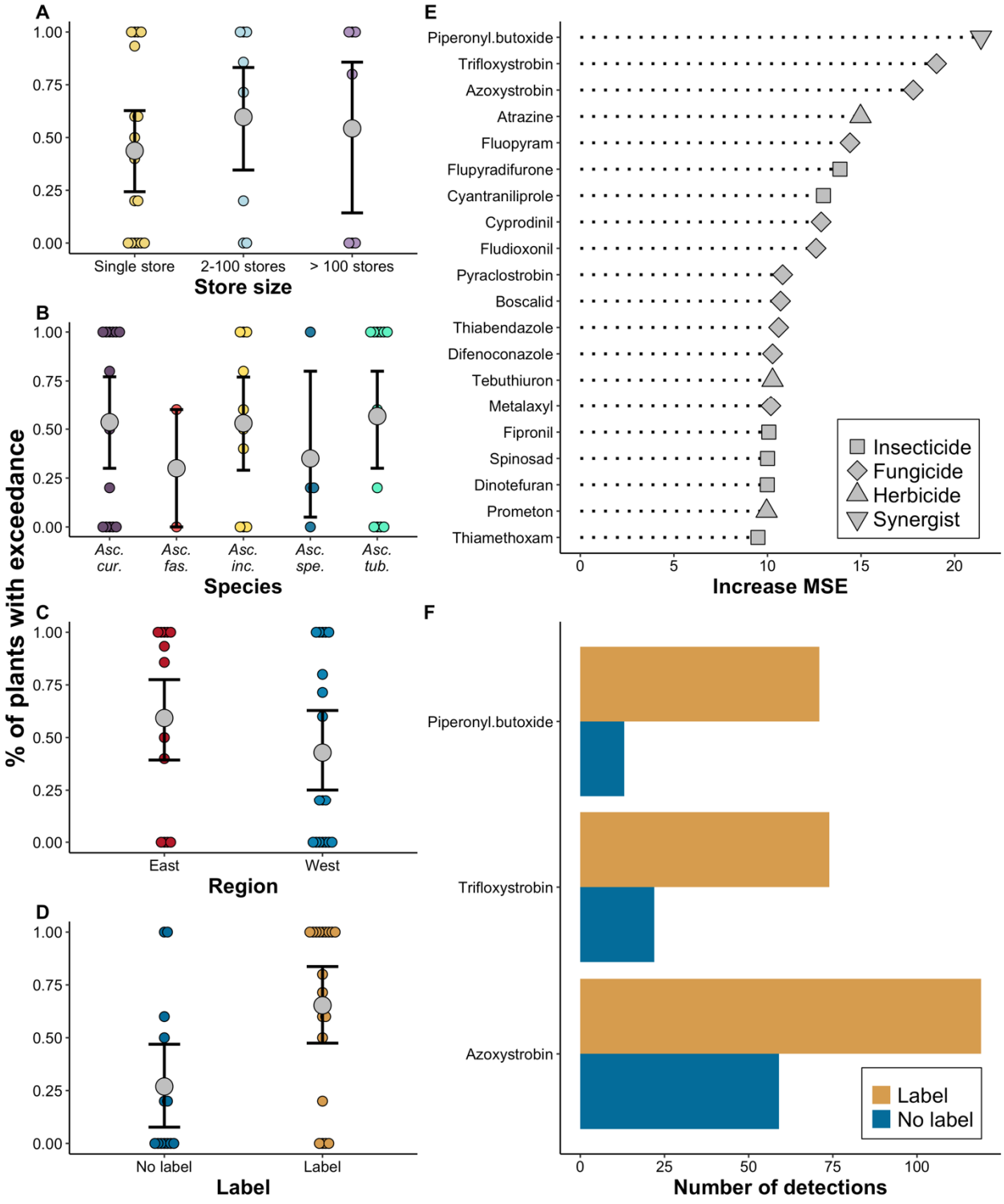
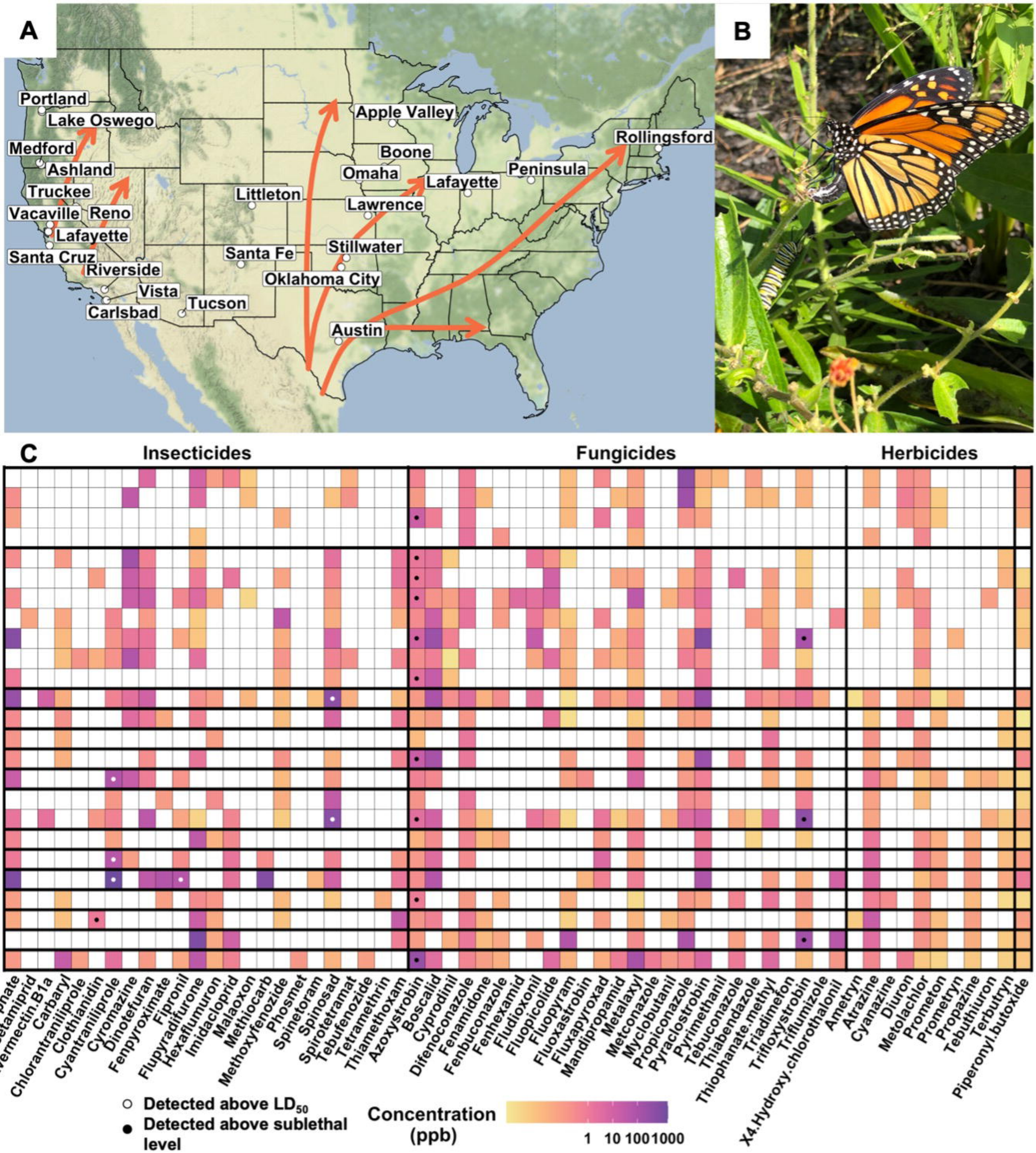


Figure 2. Summary of exceedance associations. Panels A-D show the percentage of plants from each store with at least one exceedance by store size, species, region, and wildlife label. Gray points show the mean percentage (bars are standard errors). E) Compounds that are the most predictive of whether a sample was labeled safe for wildlife based on random forest analysis. F)

Differences in the total number of detections between labeled and un-labeled samples for the three most predictive compounds.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Dinotefuran
