Integrating pest and pollinator management strategies for ornamental plant production

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ABSTRACT

Demand for wildlife-friendly plants, especially plants that support pollinators, has never been higher. This is great because producing and planting wildlifefriendly plants helps mitigate biodiversity loss, which is an increasing global concern. This demand also presents an incredible business opportunity for Florida's green industry. Unfortunately, many plants that support pollinators are also attacked by damaging insect pests. Insect pests reduce plant health and marketability, which often requires chemical intervention to produce a saleable product. Plant pests may also directly reduce the conservation value of plants by reducing plant quality and competing with pollinators. Most pollinator toxicological data focus on bees. Lepidopteran pollinators (i.e., butterflies and moths) differ in susceptibility to insecticides compared to bees and have different routes of exposure (e.g., larval leaffeeding versus nectar consumption). Consequently, products compatible with bee conservation pose an unknown risk to lepidopteran pollinators, yet both taxa are pervasive in agroecosystems and urbanized landscapes. We used the milkweedmonarch-oleander aphid system as a model to

begin developing integrated pest and pollinator management (IPPM) strategies for ornamental plant production. Our results demonstrate that all commercially used systemic insecticides targeting aphid pests have toxic effects on monarch caterpillars at some timepoint after application. However, these effects and the longevity of effects vary significantly between insecticides. More specifically, we found that acute mortality from exposure to treated milkweed for 48 hours occurs with most evaluated insecticides, but only within the first week after application. More importantly, we find that all insecticides have toxic effects from chronic exposure, which leads to monarch death at the pupal stage. Some products have this effect up to 28 days after treatment. This is notable, because it suggests that most insecticide mortality to monarchs occurs out of site and thus out of mind since monarchs typically wander away from their host plant to pupate. Results will be used to develop a certified wildlife-friendly plant production protocol that will increase the positive environmental and ecological impact of ornamental plant production.

OBJECTIVES

- 1. Determine the effects of aphid infestation on monarch conservation outcomes.
- 2. Determine the acute and chronic exposure toxicity of commonly used and proposed alternative insecticides to monarch butterfly larvae.
- 3. Evaluate the effectiveness of commonly used and proposed alternative insecticides to control oleander aphid infestations on tropical milkweed.

METHODS

The Study System

Tropical milkweed is a nonnative, herbaceous perennial plant that is widely available for purchase across the eastern and southern United States and is the most popular milkweed species for landscape and garden use. The monarch butterfly (*Danaus plexippus*, L.) is a widely recognized charismatic North American butterfly that engages in a yearly migration from the eastern United States and Canada to overwintering grounds in Mexico. Monarch larvae are dietary specialists that feed exclusively on milkweed (family Apocynaceae, subfamily Asclepiadoideae). Monarch populations have declined by over 80% in recent decades (Brower et al. 2011, Thogmartin et al. 2017, USFWS 2020a, b), which has spurred widespread conservation efforts, primarily in the form of providing larval host plants. The oleander aphid (Aphis nerii, Fonscolombe, 1841) is an important sapfeeding insect pest of milkweed and other related ornamental plants in tropical to warm temperate regions of the world. Milkweed infested with oleander aphids become chlorotic, drops leaves, and are often covered with black sooty mold, ultimately resulting in an unsaleable plant. Thus, insecticides are commonly used during production to prevent plant damage and loss. Since many insecticides are toxic to Lepidoptera, milkweed treated for aphid infestations may inadvertently control monarch larvae or other beneficial caterpillar species during plant production and after sale. Consequently, plants produced and planted for wildlife conservation purposes may have the opposite effect. This combination of factors makes the monarch butterfly-milkweedoleander aphid system ideal for developing IPPM strategies compatible with lepidopteran larvae conservation.

Objective 1 approach

An important initial question is: Do oleander aphids interfere with monarch conservation? We hypothesizedthat high-density aphid infestations would have negative effects on monarch oviposition and larval success by reducing plant quality and upregulating plant defenses. We measured the number of eggs deposited on milkweed plants by placing one adult male and one adult female in a mesh cage with one aphid-free and one aphid-infested tropical milkweed plant. Monarchs were allowed to oviposit freely and all eggs on each plant were collected and counted seven days later.

