



Impact of *Linepithema humile* and *Tapinoma sessile* (Hymenoptera: Formicidae) on three natural enemies of *Aphis gossypii* (Hemiptera: Aphididae)

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ABSTRACT

Ants frequently protect honeydew-excreting hemipterans from their natural enemies in exchange for food, and these interactions can have a negative impact on biological control. Invasive ant species, such as the Argentine ant, *Linepithema humile* (Mayr), enter into mutualisms with some hemipteran species and are generally regarded as more aggressive than native ants. Consequently, they may be more effective at protecting honeydew-producing hemipterans from natural enemy attack. We compared changes in populations of *Aphis gossypii* (Glover) tended by equal numbers of invasive *L. humile*, and the North America native *Tapinoma sessile* (Say), when exposed to the natural enemies *Chrysoperla carnea* (Stephens), *Hippodamia convergens* (Guer.) and *Aphidius colemani* (Viereck). While more *L. humile* than *T. sessile* workers foraged on plants with *A. gossypii*, both ants were equally effective at protecting *A. gossypii* from their natural enemies when aphid populations were relatively low. Using larger starting populations, *A. gossypii* were less negatively impacted by *C. carnea* or *H. convergens* when tended by *L. humile* than *T. sessile*. In a field experiment, the number of *Aphis gossypii* on plants with *L. humile* or *T. sessile* were greater than on plants without ants. We conclude that due to its higher foraging tempo *L. humile* may more effectively patrol plant surfaces supporting honeydew-producing hemipterans than *T. sessile*, which may, in part, underlie the success of this invasive ant.

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1. Introduction

Ants can foster honeydew-excreting hemipteran populations by interfering with the natural enemies of these hemipterans (Beattie, 1985; Buckley, 1987; Ness and Bronstein, 2004) and thus, biological control of pest hemipterans may be compromised as a consequence of natural enemy harassment and direct mortality from ants (James et al., 1999; Mgocheki and Addison, 2009). However, ant tending does not necessarily affect all natural enemy species (Bartlett, 1961; Barzman and Daane, 2001; Völkl and Kroupa, 1997). Among native ants, it is generally the highly aggressive and territorial species that provide honeydew-producing hemipterans the greatest protection (Buckley and Gullan, 1991; Kaneko, 2003).

Exotic, invasive ant species also often engage in facultative mutualisms with pest Hemiptera. Some species of invasive ants are particularly aggressive, behaviorally dominant and abundant, and thus may have a greater disruptive effect on biological control of these hemipterans than native ants (Holway et al., 2002; Lach, 2005; Ness and Bronstein, 2004; Styrsky and Eubanks, 2007). Large outbreaks of sometimes seemingly inconspicuous hemipterans, are

often correlated with the presence of invasive ant species such as *Anoplolepis gracilipes* (=longipes) (Smith) (Abbott and Green, 2007; O'Dowd et al., 2003), *Wasmannia auropunctata* (Roger) (de Souza et al., 1998), *Solenopsis invicta* (Kaplan and Eubanks, 2002) and *Pheidole megacephala* (Fabricius) (González-Hernández et al., 1999; Reimer et al., 1993).

The Argentine ant, *Linepithema humile* (Mayr) (Formicidae: Dolicherinae), is among the most destructive and well-studied invasive ant species (Holway et al., 2002; Silverman and Brightwell, 2008). Native to portions of South America, it has spread throughout the world to regions with Mediterranean, subtropical and mild-temperate climates (Wetterer et al., 2009). Once established, *L. humile* readily displaces most native ant species and other arthropods, with community-level consequences (Holway, 1998; Holway et al., 2002; Ward, 1987). Through suppression of hemipteran natural enemies, Argentine ants are responsible for large increases of honeydew-excreting plant pests (Bartlett, 1961; Barzman and Daane, 2001; Daane et al., 2007). Though the effects of interactions between honeydew-excreting hemipterans and invasive ants, such as *L. humile*, are apparent Grover et al. (2008) suggested that comparative studies using native ants would be particularly valuable.

