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# Target and non-target effects of insecticide use during ornamental milkweed production

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There are widespread public efforts to conserve wildlife in urbanized landscapes via the installation of nursery-grown plants that support Lepidoptera taxa. Insecticides are commonly used during nursery production to suppress key plant pests, and many products have extended periods of toxicity and affect a wide range of herbivore taxa. While there are plentiful toxicological data on bee species, predominantly the Western honey bee (*Apis mellifera* L.), little is known about how insecticides affect nonpest lepidopterans. Lepidoptera has different modes of exposure (e.g., leaf-feeding) and differences in susceptibility to insecticide target sites compared to bees. Consequently, many products compatible with bee conservation pose an uncertain risk to nonpest lepidopterans and thus may represent an under-recognized conflict with conservation efforts. Using the monarch butterfly (*Danaus plexippus*, L.), tropical milkweed (*Asclepias curassavica*, L.), and oleander aphid (*Aphis nerii*, Fonscolombe, 1841) system, we conducted leaf and whole-plant feeding assays to evaluate effects of acute and chronic monarch exposure to industry standard and alternative reduced-risk insecticides used during nursery production. We also evaluated the efficacy of these insecticides against their target pest, the oleander aphid. Our results indicate that insecticides used to control pests on ornamental milkweed can cause monarch larval mortality up to 4 wk after treatment application. Furthermore, the duration of aphid suppression is often shorter than the duration of adverse effects on monarchs. This study demonstrates a conflict between insect pest management and Lepidoptera conservation during ornamental plant production and has implications for the conservation value of ornamentals after retail sale.

*Key words:* insect conservation, integrated pest management, ornamental plant production

## Introduction

Mounting evidence indicates that biodiversity is declining globally (Biesmeijer et al. 2006, Potts et al. 2010, Cameron et al. 2011, Koh et al. 2016, Wagner 2020). Among the primary factors associated with biodiversity declines is habitat loss due to rapid urbanization (Biesmeijer et al. 2006, Goulson et al. 2015), which is characterized by the replacement of natural habitats with hardscapes and ornamental plants. The dominance of impervious surface cover and human-designed ornamental landscapes has pronounced effects on the relative abundance and diversity of resident insects (Dale and Frank 2018). Biodiversity loss is particularly concerning when it includes beneficial insects that play critical roles in the functioning of urban ecosystem processes like plant pollination (Biesmeijer et al. 2006, Potts et al. 2010, Cameron et al. 2011, Goulson et al. 2015, Dicks et al. 2021), natural pest control, nutrient cycling, and supporting other wildlife as prey items (Burghardt et al. 2008, Tallamy and Shriver 2021). Due to increasing public awareness of biodiversity loss and wildlife conservation needs, particularly in urban and residential landscapes, there is unprecedented demand for ornamental plants that support myriad wildlife like bees, butterflies, and birds (Kachatryan and Rihn 2021). Recent evidence indicates that most people value pollinator conservation and are willing to change their behavior to benefit conservation (Kachatryan and Rihn 2021). In addition, a growing body of research shows that urban and suburban landscapes, if designed and managed appropriately, can support diverse plant and animal species (Baldock et al. 2015, Somme et al. 2016, Hall et al. 2017).

Lepidoptera (i.e., butterflies and moths) play important roles in ecosystems as complementary pollinators to bees (Hahn and Brühl 2016, Cusser et al. 2021), as primary food sources for higher trophic levels like birds (Tallamy and Shriver 2021), and as regulators of plant communities through herbivory as larvae (Hairston et al. 1960, Mulder et al. 2001). One focal insect for lepidopteran conservation in North America is the monarch butterfly (*Danaus plexippus*, L.). The monarch is a widely recognized, charismatic North American butterfly species that engages in a yearly migration from the eastern United States and Canada to overwintering grounds in Mexico (eastern monarch) or along the western coast of the United States (western monarch). Monarch populations have declined by over 80% in recent decades (Brower et al. 2012, Thogmartin et al. 2017, US Fish & Wildlife Service 2020a, 2020b), potentially due to losses of breeding habitat along their migration route, spurring widespread conservation efforts. As a dietary specialist that feeds exclusively on milkweed (family Apocynaceae, subfamily Asclepiadoideae), monarch conservation efforts focus primarily on planting these larval host plants to replenish breeding habitat lost to urbanization and agriculture.

Urban and suburban residents represent a substantial potential conservation force, as over 80% of US residents live in urbanized areas (U.S. Census Bureau). Although native milkweed species, primarily planted as seed, are used for large-scale conservation plantings in many natural or agricultural areas, the nonnative ornamental, tropical milkweed (*Asclepias curassavica*, L.), is the most common choice for nursery growers and ornamental gardens in urban and suburban areas throughout the southern United States (authors' observations). Tropical milkweed is an herbaceous perennial plant native to Central and South America and Mexico. Although tropical milkweed is not recommended for use in much of North America due to its propensity to interfere with monarch migration (Faldyn et al. 2018, Majewska and Altizer 2019), its high cardenolide content (Faldyn et al. 2018, Agrawal et al. 2021), and its role as a potent vector for *Ophryocystis elektroscirrha* (OE) due to its lack of senescence (Satterfield et al. 2016), it remains the most common milkweed species for residential landscape and ornamental garden use throughout the southern half of the United States. The bias toward tropical milkweed is in part due to its aesthetic appeal, ease of propagation, and extended flowering season, and its prominence within the ornamental plant market is the primary reason we focus on this species in this study. Importantly, milkweed in nurseries and urbanized landscapes throughout much of the southern United States, including native species, is frequently attacked by a sap-feeding insect pest, the oleander aphid (*Aphis nerii*, Fonscolombe, 1841) (Blackman and Eastop 2006), which commonly occurs at high densities and causes chlorosis, leaf drop, and sooty mold accumulation. In nurseries, this results in an unsaleable plant. In addition to being an economic pest of *Asclepias* spp., recent evidence indicates that monarch butterflies oviposit 30% fewer eggs and larvae consume 50% less plant tissue on tropical milkweed infested with oleander aphids compared to aphid-free tropical milkweed (Mach et al. 2023).

