Competition between honeydew producers in an ant–hemipteran interaction may enhance biological control of an invasive pest

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Abstract

Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an invasive citrus pest in southern California, which secretes honeydew and has the potential to spread a lethal bacterial disease, huanglongbing, of citrus. In urban citrus, Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), also an invasive pest, tends honeydew-producing hemipterans. We used field data to determine whether the mutualistic relationship between *L. humile* and six established species of honeydew producers may hinder or favor the establishment of *D. citri* and its biological control with *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) in citrus via competition or mutualism for ants, respectively. In the field, *L. humile* and *D. citri* are engaged in a mutualistic relationship. Ants harvest solid honeydew secreted by psyllid nymphs and tended more than 55% of observed *D. citri* colonies. *Linepithema humile* displayed a preference hierarchy when tending honeydew producers infesting citrus. It responded equally or less intensively to *D. citri* than to other honeydew-producing species. Consequently, the mutualism between *L. humile* and *D. citri* was affected by the presence of other honeydew-producing species, and the percentage of *D. citri* colonies tended by *L. humile*. The number of ants per *D. citri* colony also decreased as the number of other honeydew producers increased. *Diaphorina citri* density was also affected by the presence of other honeydew producers. Both colony size and the number of *D. citri* nymphs counted per tree decreased as the number of other honeydew producers increased. Our results indicate that competition between honeydew producers for the mutualist ant *L. humile* may hinder the establishment of *D. citri* by possibly facilitating increased biological control.

Keywords: *Diaphorina citri*, *Linepithema humile*, *Tamarixia radiata*, ant-attendance, mutualism

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Introduction

The invasibility of a habitat by a phytophagous insect pest is determined, in part, by the presence or absence of competitors, mutualists, and natural enemies. Apart from competition for resources with resident species, new phytophagous insect pests interact with natural enemies that may harm them or with mutualists that assist establishment and spread in new habitats (Liebhold & Tobin, 2008). When invasive and resident phytophagous insects share a species of mutualist they can interact indirectly via this third species. This interaction with the mutualist may result in different outcomes for each phytophagous insect. If an increase in the density of the resident phytophagous species causes a reduction in the intensity of the mutualistic interaction with the invasive species, then the two species compete and the resident species will hinder the establishment of the invader. However, the resulting increase of the resident species might lead to a corresponding increase in the intensity of the mutualistic interaction with the invasive species (van Veen et al., 2006). The result would then be a mutualism mediated by true mutualists that favor the establishment of the invasive insect.

In a well-known mutualistic relationship, some hemipterans produce a carbohydrate-rich excretion called honeydew that is collected by ants. Honeydew producers benefit from ant attendance because predation, disease, and parasitism are reduced, and, in some species, enhanced reproduction occurs (e.g., Banks, 1962; Way, 1963; Addicott, 1979; Völkl, 1992; Flatt & Weisser, 2000). The intensity of the mutualism between honeydew-producing hemipterans and ants depends, in part, on the relative attractiveness of honeydew to ants, which may lead to competition between different species for ants in the field (Addicott, 1978; Bristow, 1984; Cushman & Addicott, 1991; Cushman & Whitham, 1991; Müller & Godfray, 1999; Pekas et al., 2011). Ants therefore may respond more intensively to more profitable resources during honeydew collection activities (Davidson, 1978; Nonacs & Dill, 1991; Bonser et al., 1998; Mailleux et al., 2000). Such differences in the reward for the foraging ant may be either larger volumes of honeydew (Bristow, 1991; Völkl et al., 1999) or higher-quality honeydew that has preferred amino acids or sugars (Cushman, 1991; Cushman & Addicott, 1991; Völkl et al., 1999; Woodring et al., 2004). As consequence of this competition between honeydew producers, the less attractive species usually suffer higher predation and parasitism (Fischer et al., 2001), and ants may use these low-quality honeydew-producing hemipterans as prey (Styrsky & Eubanks, 2007). Therefore, honeydew-producing hemipterans compete for ants and this competition may hinder the establishment of an invasive honeydew producer in a new area.

Alternatively, an invasive honeydew producer may benefit from the presence of other honeydew-producing species because their presence may increase the intensity of the mutualistic interaction between ants with the invader. For example, ants disrupt or kill parasitoids of the armored scale Aonidiella aurantii (Maskell) (Hemiptera: Diaspididade), which does not produce honeydew, as an indirect consequence of ant-attendance of neighboring honeydew producers (Teresa Martinez-Ferrer et al., 2003). Consequently, ant activity because of honeydew producers causes an increase of this armored scale (DeBach et al., 1951; Moreno et al., 1987; James et al., 1997; Pekas et al., 2010).

