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Tropical milkweed herbivore and predator dynamics in suburban South Texas

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ABSTRACT

Tropical milkweed is commonly grown to conserve pollinators, but the presence of non-caterpillar herbivores may prompt treatment. Management is limited to non-chemical methods like biological control, but potential natural enemies have not been well studied in the Lower Rio Grande Valley (LRGV). We documented the arthropod community on tropical milkweed in garden and potted settings then analyzed associations between organisms. In the garden, oleander aphids and large milkweed bugs were the primary herbivores, overlapping on seedpods. Natural enemies (lady beetles, syrphid fly larvae, and aphid parasitoids) were positively associated with oleander aphids but not milkweed bugs. The arthropod community experienced similar associations but with reduced natural enemy abundance and richness on potted plants.

Additional index words: *Aphis nerii*, *Asclepias curassavica,* Coccinellidae, *Oncopeltus fasciatus*, parasitoid, Syrphid

Planting milkweed has gained traction as a way to provide habitat for monarch butterflies (*Danaus plexippus* Linnaeus, Lepidoptera: Nymphalidae) and other pollinators (Fitzgerald, 2015; Keller Science Action Center, 2017; Meier, 2019). Within the southeastern United States, tropical milkweed (*Asclepias curassavica*) is widespread in both curated and non-garden settings because it flowers year-round, is drought tolerant, and is low maintenance (Maeckle, 2013a; Maeckle, 2013b; Haynes et al., 2015; Brower, 1961; Batalden & Oberhauser, 2015). Despite these plants being low-maintenance, homeowners worry about the presence of unwanted herbivores in their pollinator gardens (Barnes et al., 2020). Such herbivores on milkweed may include the oleander aphid (Hemiptera: Aphididae, *Aphis nerii* Boyer de Fonscolombe) and the large milkweed bug (Hemiptera: Lygaeidae, *Oncopeltus fasciatus* Dallas) (Beck et al., 1958; Braman & Latimer, 2002; Dingle, 1991; Zandt & Agrawal, 2004). Although these herbivores may infest plants in large numbers, chemical insecticide use should be limited to prevent killing the desired monarch caterpillars and pollinators (Braman & Latimer, 2002).

The natural enemy community responsible for biological control of these pests may encompass several predators and parasitoids and can be effective at managing herbivores like oleander aphids (Smith et al., 2008; Mohl et al., 2016). Predators found on milkweed include lady beetles (Coleoptera: Coccinellidae), green lacewings (Neuroptera: Chrysopidae), syrphid fly larvae (Diptera: Syrphidae), and spiders (Araneae) (Braman & Latimer, 2002; Smith et al., 2008; Baker & Potter, 2019). In addition to predators, oleander aphids can be attacked by parasitoids that can be observed via aphid "mummies" or the hardened brown skin of parasitized aphids (Hall & Ehler, 1980; Smith et al., 2008). However, the impact of natural enemies may be diminished by the presence of aphid-tending ants (Hymenoptera: Formicidae) (Bristow, 1991).

While well documented in other subtropical areas, the herbivores and natural enemies associated with tropical milkweed have not been studied in the context of the LRGV. Therefore, the goals of this study were to 1) document the herbivores and natural enemies on tropical milkweed, 2) determine if organisms were associated with each other and the same plant structures to indicate potential biological control and 3) determine if these patterns differed when plants were in a pollinator garden as opposed to isolated in pots within the same suburban area.

MATERIALS AND METHODS

The suburban garden trial (~15 m^2 est. 2018) was conducted at UTRGV, Edinburg, TX, USA (26° 18'24"N, 98°10'17"W). The rainfed garden was in partial sun and contained primarily tropical milkweed with a few non-flowering shrubs and flowering trees. Milkweed plants were trimmed by groundskeeping on 1 April 2020 but were otherwise not disturbed. A randomized selection of 20 plants was monitored weekly from 4-27 March and from 14 May to 5 June 2020. At each observation, the terminal composition of each branch and all organisms present per branch were recorded with parasitoid presence being indicated by aphid mummies (Price & Wilson, 1979). The average daily temperature was recorded from local weather stations.

