The influence of host plant species on the frequency of defensive behaviors exhibited by the Oleander aphid, *Aphis nerii*, in response to the parasitoid, *Lysiphlebus testaceipes*

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ABSTRACT. The oleander aphid, *Aphis nerii* Boyer de Fonscolombe, feeds on milkweeds in the Apocynaceae family. Characteristics including trichome density and leaf toughness vary among milkweed species. The hypothesis that host plant characteristics influence the frequency of defensive behaviors exhibited by this aphid against the parasitoid, *Lysiphlebus testaceipes* (Cresson), was tested in a laboratory experiment. Interactions between oleander aphids and *L. testaceipes* were investigated on four milkweed species with different physical characteristics. Aphids responded to parasitoid presence with spinning, kicking, bucking, a coordinated defense, and the excretion of cornicle wax. When excreted, cornicle wax reduced parasitoid foraging time regardless of host plant species. The most common response to parasitoid presence was a coordinated defense behavior on all host plant species. When spinning, kicking, bucking, and coordinated defenses were combined per host plant species, aphid defenses tended to be greater in frequency on *Cynanchum laeve* and *Asclepias incarnata* than on *A. tuberosa* with *A. syriaca* being intermediate. Our results suggest that aphids colonizing host plants with few physical defenses may exhibit a greater number of defensive behaviors than aphids on plants having rougher leaf textures and high trichome densities.

INTRODUCTION

The oleander aphid, *Aphis nerii* Boyer de Fonscolombe, feeds on members of the Apocynaceae family including common milkweed, *Asclepias syriaca* L., swamp milkweed, *Asclepias incarnata* L., butterfly weed, *Asclepias tuberosa* L., and honeyvine milkweed, *Cynanchum laeve* (Michaux) (Colvin 2011). *Asclepias syriaca, A. tuberosa, A. incarnata,* and *C. laeve* have distinctly different trichome densities with an average of 752.5, 145.5, 62.3, and 15.1 trichomes per mm^2, respectively (Colvin 2011) (Fig. 1). Other physical traits including leaf toughness and leaf texture also vary among these species.

The diversity of parasitoids utilizing *A. nerii* on these milkweed species was investigated in a two-year field study. During both years, *Lysiphlebus testaceipes* (Cresson) was the dominant primary parasitoid recovered from mummies collected from all 4 milkweed species (Colvin 2011). *Lysiphlebus testaceipes* is a key parasitoid of *A. nerii* (Stary 1970). This small (3 mm) wasp lays an egg inside the body of a host aphid. Upon hatching, the wasp larva will feed inside the aphid, eventually killing it. A parasitized aphid turns brown

and becomes a mummy (Fig. 2). Once mature, the adult *L. testaceipes* will cut a hole in the aphid mummy and exit the aphid body. Development from egg to adult requires approximately 14 days at room temperature (UC IPM 2011).

Host plant characteristics can influence interactions between insect herbivores and their natural enemies (Price and others 1980). Plant characteristics that presumably evolved to deter herbivory (e.g., leaf texture, trichomes) may instead provide some herbivores protection by interfering with the ability of natural enemies to locate and attack hosts. In a laboratory study,
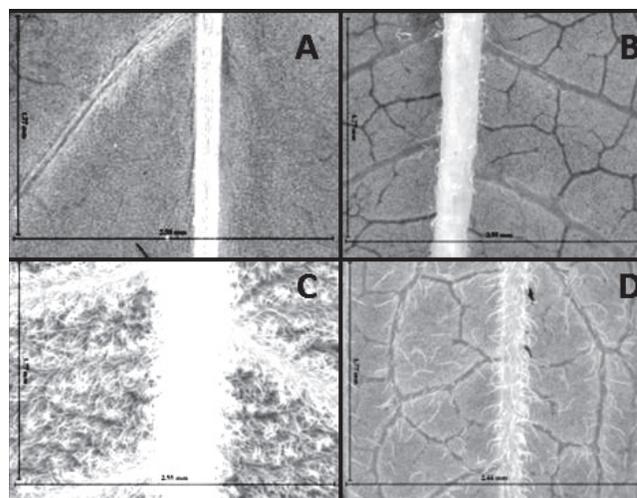
Figure 1. Variation in trichome density among leaves. A. *C. laeve*, B. *A. incarnata*, C. *A. syriaca*, D. *A. tuberosa*.