For the larval feeding study, we assigned third instar monarch larvae to three aphid density treatments: aphid-free milkweed leaves, aphid-infested milkweed leaves with sooty mold, aphids, and honeydew (i.e., "dirty" leaves), and aphid-infested milkweed leaves that had been cleaned with water to remove sooty mold, aphids, and honeydew (i.e., "cleaned" leaves). Each larva was placed in a Petri dish with one leaf and stored at 28 $^{\circ}$ C and 75% relative humidity with a 12:12 L:D diurnal cycle for seven days. A single new milkweed leaf was added to each Petri dish when > 50% of the old leaf was consumed or every 48 h, whichever came first. Total leaf area consumed (cm²) per larva was calculated. Final instar attained and larval weight were also measured after seven days.

Objective 2 approach

Three industry standard insecticides (imidacloprid, spirotetramat, and insecticidal soap) were selected based on a 2017 Florida nursery grower survey. We also selected three proposed alternatives (pymetrozine, acetamiprid, and flupyridifurone) that are labeled for aphid control on ornamental plants and suspected to provide reduced risks to lepidopteran insects. Each insecticide was applied at the labeled aphid control rate. Selected insecticides and their properties are detailed in **Table 1**.

Our first question was – Do any of these insecticides have toxic effects on monarch caterpillars due to acute exposure, or within 48 hours of feeding on treated plant tissue, and how does this change over time after treatment application? For this acute toxicity experiment, six milkweed plants were randomly assigned to each treatment. Plants were treated with the appropriate insecticide via foliar sprays at labeled rates for aphid control. To assess acute oral toxicity associated with each insecticide, we exposed third-instar monarch larvae to treated tropical milkweed leaves for 48 h. Leaves were harvested 24 h, two weeks, and four weeks after insecticide treatment and placed individually in a Petri dish with a single third-instar monarch larva and a piece of damp filter paper. Petri dishes were held at 28 °C and 75% relative humidity with a 12:12 light cycle. Larval mortality (%) and leaf area consumed were measured at 48 h in the Petri dish.

Our second question was – Do any of these insecticides have toxic effects on monarch caterpillars due to chronic exposure, or when caterpillars are allowed to feed on treated plants until they pupate and complete development, and how does this change over time after treatment application? For this chronic toxicity experiment, eighteen plants were randomly assigned to one of the six treatments and further subdivided into three cohorts of six plants that designate exposure at different time points after treatment (24 H, 2 weeks, 4 weeks). These time cohorts corresponded with times post-treatment at which monarch larvae were added to the plants and given the opportunity to feed and complete development. All plants were treated at the same time via foliar spray with the appropriate insecticide at labeled rates for aphid control. At 24 h, 2 weeks, and 4 weeks post-treatment, a single third-instar monarch larva was placed on each plant in the appropriate treatment × time cohort. Monarch mortality was assessed until all larvae either perished or reached adulthood.

Table 1. Selected insecticides and their properties.

Insecticide	IRAC mode of action	Application Rate (g or ml pesticide per 379 L H ₂ O)	Active Ingredient (g or ml per 379 L application)	Labeled for caterpillars	Honey bee contact toxicity rating	Honey bee oral toxicity rating
Pymetrozine ¹	9-B	142 g	71 g	No	Practically nontoxic	Practically nontoxic
Spirotetramat ^{1,2}	23	100 ml	24 g	No	Practically nontoxic	Practically nontoxic
Acetamiprid ¹	4-A	118 ml	11 g	Yes	Practically nontoxic	Moderate
Flupyradifurone ¹	4-D	298 ml	60 g	No	Practically nontoxic	Moderate
Imidacloprid ²	4-A	50 ml	12 g	Yes	High	High
Insecticidal soap ²	-	7393 ml	3661 g	Yes	n/a	n/a

¹Reduced Risk, US EPA ²Industry standard

Objective 3 approach

Since insecticide applications are made to milkweed to suppress oleander aphid infestations, we also evaluated the effect of each insecticide on aphid density per plant during the chronic toxicity experiment. The severity of aphid infestation was rated once weekly beginning at the time of insecticide application and continuing for the duration of the chronic toxicity 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 (approximately 50-150 aphids per terminal growth point), or 3 (>150 aphids per terminal growth point). Average aphid ratings above 1 indicate plants with infestations over the recommended treatment threshold of >50 aphids per terminal growth point.

RESULTS

Objective 1

We found that high-density oleander aphid infestations have substantial negative effects on monarch oviposition and larval health. High-density aphid infestations reduced oviposition, larval weight, and larval leaf consumption by nearly 50% (**Figures 1 & 2**). Taken together, the effects of high-density aphid infestations on monarchs have significant implications for conservation efforts in urbanized landscapes and emphasize the importance of controlling aphid infestations to preserve the conservation value of milkweed.