Insecticides are the primary approach to insect pest management during ornamental milkweed production in nurseries because they are typically the most effective and reliable pest management tools. Recent evidence indicates that many nursery-applied pesticides are still detectable in milkweed foliage at the time of retail purchase (Halsch et al. 2022). Thus, milkweed treated for aphids during plant production may threaten monarch larvae during plant production and after retail sale (Bargar et al. 2020). Integrated Pest Management (IPM) seeks to reduce key pests and their damage while minimizing negative nontarget impacts on beneficial insects. Many IPM strategies intended to protect nontarget insects that use plant resources (e.g., pollinators) rely on comprehensive toxicological data to understand the severity and duration of susceptibility of these insects to insecticides. Currently, most toxicological data and associated insecticide labeling focus on the Western honey bee (*Apis mellifera*, L.) or a select few other bee species (e.g., bumble bees). Lepidopterans can respond in markedly different ways than bees to the same insecticide, largely because they are herbivorous as larvae (Krishnan et al. 2020, 2021b). Consequently, nonpest lepidopterans have multiple potential routes of unintended insecticide exposure dependent upon their life stage (e.g., herbivorous larvae versus nectar-feeding adults), and currently, few IPM strategies account for their protection.

Due to their phylogenetic origins, lepidopterans also have different susceptibility to insecticide chemical classes and modes of action compared to bees. For example, monarch larvae exposed to *Bacillus thuringiensis galleriae* (Btg), an organic soil-derived bacterial insecticide, showed high rates of mortality in field-realistic settings (Redmond et al. 2020), despite Btg posing little risk to bees. Similarly, chlorantraniliprole, a reduced-risk insecticide (US EPA 2023) with low toxicity to honey and bumble bees (Larson et al. 2013), is highly toxic to Lepidoptera larvae (Tofangsazi et al. 2015, Krishnan et al. 2020, 2021b). Consequently, many of the insecticides compatible with bee-centric IPM practices pose an unknown or direct risk to nonpest lepidopterans (Larson et al. 2013, Redmond et al. 2020). Additionally, many of the most used and effective insecticides (e.g., imidacloprid) were developed to be toxic to lepidopteran pest species (e.g., fall armyworm, *Spodoptera frugiperda*; Eastern tent caterpillar, *Malacosoma Americanum*; cutworms, *Agrotis* spp.; among others) and thus cannot distinguish pest species from nonpest species. Despite this, beneficial nonpest lepidopterans are typically not prioritized during pest management decision-making on host plants.

As rapid urbanization continues (US Census Bureau. 2012), conserving beneficial insects and promoting insect biodiversity in the built environment is increasingly important. Current public enthusiasm and interest in conserving wildlife in places where people live and work presents a substantial opportunity for biodiversity conservation (Hall et al. 2017). Nursery-grown ornamentals are the predominant vegetation used around homes, businesses, and urban greenspaces. However, current industry-standard pesticide use during plant production conflicts with beneficial insect conservation goals in both documented and undocumented ways (Frank and Tooker 2020). Although many agricultural pests are lepidopterans, they represent less than 1% of the 14,300 documented Lepidoptera species in North America alone that fulfill many beneficial ecosystem functions (Mulder et al. 2001, Hahn and Brühl 2016, Rytkönen et al. 2019, Cusser et al. 2021, Tallamy and Shriver 2021). Therefore, evidence-based IPM strategies that protect both plants and nonpest lepidopterans are urgently needed. Using the monarch butterfly, the oleander aphid, and tropical milkweed as a model system, our objectives were to determine the acute and chronic exposure toxicity of commonly used and proposed alternative insecticides to butterfly larvae and evaluate the efficacy of these insecticides against the aphids they are intended to control in an ornamental plant production setting. Our results have implications for monarch conservation and, more broadly, Lepidoptera conservation both during ornamental plant production at nurseries and after installation into urbanized landscapes.

## Materials and Methods

### Study Organisms

Insecticide-free tropical milkweed plants were custom-ordered and purchased in 3.8 liters containers from Green Isle Gardens Nursery (Groveland, FL) for use in all experiments. Pots contained clusters of 1–3 individual plants comprised of 2–6 shoots per pot and are hereafter referred to as a single ‘plant’. Plants were maintained in a nursery setting at the University of Florida under natural light and temperature conditions for the duration of the experiments. Milkweed plants were readily colonized by naturally occurring oleander aphids before experiments began, and all plants used in each experiment avg ≥ 150 aphids per terminal shoot, which we considered high aphid densities and well above the threshold at which control measures are recommended (Xerces Society 2017).

## Monarch Larvae

Monarch eggs were obtained from a lab-maintained colony composed of stock sourced from Shady Oak Butterfly Farm in Brooker, FL, United States and wild-caught monarch butterflies in Gainesville, FL. Eggs were stored in environmental chambers at 28°C and 75% relative humidity with a 12:12 light cycle until eclosion. Neonate larvae were fed pest- and insecticide-free tropical milkweed leaves until they reached third instar, at which point they were used in experiments.

## Insecticide Selection

Three industry-standard insecticides (imidacloprid, spirotetramat, and insecticidal soap) were selected based on a 2017 Florida nursery grower survey (Daniels, unpublished data). We also selected 3 reduced-risk insecticides (pymetrozine, acetamiprid, and flupyradifurone, (US EPA 2023) that are labeled for aphid control on ornamental plants and suspected to provide reduced risks to lepidopteran larvae based on their labeled target pests or modes of action (Harrewijn and Kayser 1997, Nauen et al. 2015). Except for insecticidal soap, which relies on contact with the target insect, all selected insecticides are systemic and translaminar and move throughout the plant vasculature in the phloem and xylem (Nauen et al. 2008, Bonmatin et al. 2015). Systemic and translaminar insecticides are generally considered to be the most IPM-compatible synthetic insecticides because they reduce nontarget exposure via direct contact with insecticide residues. Each insecticide was applied at the rate corresponding with aphid control on its respective label during all trials (Table 1). All selected insecticides and their properties are detailed in Table 1. It is important to note that we used commercially formulated insecticide concentrates, rather than isolated active ingredients. Commercial formulations contain unknown inert ingredients that could have influenced our findings (Cox and Surgan 2006). Therefore, the interpretation of our results should be done with that caveat in mind.