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the number of *A. nerii* successfully parasitized by *L. testaceipes* differed significantly among host plant species (Colvin 2011). In that study, significantly more aphids were successfully parasitized (became mummies) on *C. laeve* than on *Asclepias* species tested and significantly more mummification occurred in aphids on *A. incarnata* than on *A. syriaca* and *A. tuberosa*. During that study *A. nerii* was observed to respond defensively to parasitoid presence. Previous work by Hartbauer (2010) suggests that non-lethal defensive behaviors may effectively reduce foraging efficiency of natural enemies, including predators and parasitic wasps. We hypothesized that defensive behaviors exhibited by *A. nerii* against the parasitoid *L. testaceipes* would be more frequent on milkweeds with fewer physical defenses (i.e., protective characteristics).

MATERIALS AND METHODS

Milkweed seeds were collected from natural stands of *A. syriaca* L., *A. incarnata* L., and *C. laeve* near Lexington, Kentucky. Seeds of *A. tuberosa* L. were purchased from W. Atlee Burpee & Co (Warminster, PA). After a two-week vernalization period, seeds of each milkweed species were planted three per 500 ml pot in a 1:1 Premier Pro-mix: top soil mixture. Plants were grown in a greenhouse and watered every other day.

Aphid colonies were established by transferring *A. nerii* collected from natural infestations occurring near Lexington, Kentucky, to plants in the greenhouse. Aphids underwent at least four generations before experimentation to ensure that aphids were free of parasitoids.

Several hundred aphid mummies were collected from natural *A. nerii* infestations on stands of *A. incarnata*, *A. tuberosa*, *A. syriaca*, and *C. laeve* at various locations near Lexington, Kentucky. Mummies were transferred, individually, to size 00 gelatin capsules (WebVitamins Inc., Windsor, CT) and stored at 25°C in the laboratory. Mummies were checked twice daily for parasitoid emergence. Only emerging *L. testaceipes* were used; other emerging parasitoid species were discarded. Adult *L. testaceipes* were sexed, randomly paired and mated within 24 hours of emergence. Mated females were held individually in capsules prior to experimentation (up to two hours). Females were randomly assigned to 1 of 4 aphid treatments: *A. nerii* from *A. incarnata, A. syriaca, A. tuberosa,* or *C. laeve*.

An experimental arena was prepared for each parasitoid. A single leaf corresponding to the host plant species for that treatment was collected from one-month-old milkweeds grown in a greenhouse and placed in a Petri dish (9-cm-diam.). Aphids were introduced into the Petri dish and allowed one hour to acclimate and begin feeding prior to parasitoid introduction. In all cases two gravid adults, three fourth or fifth instars, and five second or third instars of *A. nerii* were provided in each dish. A parasitoid was then placed in the center of the arena and monitored continuously for 30 minutes. Defensive behaviors exhibited by individual *A. nerii* including bucking, spinning, and kicking, as well as coordinated defenses involving more than one aphid, were recorded. Behaviors were classified similarly to those described by
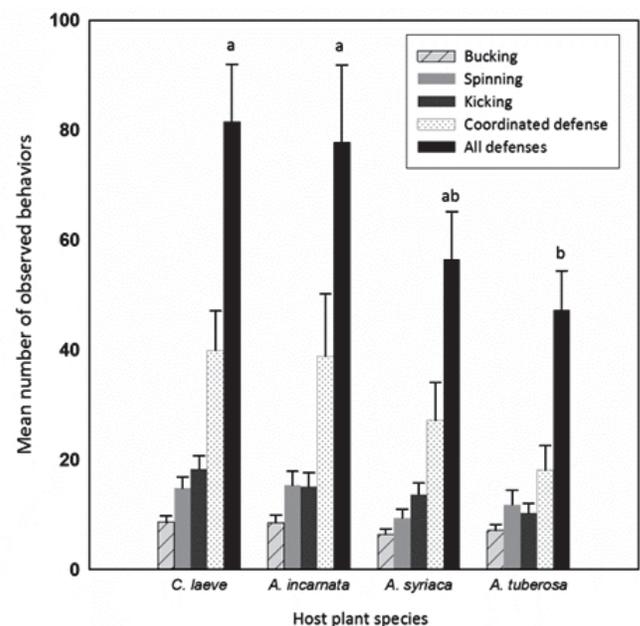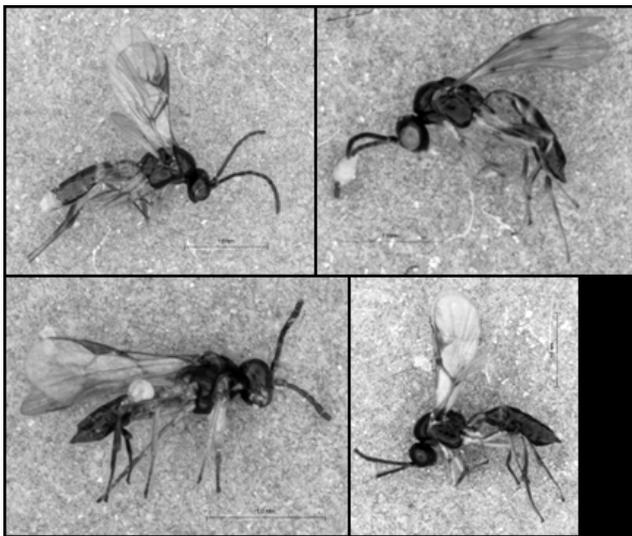

Figure 2. *Aphis nerii*. A. un-parasitized, B parasitized.