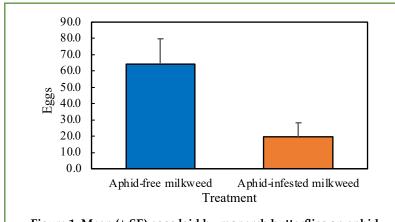


Figure 1. Mean (± SE) eggs laid by monarch butterflies on aphidfree versus aphid-infested milkweed. Different letters over bars indicates a significant difference (least-squares means, p<0.05).

Objective 2

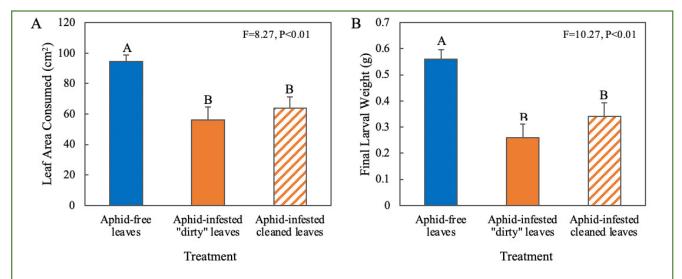


Figure 2. Mean (± SE) leaf area consumed (A) and final larval weight (B) of monarch larvae fed aphid-free milkweed leaves versus aphid-infested "dirty" leaves and aphid-infested cleaned leaves. Bars not topped by the same letter indicate significant differences (least-squares means, p<0.05).

Each of the six insecticides we evaluated resulted in either acute exposure mortality, chronic exposure mortality, or both to monarch larvae. Although spirotetramat and flupyridifurone are not labeled for caterpillar control, and pymetrozine is labeled as aphid-selective (Harrewijn & Kayser 1996), all products caused at least 40% monarch mortality at two or more of the chronic exposure test intervals. All three industry standard insecticides (imidacloprid, insecticidal soap, spirotetramat) caused at least 40% monarch mortality during two or more of the chronic exposure test intervals. Although we observed overall low mortality rates in the acute exposure trials, our chronic exposure results highlight an important and often cryptic consequence of non-target exposure to insecticides. Monarch mortality was higher when chronic exposure to treated milkweed plants was monitored for up to four weeks post insecticide application. Importantly, most of the chronic exposure mortality occurred as unsuccessful pupation, with larvae appearing to develop normally until fifth instar. This is important because it emphasizes the importance of tracking non-target impacts well past initial exposure and highlights that non-target effects of insecticides may be often out of sight and likely miss detection. Although we did not detect significant differences in mortality between treatments at 2 and 4 weeks, several insecticides caused 40% or greater mortality at both time points. These results demonstrate that insecticides commonly used during plant production pose significant risks to lepidopteran pollinators for periods well beyond application.

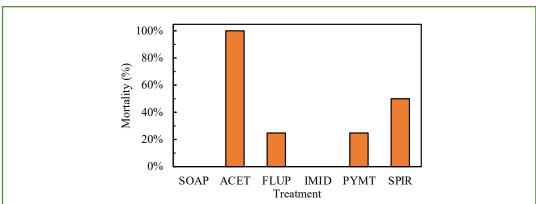


Figure 3. Acute exposure percent mortality for monarch larvae exposed to field-weathered insecticide residues for 48 h at 24 hours after insecticide application. No mortality was observed across all treatments at the 2 weeks and 4 weeks post treatment timepoints. Letters indicate significant differences between insecticides as determined by Kruskal-Wallis test with separation by post-hoc Dunn's test for multiple comparisons.

