



Ant-psyllid mutualism affects predation of *Diaphorina citri* by lady beetle larvae and abundance of psyllid natural enemies

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Abstract Ants can be involved in mutualistic relationships with honeydew producing hemipterans in which ants provide protection against their natural enemies, and, in return, hemipterans reward ants with honeydew. Such mutualism may affect population regulation of hemipterans by third trophic level predators. However, current knowledge regarding the effects of this food-for-protection mutualism of ants with Asian citrus psyllid, *Diaphorina citri*, in Florida, USA is limited. Two treatments were established in replicated small plots under field conditions in a citrus (var. Valencia) grove: (1) ants present, and (2) ants absent by exclusion with a tangle-trap sticky barrier deployed on the base of trees. Lower abundance of Coccinellidae, Araneae, and Eulophidae and corresponding higher abundance of *D. citri* were recorded in trees with ants compared to that recorded in trees without ants as measured by direct visual observations and stem tap sampling. In addition, ant-beetle behavioral interactions were directly investigated with

three species of ants [*Solenopsis invicta* Buren, *Dorymyrmex bureni* (Trager), and *Brachymyrmex obscurior* Forei]. Predation of *D. citri* nymphs by ladybeetle larvae was reduced on leaf flushes infested with *S. invicta* as compared with leaves without ants and this species caused significant direct mortality to larval beetles. Our results support the hypothesis that predation of *D. citri* by natural enemies may be reduced in citrus groves colonized by *S. invicta* than in those where ant populations are suppressed. Furthermore, our results indicate that *S. invicta* aggressively protect *D. citri* nymphs on leaves from otherwise effective potential predators, such as *Harmonia axyridis*.

Keywords Asian citrus psyllid · Predation · Biological control · Huanglongbing · Citrus greening

Introduction

Ants are a highly abundant group of arthropods in most ecosystems, and play an important role in ecosystem function and population dynamics of arthropod communities (Calabuig et al. 2014, 2015; Offenberg 2015; Anjos et al. 2021). They are generalist predators and serve as biological control agents against many pests in various ecosystems (Offenberg 2015; Anjos et al. 2021). For example, weaver ant, *Oecophylla* sp. workers were first introduced as biological control agents for citrus pests in Southern China (Huang and Yang 1987; Offenberg

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2015). Although ants are sometimes considered useful predators, they can also interfere with otherwise beneficial organisms such as pollinators, by involvement in mutualistic relationships with hemipterans (Bach 1991; Piñol et al. 2012; Offenberg 2015). This mutualistic relationship between ants and honeydew producing hemipterans has been long recognized, and widely studied (Way 1963; Kaplan and Eubanks 2005; Dao et al. 2014; Zhou et al. 2014). Nearly 45% of all ant genera are involved in relationships with hemipteran species (Oliver et al. 2008). Ants collect large amounts of honeydew from their mutualist partners and, in return, protect these hemipterans from natural enemies (Way 1963; Bach 1991; Vanek and Porter 2010; Tena et al. 2013). The honeydew is rich in carbohydrates, proteins, and amino acids, which serves as a valuable dietary component for many ant species (Way 1963; Wilder et al. 2013; Diehl-Fleig and Diehl 2018).

Ants aggressively guard their source of honeydew against intruders, such as natural enemies of their mutualist partners, and by doing so promote growth of hemipteran populations (Way 1963; Styrsky and Eubanks 2007; Diehl-Fleig and Diehl 2018). Ants can reduce the performance of predators by physically attacking larvae and adults or driving them away from hemipterans, and, as a result, these predators are likely to avoid ant-tended colonies (Oliver et al. 2008; Vanek and Potter 2010; Yoo et al. 2013). In most studies, the presence of ants was associated with increased density of honeydew-producing pests, and decreased abundance of their natural enemies was attributed to interference by ants (Bach 1991; Eubanks et al. 2002; Piñol et al. 2012; Anjos et al. 2021). For instance, densities of green scale, *Coccus viridis* Green (Hemiptera: Coccidae), the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), and the calio scale, *Eulecanium cerasorum* Cockerell (Hemiptera: Coccidae) were higher in the presence of *Pheidole megacephala*, *Solenopsis invicta*, and *Formica subsericea* (Hymenoptera: Formicidae), respectively, than on corresponding plants without ants (Bach 1991; Zhou et al. 2013; Vanek and Potter 2010). These examples illustrate the effects ant communities impart on natural enemy abundance, diversity or community structure in agroecosystems, which can also vary depending on the third trophic level predator(s) and ant species involved (Calabuig et al. 2015; Liu et al. 2020; Sanchez et al. 2020).