Figure 3. Mean ± SE number of defensive behaviors exhibited by 10 *A. nerii* per replicate and directed at a single *L. testaceipes* female over a 30 minute period (n = 20 *L. testaceipes* females [=replicates] per host plant species). Different letters (a, b) indicate significant differences among treatments (LSD, P = 0.05).

Figure 4. *Lysiphlebus testaceipes* females exhibiting hardened aphid cornicular wax. Note shortened antenna in lower two photographs.

Hartbauer (2010). Bucking involved a rapid lifting of the abdomen and was often followed by another defensive behavior. Spinning occurred when the tip of the abdomen moved in a circular direction without contacting the host plant. Kicking involved one or both hind legs and was often preceded by a jerking to one side. Behaviors were considered coordinated when kicking, spinning, or bucking were exhibited by two or more aphids (up to 10) simultaneously. Parasitoids were removed after 30 minutes. Twenty parasitoids were monitored per host plant species (with each parasitoid being presented a new arena with fresh aphids) for a total of 80 parasitoids observed. A one-way ANOVA was used to determine if the number of defensive behaviors exhibited by the aphid differed among host plant species.

RESULTS

When analyzed separately, defensive behaviors of *A. nerii* were not significantly different across host plant species (bucking: $F = 0.8$; $P = 0.49$, spinning: $F = 1.6$, $P = 0.19$, kicking: $F = 2.4$, $P = 0.08$, coordinated defense: $F = 1.7$, $P = 0.17$. In all cases, df = 3,76) (Fig. 3). However, when all defensive behaviors were combined for analysis, a marginally significant difference among aphid host plant species was found ($F = 2.6$, df = 3,76; $P = 0.06$) (Fig. 3). Aphids on *A. incarnata* and *C. laeve* appeared to exhibit more total defensive behaviors than aphids on *A. tuberosa* with *A. syriaca* being intermediate.

DISCUSSION

Parasitoid foraging behavior may be impacted by many factors, including host plant characteristics and aphid defensive behaviors (Mackauer and Volkl

1993). Milkweed species, namely *A. syriaca* and *A. tuberosa,* that have leaves with rough textures and higher trichome densities appeared to interfere with parasitoid navigation. In contrast, *A. incarnata* and *C. laeve* have fewer apparent physical defenses (Colvin 2011). Our data suggest that physical characteristics of milkweed host plants may provide the aphid with variable levels of protection from *L. testaceipes*. Increasing the frequency of defensive behaviors may compensate for the lack of protective characteristics in some host plant species.

Aphids responded to parasitoid presence by bucking, spinning, and kicking on all 4 milkweed species. However, coordinated defenses were observed most often. Hartbauer (2010) demonstrated that coordinated defenses can effectively interrupt oviposition by parasitoid wasps. Aphids occasionally responded to parasitoid presence by excreting cornicle wax. Excreted cornicle wax hardened rapidly, typically within seconds. Wax was observed on various appendages, but parasitoid antennae were most often affected (Fig. 4). When an antenna contacted wax, the parasitoid would withdraw quickly backwards while dragging the antenna. Often, the antenna became glued to leaf tissue and attempts to remove it resulted in the loss of antennal segments. Parasitoids that contacted liquid wax were distracted from foraging for several minutes while attempting to remove the material. In this study, 22.5 percent of *L. testaceipes* were exposed to aphid cornicle wax. Of these parasitoids, 61.3 percent were unable to successfully remove hardened wax material within the 30-minute observation period. Reducing the amount of time that a parasitoid actively forages may also reduce the number of successful ovipositions and thus, aphid fatality.

Trends observed in our study warrant further research investigating the potential influence of the physical characteristics of host plants on interactions between parasitoids and their insect hosts. Studies investigating tri-trophic interactions have largely focused on the chemical characteristics of host plants, particularly the role of secondary plant compounds. Physical host plant characteristics have received less attention. Our results suggest that both chemical and physical plant characteristics may affect the suitability of insect hosts for parasitoids.

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