Psyllids (Hemiptera) are honeydew producers that have high invasion potential due to their small size and high reproductive potential. In this study, we sort to determine whether the mutualistic relationship between the Argentine ant, Linepithema humile (Mayr) (Hymenoptera: Formicidae), and six established species of honeydew producers may hinder or favor the establishment of the invasive Asian citrus psyllid, Diaphorina citri Kuwayama (Hemiptera: Liviidae), in urban citrus growing in southern California via competition or mutualism. Linepithema humile is one of the most important invasive ant species in agricultural, urban and natural environments that are characterized by Mediterranean climates, including southern California (Vega & Rust, 2001). In agricultural and urban areas, L. humile maintains trophobiotic relationships with various honeydew-producing hemipterans such as brown soft scale, Coccus hesperidum L. (Coccidae), citrus mealybug, Planococcus citri (Risso) (Pseudococcidae), wooly white fly, Aleurothrixus floccosus (Maskell) (Aleyrodidae), cottony cushion scale, Icerya purchasi (Maskell) (Monophlebidae) and several species of aphids. Diaphorina citri was first detected in Florida and Texas in 1998 and 2001, respectively (French et al., 2001; Halbert & Munjunath, 2004). In southern California, D. citri was discovered in 2008 and is a significant new threat to California citrus because it spreads a bacterium that causes a lethal and incurable disease of citrus, huanglongbing (HLB) (Bové, 2006; Grafton-Cardwell et al., 2013). Adult psyllids lay eggs on citrus flush growth, and at emergence, nymphs redistribute along growing shoots. Nymphs subsequently settle, form colonies, and feeding may cause distortion and death of young leaves. Diaphorina citri has five instars, and all the nymphal stages secrete solid honeydew, but instars 1–3 produce low amounts. Field studies in Florida indicate that substantial mortality of D. citri nymphs results from generalist predators. In addition, fourth and fifth instars are also attacked by an introduced ectoparasitoid, Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) (Michaud, 2004; Qureshi & Stansly, 2009; Qureshi et al., 2009). As part of a classical biological control program targeting D. citri, more than 70,000 T. radiata sourced from the Pakistani Punjab have been released at >250 sites in southern California during 2011–2013. All of these release sites have been in urban areas where D. citri-infested citrus grow in residential gardens (Hoddle, 2013).

The occurrence of several ant-attended honeydew-producing hemipteran species on the same host plant and the recent arrival of D. citri in urban citrus in southern California provided an excellent opportunity to test whether mutualistic relationships between ants and established species of honeydew producers hindered or favored the establishment of a new invasive citrus pest, D. citri. To investigate this possibility, we first determined whether L. humile tended D. citri colonies. Once we ascertained that L. humile tends D. citri colonies, we measured and compared the intensity of this mutualistic interaction with D. citri to other honeydew producers on the same trees that were also tended by L. humile. We, then, examined whether an increase in the density of other honeydew-producing species affected the intensity of the mutualistic interaction between L. humile and D. citri. Finally, we investigated whether an increase in the density of the other honeydew producers affected the size of D. citri colonies and the total number of D. citri nymphs trees. In addition, we compared parasitism rates by T. radiata in trees with and without ant activity for the same date

Table 1. Sampling dates and sites, number of sampled trees per site and variety, and presence of Argentine ant, *Linepithema humile*, in tree canopies.

Sampling date	Locality	Street	Zip code	Citrus variety	No. of trees sampled	No. of trees with Argentine ant activity
12/10/2012	Chino	3rd St	91710	Navel	1	1
12/10/2012	Chino	4th St	91710	–	1	1
12/10/2012	Fontana	Redwood Ave	92336	Valencia	1	1
12/10/2012	Chino	Verdugo Pl	91710	Grapefruit	1	1
12/10/2012	Chino	Walnut Ave	91710	Navel	1	1
12/10/2012	Fontana	Ivy Ave	92335	Lemon	1	1
12/10/2012	Fontana	Live Oak Ave	92335	–	1	1
12/10/2012	Fontana	Sierra Ave	92337	Lemon	1	1
12/10/2012	Ontario	S Hudson Ave	91761	–	1	1
16/10/2012	Riverside	Lochmoor Dr	92507	Valencia	2	2
19/10/2012	LA	East 22nd St	90011	Lemon	1	1
19/10/2012	LA	Compton Ave	90011	Lime, Navel	2	2
19/10/2012	LA	East 23rd St	90011	Valencia	1	1
19/10/2012	LA	East 23rd St	90011	Lime	1	1
19/10/2012	LA	East 59th Pl	90003	Valencia	1	1
26/10/2012	Fullerton	Buena Vista	92833	Valencia	1	1
26/10/2012	Fullerton	Devonshire Ave	92835	Navel	1	1
26/10/2012	Fullerton	Rose Dr	92833	Navel	1	1
26/10/2012	Pico Rivera	Amistad	90660	–	1	1
26/10/2012	Pico Rivera	Beverly Rd	90660	–	1	1
26/10/2012	Pico Rivera	Melita St	90660	Navel	1	1
26/10/2012	Pico Rivera	Olympic Blvd	90660	Lemon	1	1
26/10/2012	Pico Rivera	Walnut Ave	90660	Navel	1	1
02/11/2012	Calimesa	East Ave L	92320	Lime	2	2
02/11/2012	Calimesa	East Ave L	92320	Navel	1	1
02/11/2012	Riverside	Jurupa Rd	92509	Navel, Valencia	3	3
09/11/2012	Pico Rivera	Orange St	90660	Lime	1	1
09/11/2012	Pico Rivera	Pine St	90660	Navel, Valencia	2	2
09/11/2012	Pico Rivera	Pine St	90660	Valencia	1	1
09/11/2012	Pomona	E Monterrey Ave	91767	Lemon,Valencia	2	2
09/11/2012	Pomona	Roswell Ave	91766	Navel	1	0
09/11/2012	Pomona	Roswell Ave	91766	–	1	0
16/11/2012	Azusa	S Azusa Ave	91702	Lemon	1	1
16/11/2012	Azusa	N Calvados Ave	91702	Lemon	1	1
16/11/2012	Covina	E. Gallarno Dr	91722	Lemon	1	0
16/11/2012	Covina	E. Laxford Rd	91722	Orange	1	0
16/11/2012	Azusa	N Azusa Ave	91702	Lemon	2	2
16/11/2012	Azusa	N Dalton Ave	91702	Mandarin	1	1
Total					**46**	**42**

and sampling locality. The results of these studies are presented here.