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an important pest of citrus given that it transmits a pathogenic bacterium (*Candidatus Liberibacter asiaticus*) causing the citrus disease, huanglongbing (HLB) (Bové 2006; Grafton-Cardwell et al. 2013). Current management of HLB heavily relies on insecticide sprays to suppress vector populations, which also reduces natural enemies (Monzo et al. 2014; Monzó and Stansly 2020). The key natural enemies associated with *D. citri* in Florida, USA include coccinellids, syrphids, chrysopids, spiders, and the parasitoid *Tamarixia radiata* (Waterston) (Michaud 2004; Chong et al. 2010; Monzo et al. 2014). Greater facilitation of biological control in citrus could reduce current heavy reliance on insecticides associated with HLB management (Grafton-Cardwell et al. 2013).

Ants are also highly abundant in citrus and act as generalist predators or engage in mutualistic relationships with hemipterans, such as *D. citri* (Piñol et al. 2012; Tena et al. 2013; Calabuig et al. 2015; Pekas 2015). Various species of ants in the genera *Brachymyrmex*, *Camponotus*, *Crematogaster*, *Dorymyrmex*, *Monomorium*, *Paratrechina*, *Pseudomyrmex*, *Solenopsis* and *Pheidole* are found in Florida, and have been observed collecting honeydew from *D. citri* nymphs (Michaud 2004; Chong et al. 2010; Navarrete et al. 2013). Excluding ants from psyllid habitats can facilitate biological control of *D. citri* by natural enemies (Piñol et al. 2012). For example, parasitism of *D. citri* nymphs by *T. radiata* increases by 50 to 58% when ants are excluded compared to when they are present (Navarrete et al. 2013; Milosavljević et al. 2021). However, less is known about the characteristics of ant-psyllid mutualisms in Florida, particularly in regard to the interaction between ants and lady beetle predators of *D. citri*. Greater understanding of how ant communities affect abundance of *D. citri* natural enemies in Florida may facilitate improved biological control of this important pest in a region where HLB is both endemic and highly prevalent (Coy and Stelinski 2015).

Herein, we investigated the effect of mutualism between ants and *D. citri* on population densities, behavior, and predation efficiency of third trophic level natural enemies. We hypothesized that presence of tending ants interferes with the effect of natural enemies on population suppression of *D. citri*. Our study was focused on *Harmonia axyridis* because

coccinellids are most abundant natural enemies in Florida citrus and this species in particular is dominant (Michaud 2004). Furthermore, we evaluated the behavior of *H. axyridis* in response to three different species of ants [*Solenopsis invicta* Buren, *Dorymyrmex bureni* (Trager), and *Brachymyrmex obscurior* Forei] to elucidate the behavioral mechanisms underlying these predator-ant interactions.

Materials and methods

Study site

This study was conducted in a 15 ha citrus grove at the Citrus Research Education Center (CREC), University of Florida, Lake Alfred, FL, USA (28.09° N, 81.72° W) from 8 March to 22 July 2021. The experimental site consisted of sweet orange trees, *Citrus sinensis* (L.) Osbeck, var. 'Valencia'. Trees were planted on 3 × 6 m (3 m between each individual tree within the row and 6 m between each row) and were 4–5 years old (~1.8 m tall). One of the experimental treatments required exclusion of ants from trees. However, occurrence of weeds around tree trunks in experimental plots could facilitate ant movement into the trees. We therefore mechanically removed all weeds surrounding trees in April prior to initiation of the experiment and herbicides (Monsanto Company, Marysville, OH, USA) were sprayed as spot treatments onto subsequently emerging weeds during the experiment.

Ants exclusion experiment setup

The experiment was established as a randomized complete block design with two treatments replicated four times. Each experimental block consisted of 12 trees growing in two rows. Within each block, trees were randomly assigned to one of two treatments: (1) six trees served as a control and were designated as 'ants-present' and (2) six trees were manipulated to exclude ants and designated as 'ants-absent'. All treatments replicates were separated from one another by at least one row of trees. For the 'ants-absent' treatment, an 8 cm wide band of Tanglefoot (Trècè Adhesive Division, Grand Rapids, MI, USA) sticky barrier (ca. 80 g tree⁻¹) was applied around the base of each tree trunk to prevent ants from moving into canopies.

The Tanglefoot-treated trees were inspected weekly, and the sticky barrier was reapplied if it looked dry or if ants were observed crossing the band. Control trees ('ants-present') were left untreated allowing natural ant movement into canopies. A pre-sampling of ants on randomly chosen trees within each plot confirmed no differences between ant populations on treatment and control trees prior to application of Tanglefoot (data not shown).

Assessment of ant activity and natural enemy abundance

Ant activity was assessed weekly between March and July by counting the number of ants walking on tree trunks and leaves within tree canopies. Six trees per replicate for each treatment were examined for a timed, 2 min observation period by assessing four separate quadrants representing each cardinal direction for 30 s. Observations were made between 08h30 and 12h00. Ants found during observations were collected using a thin paint brush and transferred into vials containing 70% ethanol for further identification.