## Monarch Acute Exposure

To assess acute toxicity associated with each insecticide treatment, we exposed monarch larvae to treated tropical milkweed leaves for 48 h. Milkweed plants were randomly assigned to each of the 6 insecticide treatments, with 4, 3, and 6 replicates at 24 h, 2 wk, and 4 wk post insecticide application, respectively, due to the varying availability of monarch larvae. Plants were treated with insecticide via foliar sprays at labeled rates for aphid control (Table 1). Plants did not receive any additional insecticide applications for the duration of the experiment. Leaves were harvested 24 h, 2 wk, and 4 wk after insecticide application and placed individually into a Petri dish (100 mm × 15 mm) with a single third-instar monarch larva and a piece of damp filter paper. Larvae were provided leaves ad libitum for 48 h. Petri dishes were held in an environmental chamber at 28°C and 75% relative humidity with a 12:12 light cycle for the duration of the experiments. Larval mortality was assessed 48 h after placement into the petri dish.

## Monarch Chronic Exposure

Eighteen milkweed plants were randomly assigned to each of the 6 insecticide treatment groups and further subdivided into 3 cohorts of 6 plants that designate exposure at different time points after insecticide application (24 h, 2 wk, and 4 wk). There were 4, 5, and 4 replicates at 24 h, 2 wk, and 4 wk post insecticide application,

**Table 1.** Selected insecticides and their properties

| Trade name<br>Manufacturer<br>Insecticide (%) | IRAC mode of action[d] | Application rate (g or ml pesticide per 379 liter $H_2O$) | AI (g or ml per 379 liter application) | Labeled for caterpillars | Honey bee contact toxicity rating[e] | Honey bee oral toxicity rating[e] |
|---|---|---|---|---|---|---|
| Endeavor<br>Syngenta<br>Pymetrozine[a,b] (50.0%) | 9-B | 142 | 71 | No | Practically nontoxic | Practically nontoxic |
| Kontos<br>Bayer<br>Spirotetramat[a,c] (22.4%) | 23 | 100 | 24 | No | Practically nontoxic | Practically nontoxic |
| TriStar 8.5 SL<br>Cleary Chemical<br>Acetamiprid[a,b] (8.5%) | 4-A | 118 | 11 | Yes | Practically nontoxic | Moderate |
| Altus<br>Bayer<br>Flupyradifurone[a] (17.1%) | 4-D | 298 | 60 | No | Practically nontoxic | Moderate |
| Lada 2F<br>Rotam North America Imidacloprid[c] (21.4%) | 4-A | 50 | 12 | Yes | High | High |
| Insect-killing soap concentrate<br>Safer Brand<br>Potassium salts of fatty acids[c] (49.5%) | – | 7393 | 3661 | Yes | n/a | n/a |

[a]Reduced risk (US EPA 2023).
[b]Organophosphate alternative (US EPA 2023).
[c]Industry standard, Jaret Daniels unpublished data.
[d]Insecticide resistance action committee (Sparks et al. 2020).
[e]ECOTOX knowledgebase (US EPA. 2024).

**Table 2.** Summary of Kruskal–Wallis tests for effects of insecticide treatment on monarch larval mortality at each time since treatment (24 h, 2 wk, or 4 wk)

| | | Acute exposure mortality | | | Chronic exposure mortality | | |
|---|---|---|---|---|---|---|---|
| Source | Time | df | $\chi^2$ | *P*-value | df | $\chi^2$ | *P*-value |
| Treatment | 24 h | 5 | 12.22 | 0.03 | 5 | 14.38 | 0.01 |
| | 2 wk | – | – | – | 5 | 5.22 | 0.39 |
| | 4 wk | – | – | – | 5 | 9.20 | 0.10 |

respectively, due to varying availability of monarch larvae. We added a third-instar monarch larva to each plant in the appropriate time cohort and gave it the opportunity to feed and complete development. Plants were divided in this way to ensure that larvae had sufficient plant material to complete development, to prevent overlap of multiple cohorts of caterpillars on the same plant, to exclude confounding effects of previous herbivory, and to imitate realistic levels of insecticide exposure on treated plants. No plants in the experiment experienced larval herbivory more than once. As in the acute exposure experiment, all plants were treated at the same time via foliar spray with the appropriate insecticide at labeled rates for aphid control (Table 1), and no plants received additional insecticide applications for the duration of the experiment. Monarch larval mortality was assessed every 24 h until all larvae either perished or reached adulthood. Pupae were removed and stored in an environmental chamber at 28°C and 75% relative humidity with a 12:12 light cycle and monitored until eclosion.

### Insecticidal Soap Toxicity

Milkweed plants used for our aphid-free control treatment in both monarch toxicity experiments were treated with insecticidal soap, as it is logistically impossible to keep milkweed in a nursery setting free of oleander aphids without dramatically changing other important conditions. Thus, we conducted an additional experiment to validate the use of insecticidal soap as an alternative to a no-treatment control. Fifty third-instar caterpillars were individually placed in Petri dishes (100 mm × 15 mm) and fed either insecticidal soap-treated or soap-free tropical milkweed leaves. For the soap treatment, insecticidal soap was applied to milkweed foliage daily prior to replenishing Petri dishes. We recorded total leaf area consumed, larval mortality, and larval weight at 7 and 14 days post-experiment initiation. Leaf surface area was recorded using the LI-3100c Leaf Area Meter (LI-Core Environmental, Lincoln Nebraska) before feeding and leftover material was recorded once leaves were replenished. The total leaf area consumed was then calculated by taking the surface area of the leaf added to the cup and subtracting the surface area remaining the next day. Petri dishes were held in an environmental chamber at 28°C and 75% relative humidity with a 12:12 light cycle for the duration of the experiments. The experiment was completed over a duration of 14 days.