Methods and materials

A total of 36 urban gardens with *D. citri* infested citrus were sampled in southern California from 10 October to 16 November 2012 (Table 1). Between one and three citrus trees were sampled per yard and sampling was carried out between 10.00 and 16.00 h.

To determine if *L. humile* was actively foraging in study trees, we quantified the number of ants moving past a landmark on the tree trunk during a 1-min period. We defined ant activity as a binomial (absence, presence) factor for three reasons: (i) the number of ants ascending and descending the trunk was generally very high and hard to count precisely; (ii) the trunk may not have been the only access point into trees; and (iii) citrus trees varied in size. We considered that a tree was occupied by *L. humile* when we encountered at least one ant on the trunk during our observation.

To determine the association between *L. humile* and honeydew producers, each canopy was examined for 10 min. During this time, we identified all honeydew-producer colonies at a height between 0.5 and 2 m and counted the number of attending ants per colony. Then, we recorded the species of each colony and counted the number of honeydew producers (for *D. citri* we only counted those individuals that secrete high amounts of honeydew, the 4th and 5th instars) per colony for each species. We use three ratios to analyze the ant–hemipteran association: (i) ant-attendance, (ii) absolute ant-attendance, and (iii) relative ant-attendance. We considered that a colony was attended by *L. humile* when at least one ant was tending a colony. 'Absolute ant-attendance' was calculated as the number of ants per honeydew-producer colony. Absolute ant-attendance was also used as a measure of potential honeydew preference because ants respond more intensively to a more profitable resource (Mailleux *et al.*, 2003). 'Relative ant-attendance' was calculated as the number of attending ants divided by the number of honeydew producers, and was used to measure the effectiveness of ant

protection (Itioka & Inoue, 1996; Harmon & Andow, 2007). The two latter variables were measured only for ant tended colonies.

Simultaneously, we examined 4th and 5th instar *D. citri* nymphs for signs of parasitism by *T. radiata*. Parasitized nymphs can be easily recognized by being dark brown with a flat body and beige filament-like secretions radiate from the periphery of the mummy (Chien *et al.*, 1989). Parasitism was calculated as the number of parasitized nymphs divided by the total number (alive and parasitized) of nymphs in each *D. citri* colony.

Data analysis

A Generalized Linear Mixed Model (GLMM) (Breslow & Clayton, 1993) was used to analyze the effect of hemipteran colony size and the total number of other hemipterans on ant-attendance (binomial error assumed), absolute (Poisson error) and relative ant-attendance (normal error) by *L. humile* on *D. citri* colonies. General Linear Mixed Model was also used to analyze the effect of the number of other honeydew producers (log transformed) on the size of *D. citri* colonies (normal error). In previous analyses, 'tree' was considered a random effect in all the GLMMs. A lineal model was used to analyze the effect of the total number of other honeydew producers per tree (log transformed) on the total number of *D. citri* nymphs per tree (log transformed).

Differences in ant-attendance and relative and absolute ant-attendance among honeydew-producing species were first measured by plotting all data together. GLMM was also used to test whether there were differences among honeydew-producing species in ant-attendance (binomial error) and absolute ant-attendance (Poisson error). In previous analyses, 'tree' was considered a random effect in all the GLMMs. A Kruskall–Wallis test was used to analyze differences in relative ant-attendance. All tests were run at 0.05 levels of significance. Next, we compared ant attendance (the three ratios) of *D. citri* with the rest of the honeydew producers sequentially in trees where they co-existed. Owing to the low numbers of aphids (*Aphis gossypii* and *A. spiraecola*) the data for both aphid species were combined. Kruskall–Wallis tests were used to analyze differences in absolute and relative ant-attendance and GLMs based on binomial distribution (McCullagh & Nelder, 1989). Further, we re-evaluated the significance of the explanatory variables using an F test after re-scaling the statistical model by a Pearson's χ^2 divided by the residual degrees of freedom because overdispersion was detected in GLMs.

A GLM was used to analyze the effect of ant presence on parasitism rates (binomial error assumed) of *D. citri* by *T. radiata*. The statistical software package 'R' (http://www.R-project.org) and its package lme4 were used in our analyses.

Results

Ant activity

Linepithema humile was present in 91.3% (42 out of 46) of sampled trees (Table 1).