Similarly, natural enemies in treatment plots were sampled with both visual observations and by collection on sticky traps. Visual sampling for natural enemies was conducted as described above for ants. Furthermore, four 20 × 12.5 cm translucent sticky pane traps (Great Lakes IPM Inc., Vestaburg, MI, USA) were deployed in four randomly selected trees in each treatment block. These were hung on tree branches approximately 1.0 m above ground level and replaced every two weeks from March to July. Sticky cards were returned to the laboratory for enumeration of natural enemies under a microscope.

D. citri adult and nymph densities

Populations of *D. citri* adults in all treatment blocks were monitored weekly using the tap sampling method as described by Qureshi and Stansly (2007). Twelve trees were sampled per replicate plot per week from March to July. For each tap sample, a tree branch was vigorously tapped three times with a PVC pipe directly over a horizontally placed 210 × 297 mm plastic white sheet. All *D. citri* adults found on the sheet following branch agitation were counted and recorded. *D. citri* nymph densities were monitored

in treatment plots by random collection of five leaf flushes (approx. 6–8 cm length) from twelve trees per plot every two weeks. Flush samples from each plot were placed into paper bags and transferred to the laboratory for careful examination using a stereomicroscope.

All statistical analyses were conducted in R version 3.6.3 (R Core Team 2020). Prior to analyses, data were tested for normality using a histogram, qqplot, and Shapiro–Wilk tests and we determined that our data were not distributed normally. A generalized linear mixed model (GLMM) with a Poisson distribution and a log link function was used to compare the mean number of ants and adult *D. citri* between trees treated with the Tanglefoot barrier and untreated trees. The model included ant and *D. citri* adult counts as response variables, ant exclusion treatment and sampling date as fixed effects, and tree sample as a random effect. Post-hoc pairwise comparisons were done using a Tukey procedure with the `glht` function (package `multcomp`) at a significance level of $P < 0.05$. To compare the mean number of ant species, psyllid nymph densities, and natural enemy abundance data monitored visually or with trap catch between treatments, we used generalized linear model (GLM) fitted with a log link function for negative binomial distribution.

Behavioral observations of interactions between beetle larvae and ants

The adult lady beetles, *H. axyridis*, used in this experiment were originally collected from citrus groves in Polk Co., FL, USA in the spring of 2018. They were maintained at $25\text{ }^{\circ}\text{C} \pm 1$, $56\% \pm 5\%$ RH, and a 14:10 L:D photoperiod in insect rearing cages. Beetles were fed pea aphids, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae), reared on fava broad pea plants, *Vicia faba* L. (Vermont Bean Seed Co., Randolph, WI, USA) and supplemental diet (*Ephestia* moth eggs from Beneficial Insectary). Following emergence, beetle larvae were maintained individually in a 37 ml plastic cup (Solo cup company, Highland Park, IL, USA) to prevent cannibalism.

The interactions between ladybeetle larvae and three species of ants (*S. invicta*, *D. burenii* and *B. obscurior*), with known association with *D. citri* nymphs, were quantified by direct observation on Valencia trees. We chose these three species for

observation because they were encountered most often during preliminary scouting. Observations were recorded in the citrus grove described above and made in July between 08h30 to 12h00. Prior to observations, we scouted trees to find newly emerged leaf flush that contained *D. citri* nymphs and were tended by ants from each of the three chosen species above or that contained nymphs without ants. During previous observations, we found that ant-psyllid mutualisms were typically established among *D. citri* nymphs and a single ant species per nymph cluster per leaf flush. Each leaf flush chosen for observations contained roughly 10–40 second to fifth instar nymphs. In cases where ants co-occurred with nymphs, there were typically 2–8 ants in view in or around the leaf flush containing *D. citri* nymphs. After identifying nymphs or nymphs with ants of each species, leaf flush with insects were marked with flagging tape in preparation for subsequent return for observations. All observations were made within 24 h of finding usable leaf flush with naturally occurring insects. Each ant-tended leaf with *D. citri* nymphs contained only one of the three species of ants. A single 3rd or 4th instar *H. axyridis* larva was released per leaf flush using a thin paintbrush and allowed to move freely throughout the leaf flush surface at the onset of each observation interval. These were obtained from the culture described above. Beetle larvae were starved for 24 h prior to release. Interactions between ladybeetle larvae and ants were observed for 10 min intervals and number of nymphs eaten by larvae was recorded within this period. The behaviors/outcomes recorded during beetle-ant encounters with *D. citri* nymphs were: (1) ladybeetle larvae moving away from ants or exiting from tree, (2) larvae feeding on *D. citri* nymphs, and (3) ants attacking and killing beetle larvae. In addition, predation was quantified by recording the number of nymphs consumed per beetle larva within the 10 min observational period. There were 32 replicate observations of ladybeetle larvae for each ant species, and 45 replicates of ladybeetle larvae without ants. If ants had no contact with larvae within 5 min, the observation was ended. Also, if ants abandoned the *D. citri* colony or beetle larvae moved in a direction away from *D. citri* nymphs before coming in contact with ants, observations were halted. There were five instances recorded where observations were interrupted for the above two reasons and these were not included in the analysis.