### Oleander Aphid Suppression

Concurrently with the monarch chronic exposure experiment, we rated oleander aphid density on each plant (18 plants total per each of the 6 insecticide treatments) once weekly beginning at the time of insecticide application and continuing for the 5-wk duration of the experiment. As noted in the chronic exposure experiment, all plants were treated at the same time via foliar spray with the appropriate insecticide at labeled rates for aphid control (Table 1) and did not receive additional insecticide applications for the duration of the experiment. Aphid densities were averaged across all terminal growth points per plant and ranked on an ordinal scale as 0 (no live aphids), 1 (<50 aphids per terminal growth point), 2 (50–150 aphids per terminal growth point), or 3 (>150 aphids per terminal growth point). Average aphid ratings greater than 1 indicate plants with infestations over the recommended treatment threshold of >50 aphids per terminal growth point (Xerces Society 2017).

### Statistical Analyses

Statistical analyses were conducted using R ver. 4.1.0 (R Core Team 2021). Our initial intent was to use insecticidal soap as a positive control to suppress aphids without affecting monarchs. However, due to low efficacy against aphids, all plants treated with insecticidal soap had high aphid densities for most of the study period and thus functioned more like a no-treatment control. Monarch mortality and average aphid density ratings (0–3) were analyzed using Kruskal–Wallis tests, due to low sample size, and separated by week after insecticide application to determine differences between insecticide treatments at each timepoint post-treatment. If $\chi^2$ results were significant ($P < 0.05$), we compared treatment means using Dunn's test for multiple comparisons with *P*-values adjusted using the Benjamini–Hochberg method to reduce the false discovery rate.

Insecticidal soap toxicity results were analyzed using one-way Analysis of Variance (ANOVA) with treatment (soap and no soap) as the independent variable and total leaf area consumed ($cm^2$) or larval weight (mg) as the dependent variables. Results were considered significant if $P < 0.05$. We did not analyze mortality between the 2 treatments as there were few deaths throughout the whole experiment (2 individuals in the no-soap treatment and 3 in the soap treatment).

## Results

### Monarch Acute Exposure

We observed only moderately toxic effects of our insecticide treatments on monarch larvae from acute exposure to insecticide-treated milkweed leaves, and only at the 24 h post-application time point (Table 2, Fig. 1). When exposed to treated milkweed tissue for 48 h beginning 24 h after treatment application, acetamiprid caused 100% monarch larva mortality followed by spirotetramat (50%), flupyradifurone (25%), pymetrozine (25%), imidacloprid (0%), and insecticidal soap (0%). Monarch larvae exposed to milkweed tissue treated with acetamiprid exhibited classic signs of neonicotinoid toxicity, including spasms and paralysis (Simon-Delso et al. 2015). We observed no acute exposure mortality from any of the insecticides at 2 wk or 4 wk post-treatment application.

### Monarch Chronic Exposure

Unlike our acute exposure toxicity results, we observed the highly toxic effects of all insecticide treatments on monarch larvae from chronic exposure to insecticide-treated milkweed plants (Table 2, Fig. 2). Chronic exposure mortality varied greatly between insecticide treatments at 24 h and 2 wk posttreatment, but not at 4 wk posttreatment (Table 2, Fig. 2). We observed an average of 67%, 77%, and 17% monarch mortality across all insecticide treatments at 24 h, 2 wk, and 4 wk after treatment application, respectively, as compared to 33%, 0%, and 0% across all insecticide treatments during the acute exposure mortality experiment. Four weeks after treatment application, we only observed chronic exposure monarch mortality on plants treated with pymetrozine and insecticidal soap, both of which were 50%. Importantly, most monarch mortality occurred late in larval development, in many cases as unsuccessful pupation or eclosion from pupae.

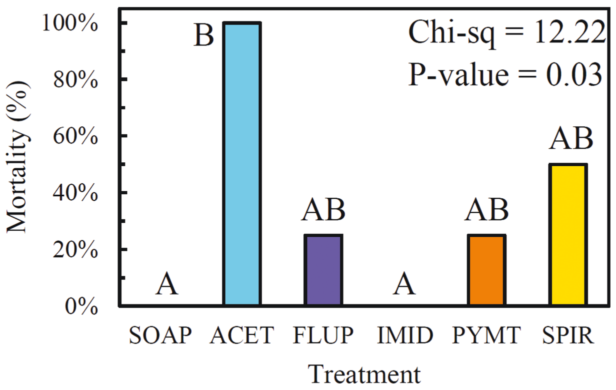


**Fig. 1.** Acute exposure percent mortality for monarch larvae exposed to field-weathered insecticide residues for 48 h at 24 h after insecticide application. Letters indicate significant differences between insecticides as separated by post hoc Dunn's test for multiple comparisons with *P*-values adjusted using the Benjamini–Hochberg method.

As we observed in the acute toxicity experiment, plants treated with acetamiprid resulted in 100% monarch mortality when larvae were placed on treated plants 24 h after treatment. However, this mortality dissipated over time, resulting in 40% mortality at 2 wk and 0% mortality at 4 wk post-treatment application. In contrast to the acute exposure trial, milkweed treated with flupyradifurone and imidacloprid resulted in 100% chronic exposure mortality at 24 h, 80% at 2 wk, and 0% mortality at 4 wk post-treatment application. Feeding on plants treated with pymetrozine resulted in 50% mortality at 24 h, 100% mortality at 2 wk, and 50% mortality at 4 wk post-treatment application. Milkweed treated with spirotetramat caused 50% monarch mortality at 24 h, 80% mortality at 2 wk, and 0% mortality at 4 wk post-treatment application. Insecticidal soap applications caused no monarch mortality 24 h post-treatment application as expected, but we did observe 80% and 50% mortality at 2 and 4 wk posttreatment, respectively, as oleander aphid densities dramatically increased on those plants. As previously determined, monarchs perform poorly on tropical milkweed with high-density aphid infestations (Mach et al. 2023). Thus, monarch mortality observed on plants treated with insecticidal soap at 2- and 4-wk post-application is confounded by the indirect effects of aphid infestation on monarch performance.