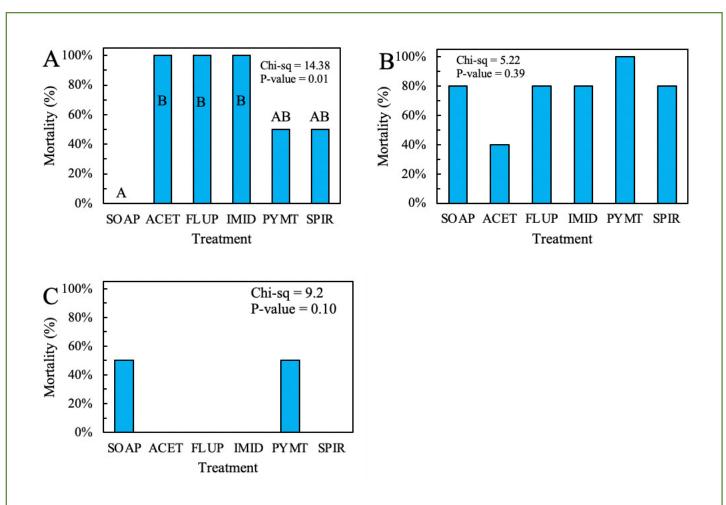


Figure 4. Chronic exposure percent mortality for monarch larvae exposed to field-weathered insecticide residues and provided the opportunity to complete development at A) 24 hours, B) 2 weeks, and C) 4 weeks after insecticide application. Letters on each bar indicate significant differences between treatments at each timepoint as determined by Kruskal-Wallis test with separation by Dunn's test for multiple comparisons.

Objective 3

Despite their commonplace use, none of the industry standard insecticides suppressed aphid densities below threshold levels for more than two weeks after one application. However, we two of our alternative products, flupyridifurone and acetamiprid, suppressed aphid densities below threshold levels for at least four weeks. Although acetamiprid is a neonicotinoid and flupyridifurone is neonicotinoid adjacent, each of these products are reduced risk insecticides and were among the best performers regarding non-target impacts to monarchs. These results suggest that nursery industry professionals should adjust their management programs to increase aphid control efficacy and reduce the required frequency of insecticide applications.

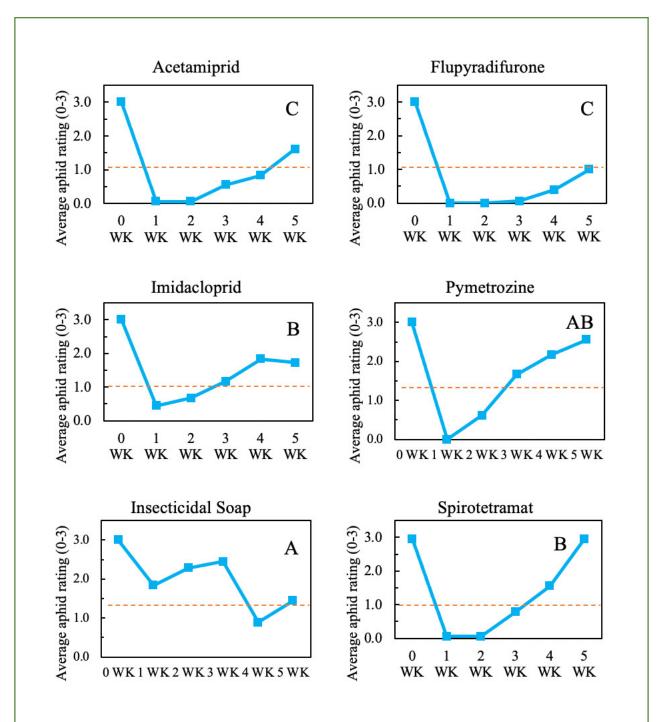


Figure 5. Average weekly aphid density rating (0-3) per treated milkweed plant during the chronic exposure trials. Treatment threshold (1) is indicated by the dashed line. Letters indicate significant differences between treatments over the duration of the experiment as determined by Kruskal-Wallis test (chi-sq = 80.076, p-value = 8.092e-16) with separation by Dunn's test for multiple comparisons.

CONCLUSIONS

Our results show that the effects of high-density aphid infestations on monarchs have significant implications for conservation efforts in urbanized landscapes. Pest-ridden milkweed provides less conservation value for monarchs as it recruits fewer monarch eggs, and those eggs will hatch into larvae with worse developmental outcomes, which in turn will result in adults with reduced fitness and migratory ability. This is especially relevant for milkweed plantings in urbanized landscapes where public conservation efforts are most prevalent, but where outbreaks of sap-feeding herbivores are also commonplace and difficult to manage. Unfortunately, insecticides used to control pests pose direct risks to monarchs. Furthermore, the duration of aphid control in these experiments was often shorter than the duration of adverse effects on monarch larvae, clearly demonstrating the conflict between aphid control and monarch larval conservation in ornamental plant production settings. Of the six insecticides tested during the chronic exposure trials, only two had a period where aphids were controlled below the treatment threshold and monarch larvae experienced no adverse effects. Additional research to develop IPPM strategies is urgently needed to ensure that milkweed and other lepidopteran host plants retain their conservation and monetary value during production.