Fenpyroximate
Fipronil
Flupyradifurone
Hexaflumuron
Imidacloprid
Malaoxon
Methiocarb
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Spinetoram
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Spirotetramat
Tebufenozide
Tetramethrin
Thiamethoxam
Azoxystrobin
Boscalid
Cyprodinil
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Fenhexamid
Fludioxonil
Fluopicolide
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Thiabendazole
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Trifloxystrobin
Triflumizole
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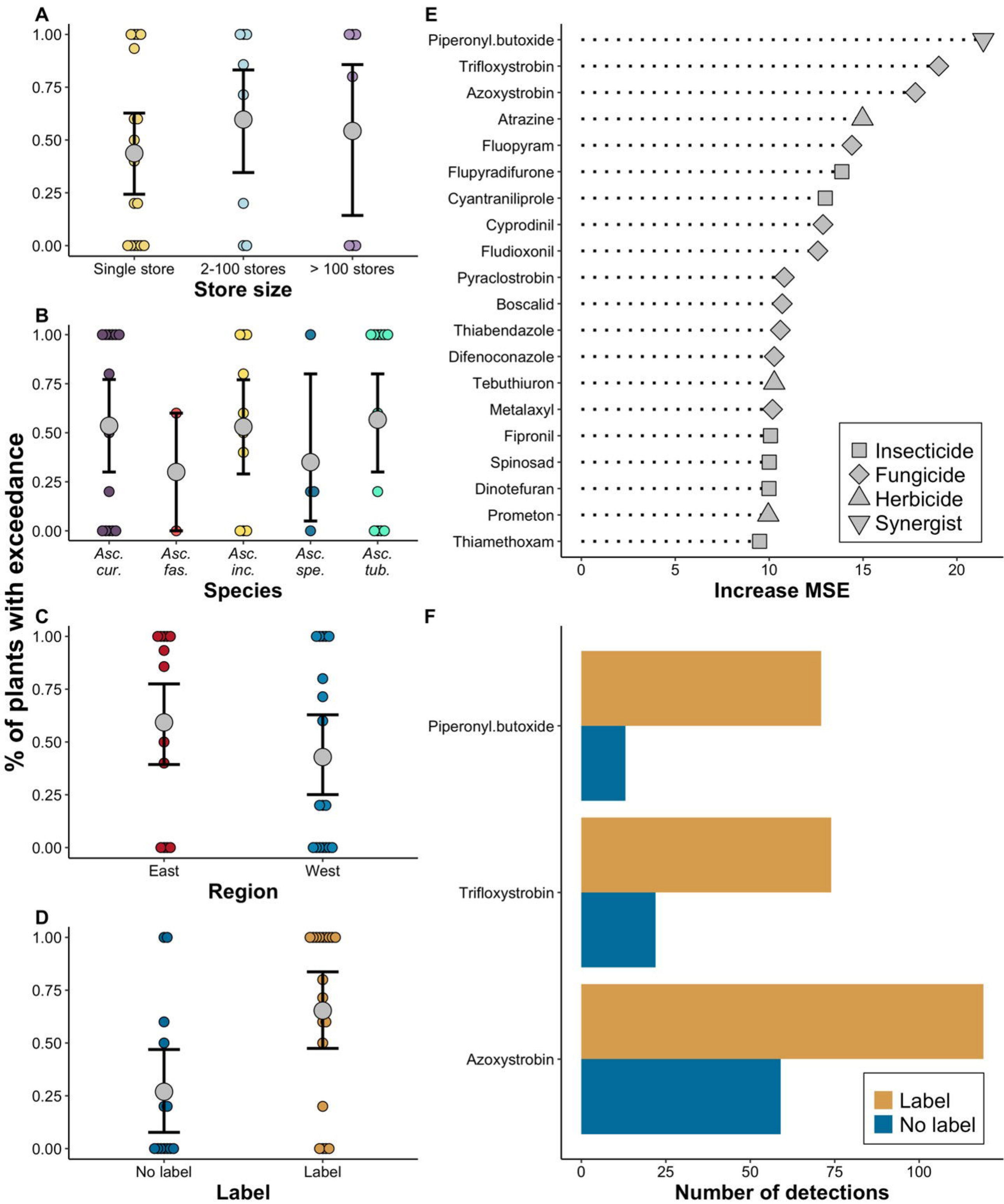
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Single store
2-100 stores
> 100 stores
Store size
B
Asc. cur.
Asc. fas.
Asc. inc.
Asc. spe.
Asc. tub.
Species
% of plants with exceedance
C
East
West
Region
D
No label
Label
Label
E
Piperonyl.butoxide
Trifloxystrobin
Azoxystrobin
Atrazine
Fluopyram
Flupyradifurone
Cyantraniliprole
Cyprodinil
Fludioxonil
Pyraclostrobin
Boscalid
Thiabendazole
Difenoconazole
Tebuthiuron
Metalaxyl
Fipronil
Spinosad
Dinotefuran
Prometon
Thiamethoxam
0
5
10
15
20
Increase MSE
Insecticide
Fungicide
Herbicide
Synergist
F
Piperonyl.butoxide
Trifloxystrobin
Azoxystrobin
0
25
50
75
100
Number of detections
Label
No label