## Insecticidal Soap Toxicity

We did not observe any detectable differences in leaf area consumed ($F = 0.611$, $P = 0.438$) or larval weight (7 days: $F = 0.012$, $P = 0.912$; 14 days: $F = 0.485$, P = 0.489) between monarch larvae

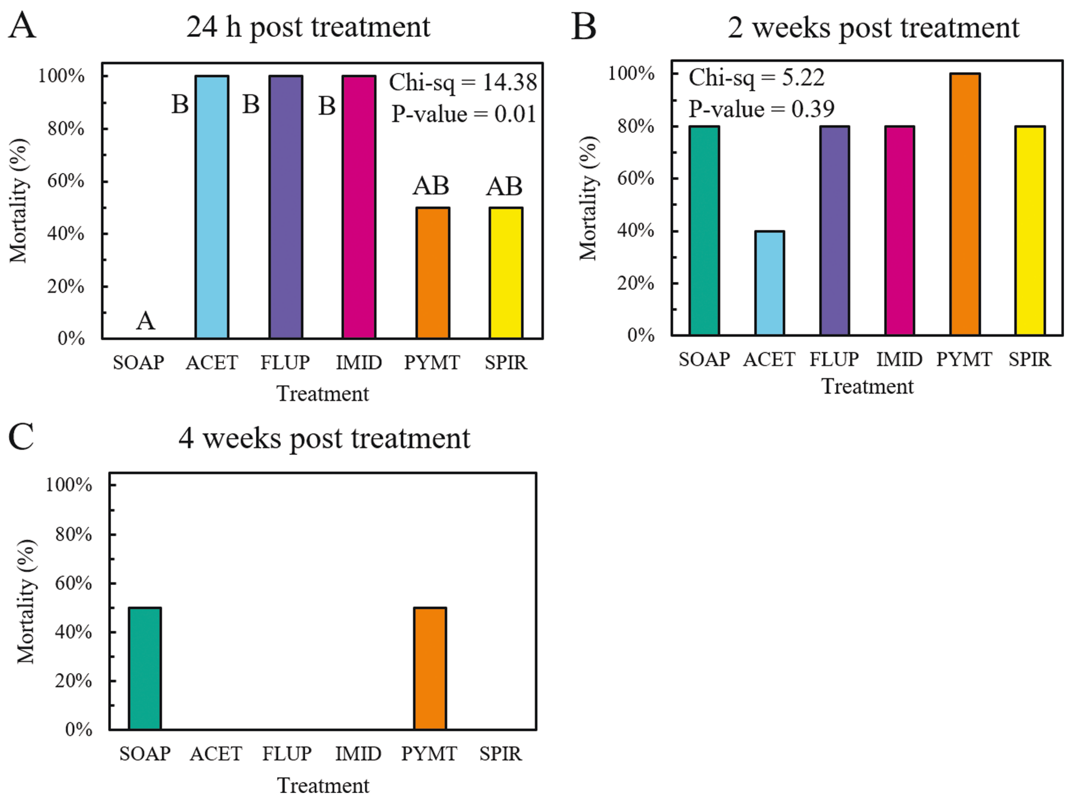


**Fig. 2.** Chronic exposure percent mortality for monarch larvae exposed to field-weathered insecticide residues at 3 time points after insecticide application (24 h, 2 wk, and 4 wk). Letters on each bar indicate significant differences between treatments at each timepoint as determined by Kruskal–Wallis test with separation by Dunn's test for multiple comparisons and *P*-values adjusted using the Benjamini–Hochberg method.

fed insecticidal soap-treated or untreated milkweed leaves over the course of the 2-wk experiment (Fig. 3). Mortality was low in the soap treatment (3 individuals out of 28) and in the no-soap treatment (2 individuals out of 27). Total leaf area consumed for the insecticidal soap treatment ranged from 85.8 to 223.9 $cm^2$ with an avg (±SE) of 158.3 (±4.8) $cm^2$ and the soap-free treatment ranged from 128.3 to 193.1 $cm^2$ with an avg of 154.3 (±3.2) $cm^2$ (Fig. 3). Monarch larval weight 7 days after experiment onset avg 461.8 (±22.7) mg (range: 194.6–717.6 mg) for the insecticidal soap treatment and 472.9 (±18.7) mg (range: 306.9–634.7 mg) for the soap-free treatment (Fig. 3). Larval weight 14 days after initiation avg 639.5 (±22.2) mg (range: 275.3–787.5 mg) for the insecticidal soap treatment and 573.6 (±25.4) mg (range: 112.9–777.7 mg) for the soap-free treatment (Fig. 3). In addition to the data described above, we observed no other differences in larvae between treatments.

### Oleander Aphid Suppression

We observed significant differences in average aphid density ratings between insecticide treatments over the course of the 5-wk chronic exposure experiment (Table 3, Fig. 4). All plants avg over 150 aphids per terminal growing point at the onset of the experiment. Every insecticide treatment except for insecticidal soap effectively reduced aphid densities below treatment threshold levels (ca. 50 aphids per terminal growth point) within 1 wk of application (Fig. 4). Duration of aphid suppression varied substantially between treatments, with flupyradifurone and acetamiprid suppressing aphid populations below the treatment threshold until 5 wk or 4 wk post-treatment application, respectively. Spirotetramat suppressed aphid densities below threshold levels for 3 wk, while imidacloprid and pymetrozine both provided 2 wk of aphid suppression. Insecticidal soap failed to reduce aphid densities below the treatment threshold level for the entire 5-wk experiment. Although oleander aphid populations on milkweed treated with insecticidal soap briefly declined at 4 wk posttreatment (Fig. 4).

## Discussion

Creating viable wildlife resources in urbanized landscapes is critical for mitigating global biodiversity loss (Somme et al. 2016, Hall et al. 2017, Kawahara et al. 2021). Ornamental plants are the primary vegetation installed and maintained in urban and residential landscapes, nearly all of which originate from nurseries. Paired with unprecedented societal interest in gardening for wildlife, ornamental plants that support wildlife represent an important component of urban conservation efforts (Somme et al. 2016, Hall et al. 2017). Unfortunately, herbivorous insect pests frequently reach damaging densities on plants during nursery production, which directly reduces the salability of ornamental plants. Thus, many nursery