Relationship between L. humile and D. citri

A total of 189 *D. citri* colonies were observed in trees with ant activity. Of these, 107 (56.61%) were tended by *L. humile*.
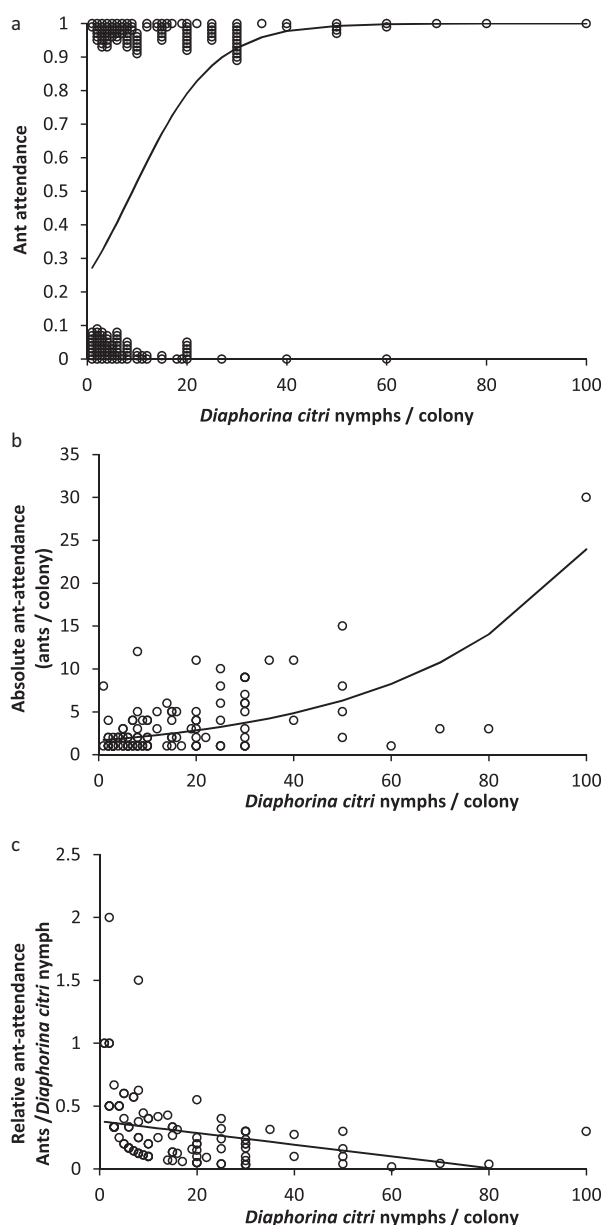

Fig. 1. Effect of colony size of the mutualism between the Argentine ant *Linepithema humile* and *Diaphorina citri* nymphs. (a) Ant-attendance [Ant attendance = $1/(1 + (1/(exp(0.12 * D. citri$ nymphs) – 1.11)))] (data are binary as colonies either were or were not ant tended, but are shown slightly vertically displaced to indicate sample sizes), (b) absolute ant-attendance [number of ants per colony = $exp(0.027 * D. citri$ nymphs +0.51)], and (c) relative ant-attendance [Number of ants per *D. citri* nymphs = 0.38 – 0.0047 * *D. citri* nymphs)].

The mean number of *D. citri* nymphs per colony was 17.58 ± 1.7 (SEM) when they were tended by ants and 7.74 ± 1 when they were not tended. The probability that *L. humile* tended *D. citri* colonies increased significantly as the number of *D. citri* nymphs per colony increased ($\chi^2_1 = 28.61$; $P < 0.0001$) (fig. 1a). When *D. citri* colonies were tended by *L. humile*, the number of attending ants per colony (absolute ant-attendance)

also increased significantly as the number of *D. citri* nymphs per colony increased ($\chi^2_1 = 88.89$; $P < 0.0001$), but the number of attending ants per nymph (relative ant-attendance) decreased ($F_{1, 72} = 8.81$; $P = 0.0041$) (fig. 1b, c).

Relationship between L. humile *and other honeydew producers*

Linepithema humile attended other honeydew-producing species within the same trees. Pooling data from all sampled trees, ants attended $86.5 \pm 2.7\%$ ($n = 156$) of all observed honeydew-producer colonies (fig. 2a). Ant-attendance varied significantly among honeydew-producing species ($\chi^2_6 = 90.84$; $P < 0.0001$). The number of ants per colony (absolute ant-attendance) and per honeydew producer (relative ant-attendance) also varied among honeydew-producing species (Number of ants per colony: $\chi^2_6 = 553.8$; $P < 0.0001$; number of ants per honeydew producer: $K = 99.49$; $P < 0.0001$) (fig. 2b, c).

When we sequentially compared ant attendance of *D. citri* nymphs with the other honeydew producers in trees where they coexisted, *D. citri* colonies were tended at significantly lower levels than the white fly *A. flocossus,* brown soft scale *C. hesperidum*, and the mealybug *P. citri* (Table 2). The number of ants per colony (absolute ant-attendance) was significantly higher in *C. hesperidum* and *P. citri* colonies than in *D. citri* colonies (Table 2). Finally, the number of ants per honeydew producer (relative ant-attendance) was significantly higher in *P. citri* colonies than in *D. citri* colonies (Table 2).

Effect of other honeydew producers on D. citri *attendance by* L. humile

The probability that *D. citri* colonies were tended by *L. humile* decreased as the number of other honeydew producers per tree increased ($\chi^2_1 = 13.5$; $P = 0.0002$) (fig. 3a). When *D. citri* colonies were attended by *L. humile*, the number of ants per colony (absolute ant-attendance) decreased as the number of other honeydew-producing hemipterans per tree increased ($\chi^2_1 = 6.64$; $P = 0.01$) (fig. 3b). However, the number of ants per *D. citri* nymph (relative ant-attendance) was independent of the number of other hemipterans per tree ($F_{1, 31} = 0.04$; $P = 0.84$).