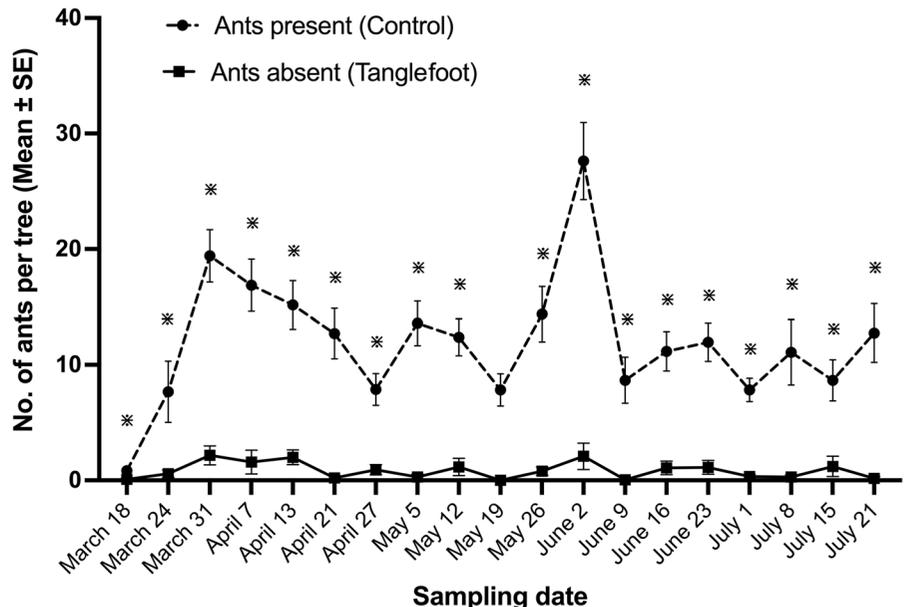
Behavioral interactions between ants and *H. axyridis* larvae were analyzed using χ^2 contingency tables, followed by Bonferroni correction for multiple comparisons in R version 3.6.3 (R Core Team 2020). Differences in predation of *D. citri* nymphs by beetle larvae in response to different ant species were determined using a generalized linear model (GLM) fitted with a log link function for negative binomial distribution, followed by Bonferroni correction for pairwise comparisons at a significance level of $P < 0.05$.

Results

Ant activity

There was a significant effect of ant treatment ($\chi^2=9.64$; $df=1$; $P < 0.001$), sampling date ($\chi^2=158.76$; $df=18$; $P < 0.001$), and their interactions on the mean activity of ants ($\chi^2=86.66$; $df=18$; $P < 0.001$). The mean number of ants occurring on trees treated with the Tanglefoot barrier ('ants-absent') was significantly lower than that on untreated control trees ('ants-present') across all the sampling dates ($P < 0.001$), except on May 19 (Fig. 1).

Fig. 1 Mean number (\pm SE) of ants observed on control (ants present) and Tanglefoot-treated (ants absent) trees. Asterisks indicate significant differences between treatments at $P \leq 0.05$



Ant species composition

There were three species of ants predominantly encountered during observations in our study. There were significantly more *Brachymyrmex obscurior* ($\chi^2=107.14$; $df=1$; $P < 0.001$), *Dorymyrmex bureni* ($\chi^2=565.90$; $df=1$; $P < 0.001$), and *Solenopsis invicta* ($\chi^2=147.43$; $df=1$; $P < 0.001$) on untreated trees than on Tanglefoot-treated trees (Fig. 2). *Brachymyrmex obscurior* was the most abundant species