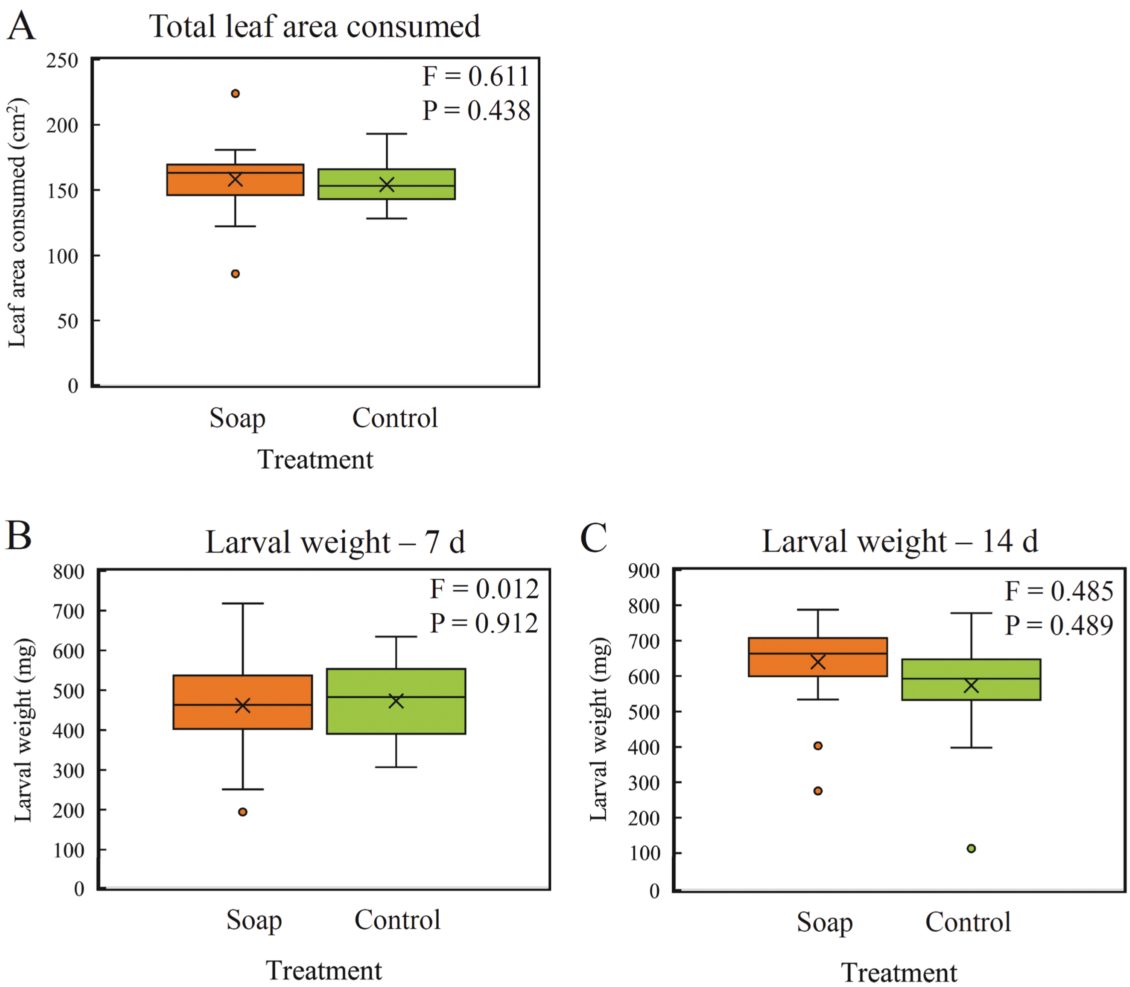


**Fig. 3.** Total leaf area consumed (A) and larval weight at 7 days (B) and 14 days (C) between monarch larvae fed either soap-treated or soap-free milkweed leaves ad libitum. Statistics were reported from a one-way ANOVA.

**Table 3.** Summary of Kruskal–Wallis tests for differences between aphid ratings for each of the 6 insecticide treatments at each time since treatment

| | | Aphid rating | | |
|---|---|---|---|---|
| Source | Time | df | Chi-sq | P-value |
| Time | Pretreatment | 5 | 5.00 | 0.42 |
| | 1 wk | 5 | 79.06 | <0.01 |
| | 2 wk | 5 | 74.82 | <0.01 |
| | 3 wk | 5 | 75.75 | <0.01 |
| | 4 wk | 5 | 42.91 | <0.01 |
| | 5 wk | 5 | 50.94 | <0.01 |

growers rely on insecticides to prevent pest damage and produce a marketable product. Mounting evidence illustrates that pesticides used during plant production can persist and may have downstream negative impacts beyond the nursery where they originate (Krischik et al. 2015, Halsch et al. 2022). This is concerning for both growers and consumers, neither of whom wish to create ecological traps by provisioning beneficial herbivores with toxic plant material once purchased plant material is installed into urban landscapes. Our study adds to the growing body of literature demonstrating that insecticides commonly used during plant production to reduce key pests can pose significant risks to nontarget beneficial insects, particularly nonpest herbivores like Lepidoptera of conservation interest, well beyond the time of application (Krischik et al. 2015, Pecenka