Effect of other honeydew producers on D. citri *colonies*

Both the number of *D. citri* nymphs per colony ($F_{1, 40} = 9.97$; $P = 0.003$) and the total number of *D. citri* nymphs per tree ($F_{1, 40} = 10.53$; $P = 0.0024$) decreased as the number of other honeydew producers per tree increased (fig. 4).

Effect of L. humile *on* D. citri *parasitism by* T. radiata

Parasitism of *D. citri* nymphs by *T. radiata* was significantly higher ($F_{1, 9} = 9$; $P = 0.015$) in trees without *L. humile* activity ($91.25 \pm 5.9\%$ [$n = 4$ trees]) than in those with ant activity ($11.57 \pm 8.5\%$ [$n = 7$]) when we compared trees sampled in localities where we encountered at least one tree without ant activity (Pomona on 9 November 2012 and Azusa-Covina on 16 November 2012. The trees sampled in Azusa and Covina were separated by less than 2.5 km).
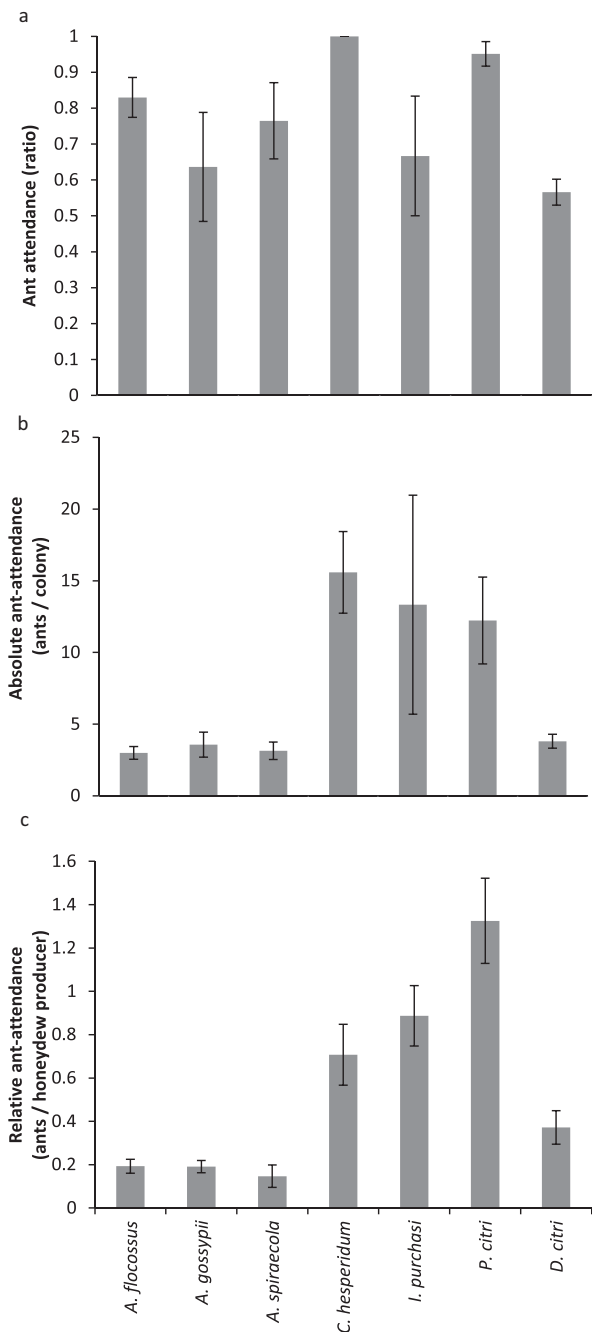

Fig. 2. Relation between *Linepithema humile* and different honeydew-producing species (wooly white fly, *Aleurothrixus floccosus*; citrus aphids: *Aphis gossypii* and *A. spiraecola*; brown soft scale, *Coccus hesperidum*; cottony cushion scale, *Icerya purchasi*; citrus mealybug, *Planococcus citri*; citrus psyllid, *Diaphorina citri*) in citrus yard trees from southern California. (a) Ant-attendance. (b) Absolute ant-attendance. (c) Relative ant-attendance.

Discussion

The invasive Argentine ant, *L. humile*, was the only ant species observed in our study and it was present in 90% of the citrus trees sampled in ten urban localities in southern

Table 2. Ant (*Linepithema humile*) attendance on *Diaphorina citri* versus other honeydew-producing species in citrus yard trees from Southern California were they coexisted. Ant-attendance (%), absolute ant-attendance, and relative ant-attendance have been compared only in those trees were hemipteran species co-existed.