Many of the native ants displaced by *L. humile* also consume hemipteran honeydew (Holway, 1998; Human and Gordon,

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1996; Ward and Harris, 2005). One of these ants, *Tapinoma sessile* (Say) (Formicidae: Dolichoderinae), is native to North America and shares many traits with *L. humile* (e.g. polydomy, polygyny, permanent nests and omnivory, including hemipteran tending) (Barbani, 2003; Buczkowski and Bennett, 2006; Passera, 1994; Smith, 1928), *T. sessile* is often dominated during encounters with many ants (Fellers, 1987) including *L. humile* (Buczkowski and Bennett, 2008; Human and Gordon, 1996, 1999). Furthermore, Addicott (1979) reported that *T. sessile* had no apparent positive effects on field populations of *Aphis varians* (Patch), and thus it may have a minor impact on the natural enemies of this aphid. *Myrmica lobicornis fracticornis* Emery and *M. Americana* Weber, were more aggressive than *T. sessile* and more effective at driving predators away from hemipteran aggregations on New York ironweed, *Vernonia noveboracensis* (L.), (Bristow, 1984). We sought to determine whether the more aggressive *L. humile* could have a greater positive effect on facultative mutualist hemipterans than *T. sessile* through the ants' impact on hemipteran natural enemies.

Of the numerous honeydew-producing hemipterans available to both *L. humile* and *T. sessile* we chose to study interactions of these ants with *Aphis gossypii* (Glover) and some of its natural enemies. *Aphis gossypii* is highly polyphagous, having been found on over 90 plant families in 40 orders, including important agricultural products such as cotton, avocado, cucumber, papaya, eggplant (Ebert and Cartwright, 1997). Furthermore, *A. gossypii* is easily reared in the laboratory (Vinson and Scarborough, 1989) and engages in facultative mutualisms with ants (Diaz et al., 2004; Kaplan and Eubanks, 2002; Vinson and Scarborough, 1989), including *L. humile* (Mondor et al., 2008; Powell and Silverman, in press) and *T. sessile* (Powell and Silverman, in press).

Aphis gossypii is attacked by numerous predators and parasitoids (Ebert and Cartwright, 1997; Zamani et al., 2007; Zarpas et al., 2007). Kaplan and Eubanks (2002) demonstrated that *S. invicta* suppressed both *Chrysoperla carnea* (Stephens) and *Hippodamia convergens* (Guer.) larval predation of *A. gossypii* in greenhouse experiments. We selected natural enemies of *A. gossypii* exhibiting different behaviors and/or modes of dispersal for our experiments, in which to identify possible ant-specific responses. The natural enemies were: a non-flying predator, larval *C. carnea*; a flying predator, adult *H. convergens* and a parasitoid, *Aphidius colemani* (Viereck). All three natural enemies are widespread in North America. *Hippodamia convergens* is native to North America (Hagen, 1962), while *C. carnea* is distributed widely across North America and Eurasia, but not South America (Henry et al., 2002). The origin of *A. colemani* was probably north India/Pakistan, but it now is a cosmopolitan species (Messing and Rabasse, 1995). Therefore, *H. convergens* and *C. carnea*, but not *A. colemani*, may have had longer to co-evolve with *T. sessile* and thus may have developed mechanisms to overcome detection and attack by *T. sessile*, such as chemical mimicry (Liepert and Dettner, 1993; Völkl and Mackauer, 1993). We tested whether these natural enemies, particularly *H. convergens* and *C. carnea* will cause less *A. gossypii* mortality when *L. humile* is present because *L. humile*, rather than *T. sessile*, may more aggressively defend *A. gossypii*. Also, *L. humile* may be more effective in protecting *A. gossypii* against natural enemies that are outside of this ant's native range.

2. Material and methods

2.1. Organisms

Source populations for *T. sessile* and *L. humile* were taken from several collections of multiple, discrete geographic, urban locations. *Linepithema humile*, *T. sessile* and *A. gossypii* were all maintained in culture in the laboratory at 27 °C and 70% RH. *Tapinoma*

sessile and *L. humile* were confined in fluron-coated trays (200 × 250 × 75 mm) and provided petri dish nests (150 mm diameter) filled with moistened grooved plaster. Ants were fed 25% sucrose, artificial diet (Bhatkar and Whitcomb, 1970) and freshly killed German cockroaches, *Blattella germanica* (Blattaria: Blattellidae). *Aphis gossypii* were obtained from BASF Co. (Research Triangle Park, NC) and reared on approximately 50 cotton (*Gossypium herusitum*) cotyledons (ca. 2 weeks old) in plastic containers (150 mm diameter) w/moisture-control potting soil. Aphids were transferred every 10 days to fresh plants. *Chrysoperla carnea* and *H. convergens* were obtained from Biconet (Brentwood, TN) and *A. colemani* was obtained from Arbico Organics (Tucson, AZ). *Chrysoperla carnea* were received as eggs and were subsequently raised on *A. gossypii* until the beginning of the third stadium (length ~4 mm). Early stadium lacewing larvae are not effective predators of aphids when ants were present (Grover et al. 2008). *Hippodamia convergens* were received as adults and females were immediately used in our experiments. *Aphidius colemani* arrived as mummies (parasitoid pupae encased in dead aphids), which were separated into gel capsules (#2, ~10 mm in length) until adult emergence. After emergence the wasps were sexed and randomly paired. Copulation was observed in all pairings and female wasps were used in experiments 24 h after the initial pairing.