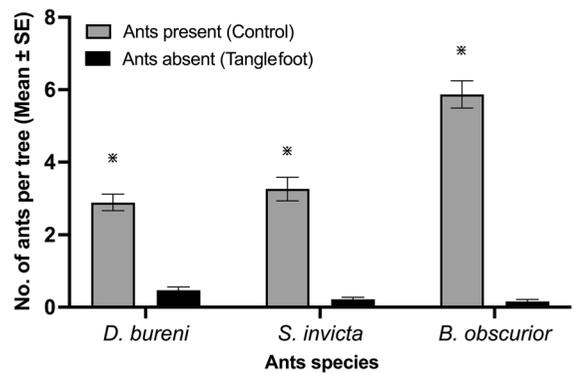


Fig. 2 Mean number (\pm SE) of ants from three different ant species (*Dorymyrmex bureni*, *Solenopsis invicta* and *Brachymyrmex obscurior*) observed on control (ants present) and Tanglefoot-treated (ants absent) trees. Bars with asterisks indicate significant differences between treatments within species at $P \leq 0.05$

with an average of 5.87 ± 0.38 individuals counted per tree per 2 min observation per week. This was followed by *S. invicta* (3.26 ± 0.32 per week) and *D. bureni* (2.89 ± 0.22 per week) in untreated citrus trees (Fig. 2). The Tanglefoot barrier effectively excluded ants from treated trees during the sampling period (Fig. 2).

D. citri adult and nymph densities

There were significant effects of ant treatment, sampling date, and their interaction on the mean number of adult *D. citri* counted by tap sample ($\chi^2=69.17$; $df=18$; $P<0.001$). The number of adult psyllids counted in the Tanglefoot-treated trees was significantly lower than in untreated control trees on 7, 21, 27 April, 12, 19, 26 May, 2, 23 June, 1, 8, and 21 July ($P<0.05$) (Fig. 3). Similarly, the mean (\pm SE) number of *D. citri* nymphs in trees treated with Tanglefoot (54.4 ± 5.2) was significantly lower than in untreated trees (77.6 ± 7.4) that contained ants ($\chi^2=4.92$; $df=1$; $P=0.026$).

Abundance of natural enemies

During visual observations, a wide variety of *D. citri* natural enemies were observed and were categorized into to six taxonomic groups: Coccinellidae, Syrphidae, Chrysopidae, Dolichopodidae, Eulophidae, and Araneae (Fig. 4a). The most frequently

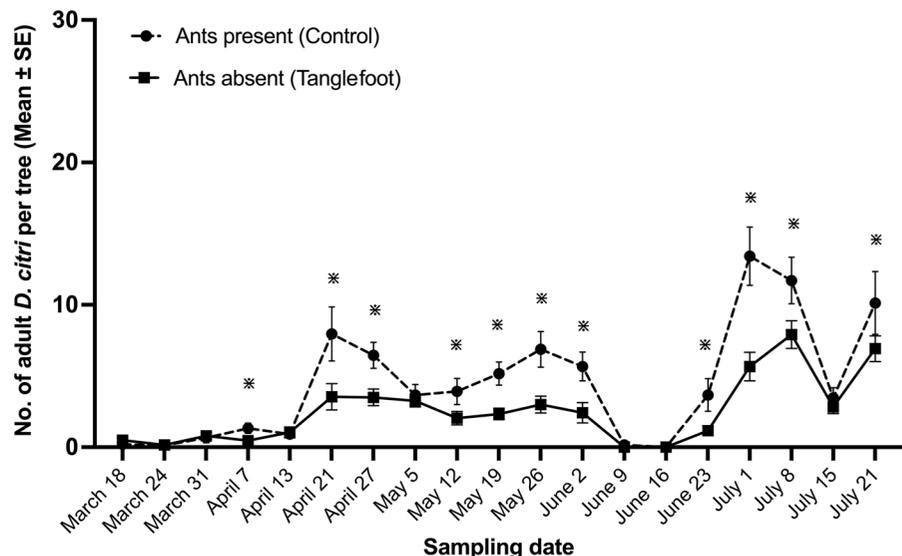
observed arthropods were in the Coccinellidae and Araneae (Fig. 4a). Significantly fewer coccinellids ($\chi^2=13.57$; $df=1$; $P<0.001$), spiders ($\chi^2=7.73$; $df=1$; $P=0.005$), and eulophids ($\chi^2=10.05$; $df=1$; $P=0.001$) were counted in untreated control trees ('ants present') than in those where Tanglefoot was used to exclude ants ('ants absent') (Fig. 4a). However, no significant differences ($P>0.05$) were found between treatments for relative densities of the Syrphidae, Chrysopidae, and Dolichopodidae (Fig. 4a).

Overall, the same taxonomic groups were observed in treatment plots as recorded by captures on sticky traps (Fig. 4b). There were no statistical differences ($P>0.05$) between mean captures of natural enemies on traps in Tanglefoot-treated and untreated trees within each of the taxonomic groups (Fig. 4b).