D. citri versus Hemipteran	Ant-attendance (%)			Absolute ant-attendance			Relative ant-attendance		
	D. citri	Hemipteran	GLM semi-binomial	D. citri	Hemipteran	Kruskal–Wallis	D. citri	Hemipteran	Kruskal–Wallis
A. flocossus	35.8±8.3 (18)	87.2±7.6 (18)	$F_{1, 34}=10.1; P=0.003$	1.40±0.21 (11)	3.18±0.88 (16)	$K=3.69; P=0.055$	0.43±0.18 (11)	0.22±0.06 (16)	$K=1.07; P=0.3$
A. gossypii and A. spiraecola	58.2±12.2 (10)	82.1±10.1 (10)	$F_{1, 17}=0.71; P=0.41$	2.16±0.52 (8)	3±0.51 (9)	$K=1.03; P=0.31$	0.26±0.07 (8)	0.51±0.22 (9)	$K=0.04; P=0.85$
C. hesperidum	48.6±9.6 (12)	100 (12)	$F_{1, 22}=13.1; P=0.002$	1.18±0.28 (10)	12.8±3 (12)	$K=15.7; P<0.001$	0.4±0.07 (10)	0.78±0.2 (12)	$K=2.3; P=0.13$
P. citri	45.7±7.4 (18)	94.4±0.06 (18)	$F_{1, 34}=12.2; P=0.001$	2.03±0.61 (15)	10.3±2.9 (17)	$K=10.9; P=0.001$	0.42±0.14 (15)	1.43±0.25 (17)	$K=14.72; P=0.001$

California. As previously documented, *L. humile* has excluded native ants in these residential areas and monopolized sugar sources in the form of hemipteran-produced honeydew (Vega & Rust, 2001). This aggressive and territorial ant provides excellent protection to honeydew producers infesting citrus (Moreno *et al.*, 1987; Klotz *et al.*, 2004; Shik & Silverman, 2013) and the recently introduced *D. citri* has not been an exception to the tending behaviors of this ant. We consider ant tending of *D. citri* an example of an invasional meltdown, where the invasive ant *L. humile*, has facilitated, in part, the invasion of *D. citri* by protecting it from natural enemies through the development of a mutualistic relationship.

During our field observations, *L. humile* harvested solid honeydew secreted by psyllid nymphs and was observed returning it to nests using its mandibles. In return, *L. humile* guarded and tended more than 55% of the *D. citri* colonies. The intensity of this mutualism depended on psyllid density. Specifically, the probability that *L. humile* tended a *D. citri* colony and the intensity of the mutualism depended on the size of the colony. This relationship is probably due to the larger amount of honeydew secreted by larger colonies. Thus, the probability that a colony was tended by ants was almost 100% when colonies had more than 20 nymphs, whereas less than 50% of *D. citri* colonies with ten nymphs or fewer were tended. The intensity of this relation may be higher during later hours since ants increase their activity in citrus canopies at the end of the day to collect honeydew (Pekas *et al.*, 2011), here we conducted observations from 10:00 to 16:00. The pattern of density dependence described herein as well as its subsequent effect in hemipteran survival has been observed in other hemipteran–ant interactions (Addicott, 1978; Cushman & Whitham, 1989, 1991; Breton & Addicott, 1992; Itioka & Inoue, 1996; Morales 2000; Billick & Tonkel, 2003). However, the size of the colony was not the only factor that affected the mutualism between *L. humile* and *D. citri*.