A potted plant trial was conducted at 3 suburban locations separated by a minimum of 3 km and within 6.5 km of the garden plot. Each location had 2 5-gallon pots filled with a combination of organic potting soil and garden soil, each containing 6 plants (0.3-0.5 m tall) transplanted as seedlings from the unsampled areas of the garden plot. Upon transplanting, plants were inspected, and arthropods removed. Each pot was inoculated with 20 oleander aphids from a parasitoid-free laboratory colony then maintained in the lab for 3 days before placement. Once relocated, plants were placed on hardscaped walkways or porches in partial sun and watered as needed. Plants were observed 48 times from 19 April to 14 June 2020, ending 64 days post-inoculation. All herbivores, natural enemies, and branch composition were recorded as above.

All statistical analyses were conducted in R 4.0.2 (R Core Team, 2020). Differences were determined using ANOVA and Tukey HSD tests. General Linear Mixed Models (GLMMs) using the lme4 package were used to determine interactions of herbivore and natural enemy abundances per branch (Bates et al., 2015). For all models, month, time point, branch composition, the total number of branches per plant, and the proportion of branches classified as leaves were included. Significance values were estimated using Satterthwaite approximation with the lmerTest package (Kuznetsova et al., 2017). Posthoc comparisons were conducted with a Tukey estimation in the emmeans package or odds ratios (OR)(Lenth, 2020).

RESULTS

Suburban garden. While the proportion of branches categorized as flowers did not change between months, the proportion of leaves and seedpods did (S. Fig. 1). We consistently found oleander aphids, large milkweed bugs, lady beetles, syrphid fly larvae, and aphid parasitoids. Lady beetles were primarily *Cycloneda munda* Crotch adults and larvae, though *Cryptolaemus montrouzieri* Muls. larvae were occasionally observed on nearby plants. Spider and ant presence on plants were sporadic, with most being crab spiders (Thomisidae) and *Brachymyrmex depilis* Emery. Spider mites (Trombidiformes: Tetranychidae) were documented on plants in both March and May. Four queen caterpillars (Lepidoptera: Nymphalidae, *Danaus gilippus* (Cramer)) were observed in March; monarch caterpillars were not seen at any time. Isopods were found consuming stems and leaves of nearby plants, particularly along the ground in shady areas (S. Fig. 2); to our knowledge, this is the first docu-

Table 1. General linear mixed model parameter estimates for suburban garden observations of arthropod herbivore or natural enemy abundance per branch with P-values <0.05 denoted by *.

Variable	Milkweed Bugs	Oleander Aphids	Lady Beetles	Syrphid Fly Larvae	Aphid Parasitoids
Temperature	0.020	0.032	0.230	0.685*	0.310
Month (May)	-0.414	0.600	-0.210	1.627	-2.405*
Time	0.164	-0.236	-0.065	-0.971	-0.470
No. Branches	-0.005	0.016	0.086	0.186*	-0.003
Flowers	-1.026*	0.546	-0.112	-0.195	-0.652
Seedpods	-0.848*	1.681*	-2.712*	0.595	-1.111*
Prop. Leaves	-1.573	0.923*	0.266	-3.361	1.462
Milkweed Bugs		0.232	-0.738	-21.745	-0.360
Aphids	0.052*		0.094*	0.106*	0.076*
Parasitoids	-0.043	0.103*	0.060	-0.016	
Syrphid Flies	-12.908	1.095*	0.899*		0.207
Lady Beetles	-1.295	0.615		0.862*	1.368*
Ants (present)	1.549	0.112	2.021	-19.040	1.161
Mites (present)	0.225	-1.502	-21.499	0.632	-1.900

mented isopod herbivory on milkweed plants.

Aphid abundance was correlated with branch categories and milkweed bugs, potentially due to overlap in plant preferences (Table 1). Seedpod presence and a greater proportion of leaves increased aphid abundance whereas branches with leaves then seedpods had the most milkweed bugs (S. Table 1). Natural enemies were positively associated with aphids and each other and were also more likely to occur on plant leaves or plants with more branches (Table 1).

Potted plants. Potted plants generally maintained one branch for the first 20 days and 2 branches until 30 days post-inoculation. Initial branches began flowering at 20 days post-inoculation when the second branch had been added, with a large portion of plants not flowering until near the end of the observation period. The observed arthropods were less abundant (except aphids) and diverse on potted plants than in the garden. Oleander aphids and spider mites were the only herbivores, while syrphid fly larvae and aphid parasitoids were the main natural enemies as few spiders and no lady beetles or large milkweed bugs were observed at any location.