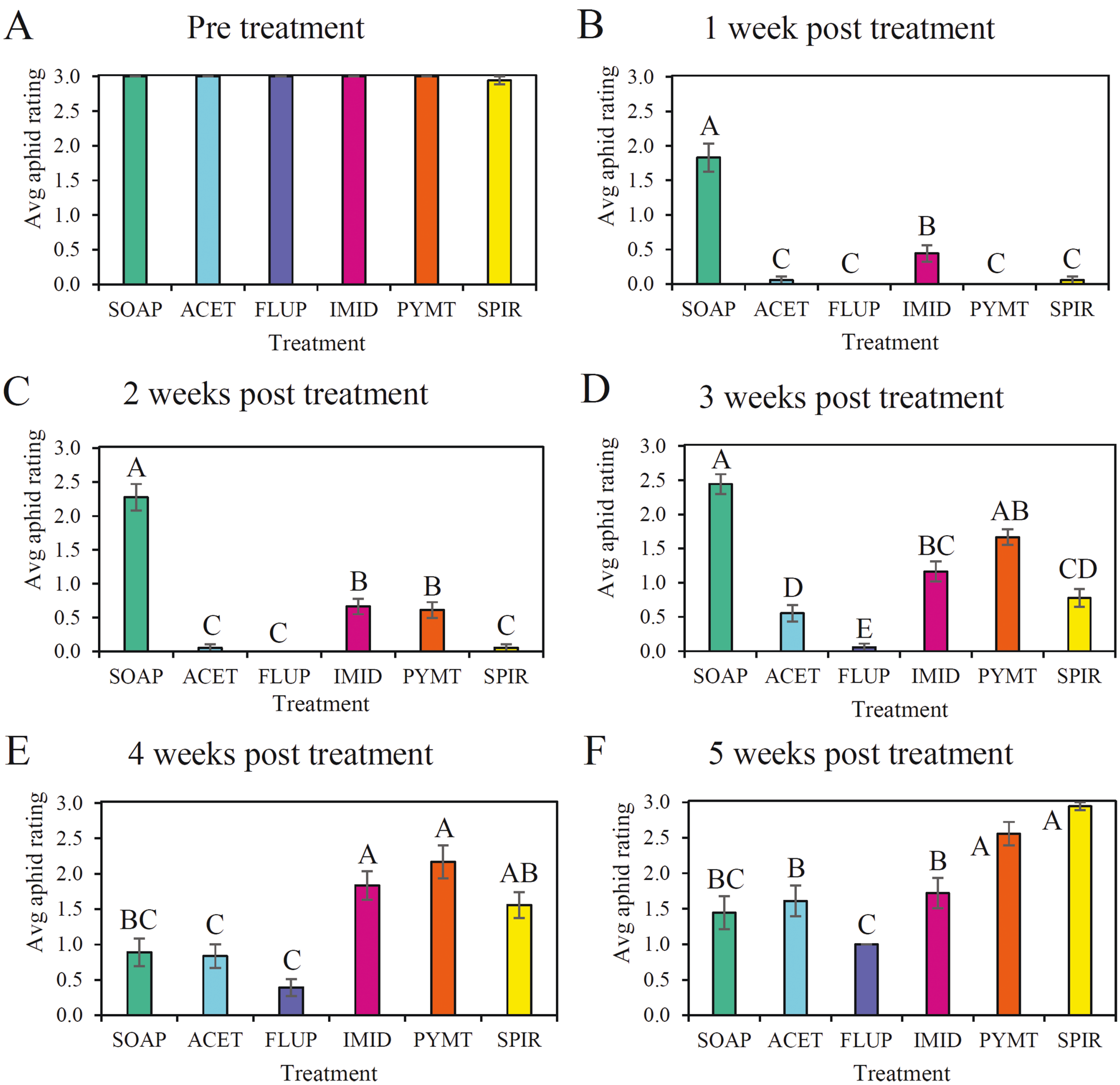


**Fig. 4.** Average weekly aphid density rating (0–3) per treated milkweed plant during the chronic exposure trials. The letters above bars indicate significant differences between treatments at each time point as determined by Kruskal–Wallis test with separation by Dunn's test for multiple comparisons and *P*-values adjusted using the Benjamini–Hochberg method.

and Lundgren 2015, Bargar et al. 2020, Krishnan et al. 2020, 2021a, Redmond et al. 2020, Knight et al. 2021, Krueger et al. 2021, Wilcox et al. 2021).

Our results indicate that insecticides used during ornamental milkweed production to control oleander aphids can cause adverse effects on monarch butterfly larvae up to 4 wk after a single treatment application. Although we observed overall low mortality rates in the acute exposure trials, our chronic exposure results highlight a more realistic, important, and often cryptic consequence of nontarget exposure to insecticides. Monarch mortality was notably higher during chronic exposure to treated plants. Importantly, most chronic exposure mortality occurred as unsuccessful pupation or eclosion as an adult butterfly, with larvae appearing to develop normally until the fifth instar, similar to other descriptions of pupal-stage mortality in monarchs (Bargar et al. 2020, Krishnan et al. 2020, 2021b). Mortality at this stage of development can be obscure and difficult to detect in situ because monarch larvae typically leave their host plants to pupate in a separate location. This finding emphasizes the importance of tracking nontarget impacts well past initial exposure and highlights that nontarget effects of insecticides may occur out of sight and thus miss detection by observers.

Each of the 6 insecticides we evaluated caused either acute exposure mortality, chronic exposure mortality, or failure to control aphids to such a degree that aphid-induced mortality occurred. We predicted that our proposed alternative insecticides, spirotetramat, flupyradifurone, and pymetrozine, would have minimal impacts on monarchs since none are labeled for caterpillar control and pymetrozine is even labeled as aphid- and whitefly-selective (Harrewijn and Kayser 1997). However, all alternative products resulted in at least 40% monarch mortality at 2 or more of the chronic exposure test intervals. All 3 industry-standard insecticides (imidacloprid, insecticidal soap, and spirotetramat) were also associated with at least 40% monarch mortality during 2 or more of the chronic exposure test intervals. The observed mortality on plants treated with insecticidal soap at 2 and 4 wk can be explained by the indirect effects of observed high aphid densities on these plants and consequent effects on plant health and defense (Mach et al. 2023). This is supported by our insecticidal soap toxicity trial which showed no soap-induced mortality or effects of frequent soap applications on monarch herbivory. Our observed 0% mortality on soap-treated plants during the 48 h evaluation period in our acute and chronic exposure experiments, before aphid densities increased, further support that insecticidal soap was not toxic to monarch larvae but was inadequate for preventing aphid outbreaks. The only other insecticide that caused monarch mortality at 4 wk posttreatment also experienced high aphid densities after 3 wk (pymetrozine, Fig. 3), implying that mortality may been in part associated with high aphid densities rather than, or in addition to, the direct effects of the insecticide. Combined, our toxicity results indicate that without proper planning, intentional product selection, and application timing, current industry standards and our proposed alternative insecticides are unsuitable for use on plants intended for Lepidoptera conservation within 4 wk of a single application.