Diaphorina citri competes for *L. humile* with other honeydew-producing species that feed on citrus. In this competition, *L. humile* responded equally or less intensively to *D. citri* than to other honeydew-producing species. Generally, ants respond more intensively to honeydew resources more predictable in procurement (Noe & Hammerstein, 1994; Bonabeau *et al.*, 1997) and value (Davidson, 1978; Nonacs & Dill, 1991; Bonser *et al.*, 1998; Mailleaux *et al.*, 2000). Such differences in value to foraging ants may be due to either larger volumes of honeydew (Bristow, 1991; Völkl *et al.*, 1999), or the presence of preferred amino acids or sugars in the honeydew (Völkl *et al.*, 1999; Woodring *et al.*, 2004). The sugar composition and concentration of the honeydew secreted by the main citrus honeydew-producing species are known (Tena *et al.*, 2013). However, similar studies do not appear to have been completed for *D. citri* honeydew. When we compared the sugar composition of honeydew obtained by Tena *et al.* (2013) and ant preference herein, we could not find any relationship. Considering the strong correlation between amino acid and sugar concentrations (Woodring *et al.*, 2004), it does not seem that these components played a crucial role in the preference of *L. humile*. On the other hand, *L. humile* tended more intensively colonies that reliably secreted honeydew. Colonies of *P. citri* and *C. hesperidum* last for several months and even years on citrus, whereas *D. citri*, aphids and *A. flocossus* colonies may last less than 1 month making the latter a relatively unreliable honeydew source. This distinct hierarchy shown by *L. humile* is similar to that of the dominant and native ants *Lasius grandis* (Formicinae) and *Pheidole pallidula* (Nylander) in
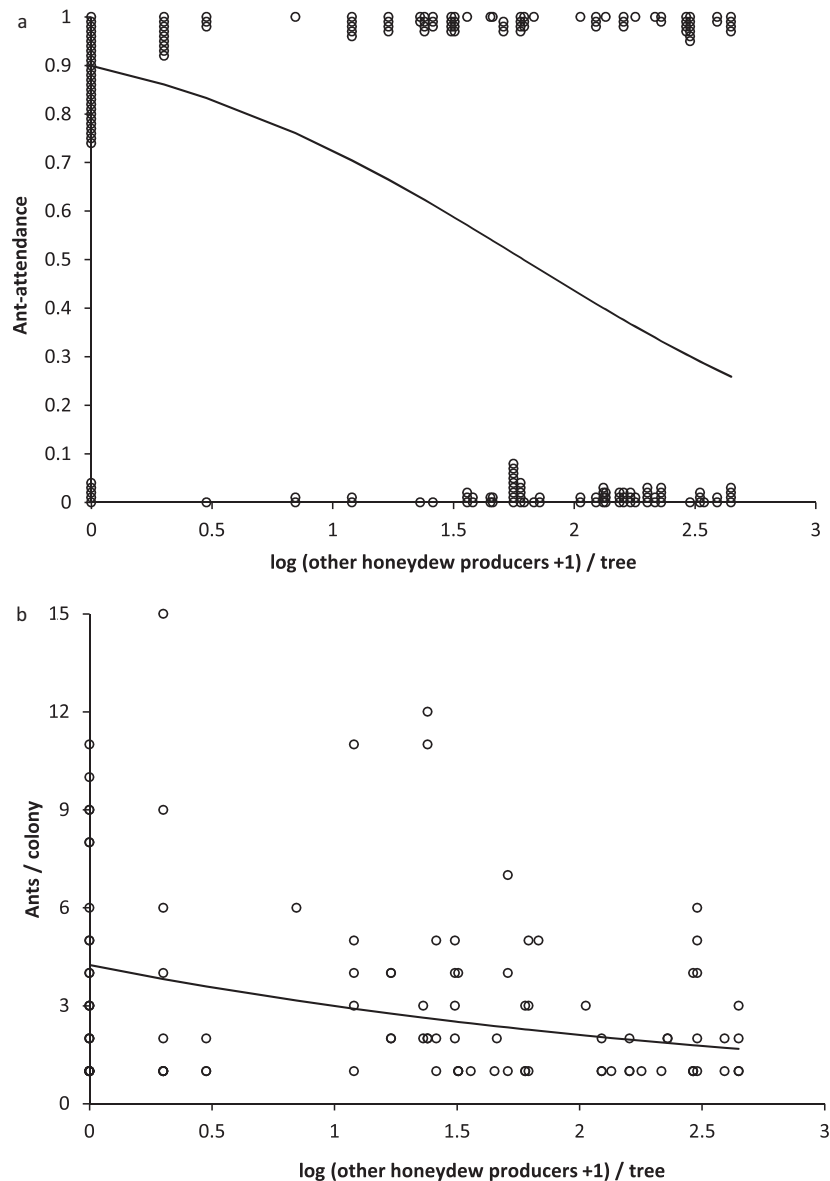
Fig. 3. Effect of other honeydew producers on: (a) ant-attendance [Ant attendance = $1/(1+ (1 /(\exp (-1.22 * (\log$ (Other honeydew producers +1) +2.19)))] (data are binary as colonies either were or were not ant tended, but are shown slightly vertically displaced to indicate sample sizes) and (b) absolute ant-attendance of *Diaphornia citri* colonies by *Linepithema humile* [Ants per *D. citri* colony = $\exp ((-0.35 * \log$ (Other honeydew producers +1) +1.44].

Mediterranean citrus (Pekas *et al.*, 2011). Considering these results and the vegetative parts where these hemipterans form colonies, one might expect that the relatively short lived *D. citri* colonies could establish mutualisms with aphids. This is because these hemipterans form colonies in the same vegetative organs (tender new flush) and might benefit by mixing their colonies and increasing the amount of honeydew secreted together to attract ants (van Veen *et al.*, 2006). However, our field observations in urban citrus in southern California suggest that these herbivores avoid each other and they do not benefit from common ant attendance. We only encountered two colonies of *D. citri* (out of the 189) and aphids within the same flush. This lack of mutualism might be also

explained by the fact that competition for tender new flush (limited resource) between aphids and psyllids is stronger than the potential benefits of a mutualism relationship.