Oleander aphid abundance increased over time with cyclical fluctuations at all locations (S. Fig. 3). Aphid abundance increased with flower presence (S. Table 2; $P<0.001$) and ant attendance ($P<0.001$) but was negatively impacted by the presence of mites (S. Table 2; $P=0.012$). Ants were mostly found at one location (A) and were more likely to be present when aphids ($P=0.005$) and flowers were also present ($P<0.001$). Syrphid fly larvae abundance increased with aphid abundance (S. Table 2; $P<0.001$), but like ants, was negatively impacted by the presence of mites ($P=0.001$). Spider mites were observed at all three locations, with the majority split between locations A and C. Parasitoids were only found at location B and were positively associated with aphid abundance ($P=0.001$).

DISCUSSION

The arthropod community on tropical milkweed was primarily composed of oleander aphids, large milkweed bugs, aphid parasitoids, lady beetles, and syrphid fly larvae and was like that of other locations and milkweed species (Braman & Latimer, 2002; Smith et al., 2008; Baker & Potter, 2019). The two herbivores exhibited overlapping preferences in plant structures like leaves and seedpods. Generally, as the number of branches per plant increased in the garden, so did the abundance of all organisms, potentially through increased resource apparency (Cohen & Brower, 1982; Stanton et al., 2016). The lack of apparency of the potted plants may also have influenced the abilities of the organisms to find the plants (Zalucki & Kitching, 1982; Nail et al., 2015). The reduced number of species on potted plants compared to the garden and the location-related variation found may have also been related to relative proximity and quantity of source green spaces (Rocha & Fellowes, 2020; Korányi et al., 2020; Parsons & Frank, 2019).

When we analyzed the herbivore-natural enemy relationships, oleander aphids but not large milkweed bugs were associated with natural enemies. Prior work in urban spaces has indicated that predator abundance often increases with aphid abundance (Rocha & Fellowes, 2020; Bächtold & Del-Claro, 2013), and the observed natural enemy community within our suburban garden acted similarly. Further, while aphid abundance in the garden plot was unaffected by ant attendance, aphids on potted plants were positively associated with ant presence. Ant presence on potted plant branches was also greater on flowers than leaves (S. Table 2), as previously observed with Argentine ants (*Iridomyrmex humile* Mayr) tending oleander aphids (Bristow, 1991). Although we observed a negative association of syrphid fly larvae with ants on the potted plants, we did not see an association of ants with natural enemies in the garden (Rocha & Fellowes, 2020). The difference in these interactions may be due to the garden providing ants with food sources other than aphid honeydew that might have disincentivized ants from venturing onto plants (Rabelo & Francini, 2014; Wäckers et al., 2017; Win et al., 2018).

Our study found a consistent presence of oleander aphids on tropical milkweed in the LRGV with the corresponding natural enemy community and large milkweed bug presence varying with garden versus potted plants. Aphid abundance was positively associated with seedpods and floral buds, overlapping with large milkweed bugs. Natural enemies were positively associated with aphid abundance and each other but had no interaction with milkweed bugs. Within the suburban garden setting, plants were more likely to be colonized by natural enemies as well as large milkweed bugs, queen butterfly caterpillars, and isopods. In the more isolated pots, plants experienced an increased number of spider mites and aphid-tending ants and a decrease in natural enemy abundance and richness. Given these data, oleander aphids in the LRGV have several potential natural enemies that may be used as biological control dependent on the plant's isolation. However, more work evaluating plant health and aesthetics resulting from these community dynamics needs to be conducted over more seasons and LRGV locations.