Behavioral observations of beetle larva-ant interactions

Ant species had a significant effect on the recorded behavioral outcome of *H. axyridis* larvae ($\chi^2=69.15$; $df=4$; $P<0.001$). Behavioral outcomes of ant-beetle interactions are summarized in Table 1. *S. invicta* were observed attacking and killing ladybeetle larvae, whereas the other two species of ants did not kill any beetle larvae. Beetle larvae that encountered *D. bureni* or *B. obscurior* either moved away from these ant species or consumed *D. citri* nymphs in the presence of these ants without interruption.

Fig. 3 Mean number (\pm SE) of *Diaphorina citri* adults counted per tap sample from control (ants present) and Tanglefoot-treated (ants absent) trees. Asterisks indicate significant differences between treatments at $P \leq 0.05$



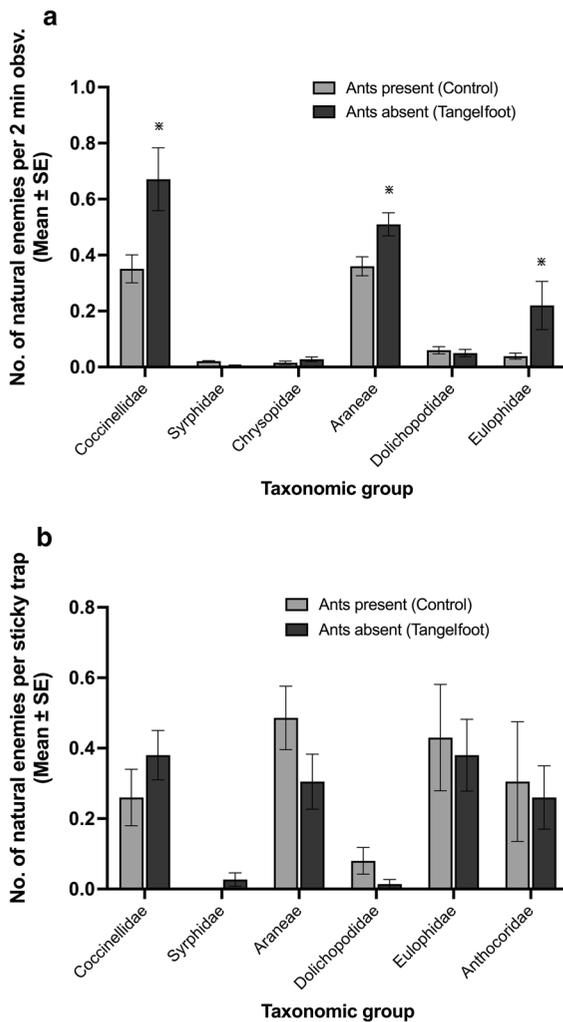


Fig. 4 **a** Mean number (\pm SE) of arthropods per taxonomic group observed visually per 2 min intervals in control (ants present) and Tanglefoot-treated (ants absent) trees. **b** Mean number (\pm SE) of arthropods per taxonomic group trapped on sticky cards in control and Tanglefoot-treated trees. Bars with asterisks indicate significant differences between treatments at $P \leq 0.05$

There was a significant effect of ant species present on leaf flush on the number of *D. citri* nymphs consumed by *H. axyridis* larvae ($\chi^2 = 44.58$; $df = 3$; $P < 0.001$). Significantly fewer *D. citri* nymphs were consumed by *H. axyridis* in the presence of *S. invicta* compared to any of the other treatments. However, there was no difference in psyllid consumption on leaves where *B. obscurior* or *D. bureni* were present versus absent (Table 2).

Discussion

The goal of this investigation was to determine how ants occurring in a citrus grove habitat in central Florida, USA may impact predation of *D. citri* by natural enemies. The primary hypothesis tested was that ants tending *D. citri* for honeydew decrease natural predation of psyllids. Testing this hypothesis in the field required us to manipulate ant abundance to prevent establishment of ant-psyllid mutualisms in certain trees. The Tanglefoot barrier proved to be very effective in preventing ants from entering tree canopies. The activity of ants was, on average, 12 times lower in Tanglefoot-treated than untreated trees throughout the investigation. However, the effectiveness of the Tanglefoot barrier waned over time due to wash off from rain and irrigation, requiring monthly re-application to consistently exclude ants.

There were three predominant ant species consistently observed in association with *D. citri*; *B. obscurior* was the most commonly observed species visiting *D. citri* nymphs in tree canopies followed by *S. invicta* and *D. bureni*. These three ant species were previously reported tending *D. citri* (Michaud 2004; Navarrete et al. 2013). We also observed these species of ants carrying honeydew away from tended *D. citri* during our visual observations. Honeydew acquired from hemipterans is a critical resource for multiple ant species and this carbohydrate rich resource is used as a supplement for colony growth and maintenance (Zhou et al. 2014; Liu et al. 2020). Wilder et al. (2013) found that the number of workers and brood size of *S. invicta* were greater when provided with honeydew and insect prey compared to colonies receiving insect prey only. Similarly, colonies of *D. bureni* with access to honeydew and insect prey had twice as many workers and brood than those with access to insect prey alone (Wilder et al. 2013; Zhou et al. 2017).