Interestingly, our results demonstrated that the presence of other honeydew-producing species distracted *L. humile* from *D. citri* colonies. The intensity of the mutualistic interaction of *L. humile* and *D. citri* measured as ant-attendance and absolute ant-attendance decreased as the density of other honeydew producers increased. However, relative ant-attendance was independent of the density of other honeydew-producing species. Several studies on ants from the genera *Lasius* and *Formica* have successfully used the relative ant-attendance ratio to measure the effectiveness of ant protection (Banks,
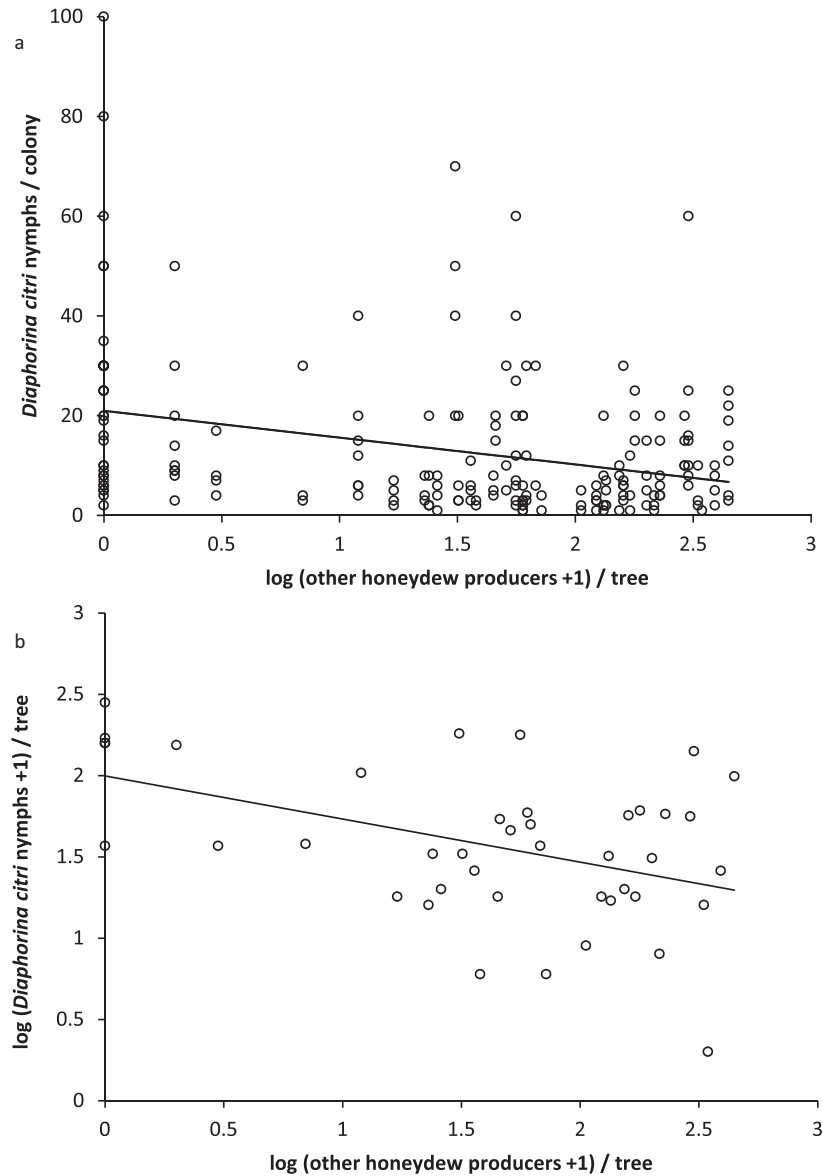
Fig. 4. Effect of other honeydew producers on *Diaphorina citri* density: (a) *D. citri*-colony size [*D. citri* nymphs per colony = 20.97 − (5.38 * (log (Other honeydew producers +1))]; (b) Total *D. citri* nymphs per tree [log (*D. citri* nymphs per tree +1) = 1.99 − (0.27 * log (Other honeydew producers +1))].

1962; Breton & Addicott 1992; Itioka & Inoue, 1996; Morales, 2000; Harmon & Andow, 2007). For example, in the mutualism aphids-*Lasius neograndis* (Way), low relative ant-attendance allowed predators to approach aphid colonies from the perimeter and feed without detection because ants were concentrated in the midst of the aphid colonies, whereas they move around the perimeter and detected predators when the number of ants per hemipteran is high (Harmon & Andow, 2007). Although we recognize that a higher relative ant-attendance ratio will facilitate the protection of *D. citri*, we also consider that this ratio might not measure ant protection of *D. citri* by *L. humile* as accurately as it did in previously tested mutualisms for two reasons. First, *L. humile* is more aggressive than ants in the genera *Lasius* and *Formica* and the degree

of protection provided by tending ants depends also on their aggressiveness (Bristow, 1984; Buckley & Gullan, 1991; Kaneko, 2003; Styrsky & Eubanks, 2007). Second, the structure of *D. citri* colonies affords easy protection by ants. Most of *D. citri* nymphs settle along tender green twigs which can allow just one *L. humile* to effectively patrol an entire colony within 2–10 s (Tena pers. obs.). Thus, high densities of other honeydew producers in citrus trees might negatively affect the protection of *D. citri* colonies even though the density of other honeydew producers did not affect the relative ant-attendance of *D. citri* colonies by *L. humile*.

In fact, the presence of high densities of other honeydew-producing species in citrus trees reduced not only the intensity of the mutualistic interaction of *L. humile* with *D. citri*, but also

the density of *D. citri*. Consequently, indirect competition between honeydew producers for *L. humile* may hinder the establishment of *D. citri* and facilitate its biological control at least during October–November in southern California when flush densities and, consequently, *D. citri* densities are relatively low. Although we have demonstrated the effect of this competition on *D. citri*, we have not analyzed the mechanisms that have generated the decrease of *D. citri* densities. The most obvious hypothesis is that natural enemies attacked nymphs more successfully when *D. citri* colonies are not tended by ants. Our results indicated that parasitism by *T. radiata* is ~80% greater when *L. humile* is absent. Although our conclusions about natural enemy effectiveness are based on small samples, this hypothesis is supported by previous studies, which have elegantly demonstrated that, as consequence of competition for mutualistic ants, the less preferred honeydew-producing species are not tended effectively by ants and their populations subsequently decrease because of increased attacks by natural enemies (Cushman & Whitham, 1991; Fisher *et al.*, 2001). In the case of *D. citri*, it is well known that generalist predators (e.g., coccinellids) are the main natural enemies of *D. citri* (Michaud, 2004; Qureshi & Stansly, 2009) and it has been recently demonstrated that predation rates decrease in the presence of ants (Peña *et al.*, in prep). In our study, we did not measure predation, but our results showed that *D. citri* colonies suffered higher attacks from *T. radiata* when *L. humile* was absent. Controlling ants may be important when establishing *T. radiata* in southern California as part of an emerging classical biological control program targeting *D. citri*.

Acknowledgements

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