Some of the most well-studied insecticides in the context of monarchs are neonicotinoids, which include 2 of our evaluated products, imidacloprid and acetamiprid (Krischik et al. 2015, Pecenka and Lundgren 2015, Bargar et al. 2020, Krishnan et al. 2020, Knight et al. 2021, Wilcox et al. 2021). Neonicotinoids have been shown to cause arrested ecdysis, reduced larval weight, and acute mortality in monarch larvae (Krischik et al. 2015, Pecenka and Lundgren 2015, Bargar et al. 2020, Krishnan et al. 2020). These products are among the most widely used insecticides in ornamental crop production (Douglas and Tooker 2015, Kachatryan and Rihn 2021, Krishnan et al. 2021) and mounting evidence points to negative nontarget impacts associated with their use (Frank and Tooker 2022). Imidacloprid is one of the most frequently used ornamental insecticides (Douglas and Tooker 2015) because of its efficacy against sap-feeding pests and extremely low cost. Acetamiprid, one of our proposed industry alternatives, is categorized by the US Environmental Protection Agency as a reduced risk because of its documented lower risk to nontarget wildlife and natural resources (US EPA 2023). Therefore, we predicted it would be safer for monarchs relative to conventional standards like imidacloprid. Although both of our evaluated neonicotinoids had pronounced toxic effects on monarchs, our results provide further evidence against using imidacloprid in ornamental plant production for lepidopteran host plants. Specifically, imidacloprid caused 80% monarch mortality 2 wk after treatment application and the next week aphid densities had reached the treatment threshold, suggesting that a subsequent application would be needed, perpetuating monarch toxicity at or above observed levels. Importantly, adult female monarchs do not discriminate between imidacloprid-treated and imidacloprid-free plant material (Mullins et al. 2021), resulting in potential exposure of highly vulnerable early-instar larvae to toxic insecticide residues if they encounter treated plant material.

Nursery growers need to maintain low pest densities to produce saleable plant material. Our results shed light on the efficacy of industry standard and alternative insecticides targeting a key sap-feeding insect pest group. We identified 2 industry alternative products, flupyradifurone and acetamiprid, that suppressed aphid densities below the treatment threshold for at least 4 wk from 1 application. Although acetamiprid is a neonicotinoid and flupyradifurone is neonicotinoid-adjacent (same primary mode of action, Nauen et al. 2015), both are reduced-risk insecticides (US EPA 2023) and were among the best performers regarding aphid suppression and nontarget impacts to monarchs. Both products were highly toxic to monarch larvae within the first 2 wk following application, but this toxicity completely dissipated by week 4 when aphids were still below threshold levels. Although additional research is needed to validate specific grower recommendations, such windows of toxicity could possibly be accounted for during plant production and distribution with proper timing of insecticide applications. For example, a milkweed cohort could be treated with flupyradifurone, held for 2 wk, and then distributed for retail sale with an increased likelihood of low pest levels and minimal nontarget effects on monarchs after plant sale and installation into landscapes. Such product selection and application tactics may allow growers to improve aphid suppression, reduce insecticide applications, and thus reduce nontarget impacts to beneficial Lepidoptera. However, additional research is needed to validate these potential protocols since we did not test toxicity to eggs or early instars, which can be more sensitive to insecticide residues than the third instar larvae we used in our experiment.

Unfortunately, the duration of aphid suppression provided by most of the insecticides we evaluated was shorter than the duration of adverse effects on monarch larvae. As mentioned above, only acetamiprid and flupyradifurone had a period of no monarch mortality and effective aphid control. Despite their common use, all of the industry standard insecticides caused some degree of monarch mortality for the duration of time that they suppressed aphid densities below threshold levels (Fig. 3). Based on our aphid density evaluations, most products we tested would have required re-treatment of either the same or a different insecticide after week 1 (insecticidal soap), week 2 (imidacloprid, pymetrozine), or week 4 (spirotetramat) while also causing monarch mortality during the first

2 wk post application. This is important because growers reapply insecticides at or before aphid populations reach the aphid density threshold, which would perpetuate or intensify the acute and chronic nontarget effects on monarchs. Also concerning is that only 1 of the 3 industry-standard insecticides provided aphid control for more than 2 wk (spirotetramat), suggesting that subsequent applications of commonly used insecticides would fall well within the monarch toxicity window. Although ornamental plants likely provide limited conservation value in nursery settings, residual effects of these treatments after sale are important to consider, especially since earlier larval instars may be more sensitive to insecticide residues than the third instar larvae we tested. Further, multiple applications of either the same or different insecticide active ingredients may potentially exacerbate the toxicity or longevity of our observed nontarget toxicity to Lepidoptera due to additive or synergistic effects, but little is known about the true extent of this risk.

High oleander aphid densities and the insecticides used to control them on ornamental milkweed independently threaten monarch butterfly conservation in urban and residential landscapes. Monarch larval survival is already low in nature (7–10% from egg to fifth instar) (Nail et al. 2015), and adding insecticide- and aphid-induced mortality could deal a critical blow to monarch butterfly populations. Therefore, IPM programs should balance the benefits of aphid suppression on monarchs and plant marketability with the risks that insecticides pose to monarch larvae. Our results clearly show that some commonly used insecticides have nontarget adverse effects on monarch larvae for up to 4 wk after application via foliar spray. Importantly, for several products, adverse effects persisted beyond the period of aphid suppression, meaning that our illustrated risk to monarchs in nursery settings is likely conservative compared to real-world insecticide use. Moreover, half of the insecticides we evaluated are not labeled for caterpillar control, yet they had highly toxic effects on monarchs, illustrating a need for evidence beyond the product label to guide pest management on larval host plants. Although this study provides evidence of potential impacts within a nursery production setting where conservation is not the goal, our observed residual toxicity, and our results are concerning when combined with recent evidence that plants from retail garden centers are contaminated with insecticide residues (Halsch et al. 2022). Given that many of these products are routinely applied via soil drench to take full advantage of their systemic properties and minimize nontarget exposure via insecticide sprays, and residues from insecticides applied in this method can persist for months or even years (Bonmatin et al. 2015, Mach et al. 2018), future research should investigate the effects of different application methods on toxicity to lepidopterans of conservation concern. With growing public interest in insect conservation and subsequent increases in the production and sale of Lepidopteran larval host plants, more research is needed to generate evidence-based production and maintenance IPM practices that provide and sustain plant material that is compatible with wildlife conservation goals.

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## Author Contributions

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