Mutualistic food-for-protection relationships between ants and hemipterans can facilitate outbreaks of the herbivore (Way 1963; Pekas 2015). Our investigation indicates that densities of both *D. citri* adults and nymphs were higher in trees where ants naturally occurred than when they were excluded by manipulating the environment. A parsimonious hypothesis explaining the effect of ant exclusion on *D. citri* populations is that predation of psyllids increased in the absence of guarding ants. For example, Calabuig

Table 1 Outcomes of behavioral interactions between various ant species and *Harmonia axyridis* larvae during 10 min intervals of observation on citrus leaf flush in the field

| Ants species | Outcome of interaction between <i>H. axyridis</i> larvae and ants | | | |
|-------------------------------|---|-----------------------------|--|---------------------------------------|
| | No. trials | Larvae ran away/ dropped | Larvae ignored and continue to feed | Larvae were attacked and killed |
| <i>Dorymyrmex bureni</i> | 32 | 19a | 13a | 0b |
| <i>Solenopsis invicta</i> | 32 | 8a | 0b | 24b |
| <i>Brachymyrmex obscurior</i> | 32 | 14a | 18b | 0b |

Numbers within the column followed by different letters are significantly different (Bonferroni correction at significance level of $P < 0.05$)

Table 2 Predation of *Diaphorina citri* nymphs by *Harmonia axyridis* larvae during 10 min observations in the field

| Treatment | Mean number (\pm SE) of <i>D. citri</i> nymphs consumed |
|-------------------------------|---|
| Control (no ants) | 1.51 \pm 0.29a |
| <i>Dorymyrmex bureni</i> | 0.84 \pm 0.23a |
| <i>Brachymyrmex obscurior</i> | 1.03 \pm 0.26ab |
| <i>Solenopsis invicta</i> | 0 \pm 0b |

Means within the column followed by different letters are significantly different (Bonferroni correction at significance level of $P < 0.05$)

et al. (2015) observed higher hemipteran densities in citrus when ants were present, which was explained by reduced abundance of natural enemy species. Similarly, higher densities of a soft scale, *Coccus hesperidum* L. (Hemiptera: Coccidae), and an armored scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) were associated with low densities of beneficial insects on ant-infested citrus trees (James et al. 1999). Congruently, Zhou et al. (2017) recorded greater colony growth rate of mealybugs, *Phenacoccus solenopsis* (Tinsley), in the plots with *S. invicta* compared to plots where fire ants were excluded.

In Florida, there is a great diversity of natural enemies, particularly predators, affecting *D. citri* populations, which can inflict near 100% mortality on *D. citri* nymphs during the citrus growing season (Qureshi and Stansly 2009; Monzo et al. 2014). Using both timed observations and trapping in citrus trees, we frequently collected known natural enemies of *D. citri* belonging to five taxonomic groups of insects as well as spiders. Our results indicate that not all groups of natural enemies were affected to the same degree by occurrence of ant-psyllid mutualisms. The

ant-*D. citri* mutualism had the greatest effect on coccinellids, spiders and eulophids, by decreasing their population size as indirectly measured by sampling. Similarly, James et al. (1999) observed more predatory beetles, lacewings, parasitic wasps, spiders, and predatory mites on ant-free than ant-infested citrus trees grown in Australia. Furthermore, ant foraging reduced occurrence of beneficial arthropods in citrus trees by up to 75%, with the greatest effects observed during spring and summer (James et al. 1999). Likewise, higher densities of ladybeetles, spiders, and big-eyed bugs were recorded in cotton fields where fire ants were removed with insecticide-treated bait compared to untreated controls (Eubanks et al. 2002). Syrphids and chrysopids are particularly important for regulation of *D. citri* populations, accounting for up to 86% of total predation (Kistner et al. 2016). However, members of these two groups were observed infrequently among trees in both treatment groups evaluated here and thus our investigation was unable to demonstrate how ant-*D. citri* mutualism affect their abundance. Furthermore, there was no apparent effect of ant exclusion on abundance of dolichopods or anthocorids.