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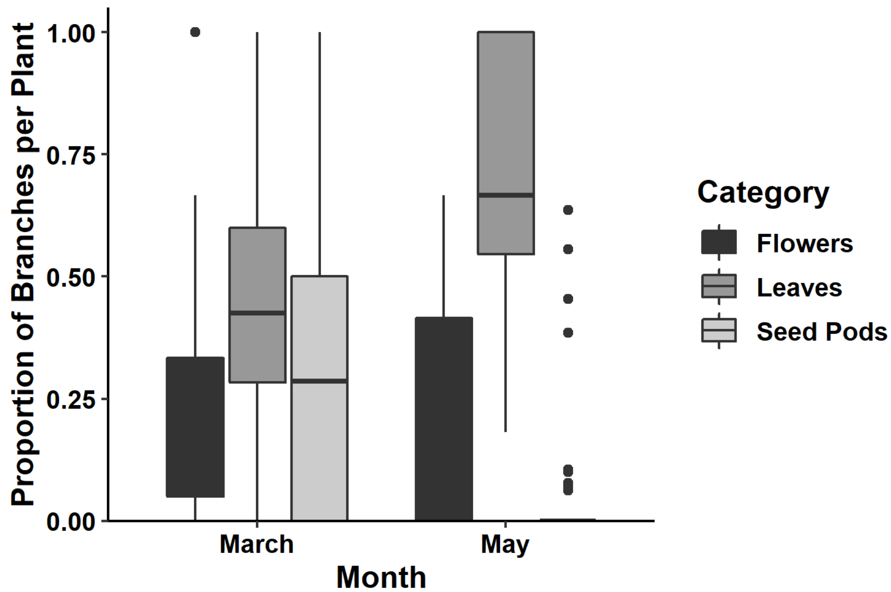
S. Table 1. Means and standard errors of observed organisms per branch in the suburban-based on branch category – leaves, flowers, or seedpods. Letters indicate significant differences between branch categories for each organism using Tukey HSD comparisons (α = 0.05) with all observation times and plants pooled.

Organism	Leaves	Flowers	Seedpods
Milkweed Bugs	0.125 ± 0.019 a	0.053 ± 0.025 a	0.073 ± 0.023 a
Aphids	1.302 ± 0.277 c	2.726 ± 0.362 b	4.173 ± 0.341 a
Lady Beetles	0.023 ± 0.007 a	0.035 ± 0.010 a	0.023 ± 0.009 a
Syrphid Flies	0.008 ± 0.009 b	0.027 ± 0.012 ab	0.064 ± 0.011 a
Parasitoids	0.495 ± 0.108 a	0.473 ± 0.141 a	0.327 ± 0.133 a

S. Table 2. General linear mixed model parameter estimates with Poisson distribution and *P*-values for inoculated potted plant branch observations of oleander aphid and syrphid fly larvae abundance. "Threshold Met" indicates GLMM results with binomial distribution when an observed branch had greater than 50 aphids, meeting the treatment threshold. "No. Branches" and "Prop. Leaves" are plant-level metrics rather than branch-level. Variables with no data were not included in the final model following model selection.

	Aphids		**Threshold Met**		**Syrphid Flies**	
Variable	Estimate	*P*-value	Estimate	*P*-value	Estimate	*P*-value
Temperature	0.046	0.035	0.405	0.002	-0.035	0.365
Days	0.116	0.000	0.164	0.075	0.131	0.000
$Days^2$	-0.001	0.000	-0.002	0.090	-0.002	0.000
No. Branches	0.129	0.352	-1.395	0.131	0.439	0.066
Flowers	0.643	0.000	1.880	0.049	-0.866	0.001
Prop. Leaves	2.479	0.000	-0.243	0.922	2.074	0.029
Aphids					0.028	0.000
Parasitoids	0.144	0.000	-15.890	0.997	-0.007	0.963
Syrphid Flies	0.312	0.000	0.984	0.003		
Ants (present)	0.671	0.000	3.713	0.001	0.581	0.162
Mites (present)	-0.391	0.012	-17.870	0.999	-2.481	0.001

S. Fig. 1. The proportion of branches per plant that apically end in flowers (*P*=0.559), leaves (*P*<0.001), or seedpods (*P*<0.001) in March compared to May 2020. Boxplot lower, median, and upper limits represent the 25th, 50th, and 75th quartiles, respectively; points represent outliers.



S. Fig. 2. (A) Isopod herbivory on tropical milkweed stems and (B) leaves on plants near but not included in the study at the suburban garden plot in March 2020.

S. Fig. 3. Oleander aphid, aphid parasitoid, and syrphid fly larvae abundance per branch for 64 days after initial potted plant inoculation with 20 aphids. The three locations are indicated by different colors. The colored, solid lines represent loess curves fit to each location. The aphid treatment threshold (50 aphids/branch) is indicated by the dashed gray line.