The parasitic wasp, *T. radiata*, is another important natural enemy of *D. citri* which established in Florida after a classical biological control release program (Qureshi and Stansly 2007). Due to food-for-protection mutualism, we anticipated to observe a decrease in parasitic wasps in the presence of ants compared with plots where ants were excluded. As predicted, we found a significant effect of ant removal on abundance of this species, which is also congruent with previous investigations. For instance, parasitism of the mealybug, *P. solenopsis* (Tinsley), by *Aenasius bambawalei* Hayat

(Hymenoptera: Encyrtidae) was comparatively lower when mealybugs were tended by *Tapinoma melanocephalum* (Fabricius) or *Paratrechina longicornis* (Latreille) (Hymenoptera: Formicidae) than for untended mealybugs (Liu et al. 2020). Also, parasitism of California red scale, *A. aurantia*, decreased in organic lemon with increasing density of Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) (Yoo et al. 2013). Similarly, parasitism of *D. citri* increased by 80% in the absence of Argentine ant in Southern California (Tena et al. 2013).

Ant species may determine the degree of specificity of ant-hemipteran interactions, as well as levels of aggressiveness/tolerance of ants toward intruding species (Pekas 2015; Liu et al. 2020). In our observations of ant response to larval beetles, we found that *B. obsucior* and *D. bureni* were not as aggressive toward *H. axyridis* larvae as compared with the behavior of *S. invicta*. Although some *B. obsucior* and *D. bureni* intentionally drove away ladybeetle larvae after encounter, and occasionally interrupted feeding activities of larvae, this only occurred in 40–60% of observed encounters. Similarly, larval syrphids and the coccinellid, *Adalia bipunctata* (Linnaeus), were in some instances attacked or driven away from aphids by *L. niger* ants (EL-Ziady and Kennedy 1956). Liu et al. (2020) showed that *H. axyridis* adults avoided spaces in foraging arenas that contained *T. melanocephalum* or *P. longicornis*, and also laid fewer eggs in arenas containing either of these ant species. In other cases, we observed individual *B. obsucior* or *D. bureni* climbing onto and over *H. axyridis* larvae without consequence and beetles behaved otherwise normally by continuing to feed on *D. citri* nymphs uninterrupted.

However, the interactions we observed between *H. axyridis* larvae and *S. invicta* were distinctly more antagonistic than with the two other species observed; this fire ant species is known to attack native ant species as well as beneficial insects (Eubanks et al. 2002; Wilder et al. 2013). In our study, *H. axyridis* larvae were unable to escape attacks from *S. invicta*. We considered a beetle larva attacked if ants attempted to sting or bite it. Upon attack from an individual *S. invicta*, ladybeetle larvae released defensive chemicals. However, this defensive secretion did not repel *S. invicta*, which successfully killed 75% of *H. axyridis* larvae among the 32 replicates observed. No

beetle mortality was observed during interactions of *H. axyridis* with the two other ant species, which were also noticeably less aggressive.

In addition to behavioral observations, we also directly quantified predation of *D. citri* nymphs by *H. axyridis* larvae in the presence or absence of ants in the field. Our results indicate that ladybeetle larvae consumed more *D. citri* nymphs from colonies that were not tended by *S. invicta* as compared with those where ants of this species were present. However, ant tending by individuals of the other two species did not affect this predation rate. *S. invicta* is known to aggressively protect the exotic honeydew-producing mealybug, *P. solenopsis*, by deterring their natural enemies (Zhou et al. 2013), with reports of significantly reduced predation rates in the presence of this ant mutualism (Kaplan and Eubanks 2005). In greenhouse experiments, Eubanks et al. (2002) observed that survival of ladybeetles (*Coccinella septempunctata* L., and *Hippodamia convergens* Guérin-Ménéville) (Coleoptera: Coccinellidae), and green lacewing larvae (*Chrysoperla carnea* Stephens) (Neuroptera: Chrysopidae) was 50% and 38% lower, respectively, in the presence of *S. invicta* than in ant-free controls.

Our investigation is congruent with previous reports from other regions indicating that food-for-protection mutualistic relationships between ants and *D. citri* enhanced population growth of this phytopathogen vector, which was likely caused by ant interference with activity of natural enemies. In general, excluding ants promoted establishment of natural enemies, with concomitant reduction in psyllid populations. Interactions between ants and third trophic level predators play an important role in dictating trophic structure (Kaplan and Eubanks 2005). Our direct behavioral observations revealed that ants directly interfered with predators of *D. citri* (sensu functional response) and inflicted significant mortality on predators, which likely accounts for their overall effect on arthropod assemblages in trees. However, the effect of ant-psyllid mutualisms was not consistent among species, with the invasive fire ant, *S. invicta*, displaying the most aggressive behaviors and causing the greatest mortality of predators. Our results indicate that these ant-mediated interactions with biological control agents of *D. citri* may result in consequential economic costs to citrus growers in Florida. The mutualism between ants and honeydew producing hemipterans appears to reduce predation of

D. citri by natural enemies, and thus promotes population growth of this phytopathogen vector. Incorporation of directed ant exclusion into a comprehensive management program for *D. citri* may improve HLB management in citrus, particularly in areas where the fire ant, *S. invicta*, is established.

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Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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