2.2. Laboratory assessment of aphid protection by ants

Ants (100 workers and 1 queen) were placed in Fluon[®]-coated plastic containers (200 × 250 × 75 mm) with Petri dish nests (75 mm) filled with moist grooved plaster covered with a piece of linoleum. Ants received water (25 ml), artificial diet (~100 µl) and two freshly killed adult female *B. germanica*, weekly. We transferred a mixture of 100 nymphal and adult *A. gossypii* to individual cotton cotyledons (~75 mm ht.) held in 473 ml plastic cups. Ten replicates were performed per ant species per treatment. Each natural enemy species assay was performed consecutively. Treatments were as follows: (1) aphids plus ants plus natural enemies, (2) ants plus aphids without natural enemies, (3) aphids plus natural enemies without ants, and (4) aphids without ants or natural enemies. The number of ants and aphids on the plants was recorded once daily. After the initial aphid transfer, a single predator or parasitoid was released onto the plant. Treatments receiving *H. convergens* or *A. colemani* were enclosed in a wooden framed cage (300 × 300 × 600 mm) surrounded by 150µm² viral mesh. We recorded aphid numbers and ant foraging activity until *C. carnea* pupation, adult *A. colemani* progeny emergence or if aphid numbers reached zero in control treatments with no ants, but predators present. We also recorded ant worker survival at the end of the experiment.

Aphid population size can influence an ant colony's ability to protect its mutualist partner against natural enemy attack (Breton and Addicott, 1992). Therefore, we conducted the above experiments again, however, allowing for aphid population growth prior to natural enemy release. Once the aphid population growth rate started to slow and approach zero (~10–20 days, depending on the trial) we released natural enemies, except for *A. colemani*, given the limited number of aphids a single wasp can parasitize (Zamani et al., 2007).

2.3. Field assessment of aphid protection by ants

We examined the ability of *T. sessile* and *L. humile* to protect *A. gossypii* from a suite of naturally-occurring natural enemies in a field planted with cotton. We conducted our trial within a 10m² plot in Clayton, NC (NCSU research station) using plants (ca. 30 cm ht) in five rows spaced 60–70 cm apart. Each plant (15 replicates) received 100 workers and one queen of either ant species housed in a 50 ml screw-capped plastic centrifuge tube one-half

filled with water and plugged with cotton. The lid was fitted with plastic tubing (200 mm × 2 mm id × 4 mm od). The containers were submerged at a 45° angle ca. 200 mm within the soil adjacent to each plant. Tubing from the containers was connected to the main stem of the cotton plant ~75 mm above the soil surface. We also affixed a 1.5 ml microcentrifuge tube filled with 100 µl of artificial diet to the same junction point to provide additional nutrition for the ants. A semi-permeable shade cloth was placed on the ground within the plot, which allowed water from rainfall to penetrate, yet prevented soil from splashing onto the plants. A ~50 mm band of Tanglefoot® (Tanglefoot Co., Grand Rapids, Michigan) below the end of the affixed tubing prevented ant escape. We placed 1000 aphids onto the newest plant growth. *Aphis gossypii* survival without ants, predators or parasitoids was determined on plants covered with 150 µm² viral mesh. Aphids were counted daily for eight days, at which time aphids were no longer detected on any plants. We counted the number of the ants and recorded any potential aphid predators present on plants. Data were recorded the same time each day (800–1000 h, high midday-afternoon temperatures retarded all ant/predator activity). Treatments in this experiment were (1) *L. humile* or *T. sessile* and natural enemy access to *A. gossypii*; (2) natural enemy access to *A. gossypii* w/supplemental diet and Tanglefoot, to ascertain any effect the supplemental diet had in attracting natural enemies and to see if the Tanglefoot kept predators away; (3) natural enemy access to *A. gossypii*; (4) *A. gossypii* only (natural enemy or ant access excluded by viral mesh).

2.4. Statistical analysis

All data sets with the number of ants, field predators and proportional change in aphids through time were analyzed in SAS 9.1.3 using a PROC MIXED repeated measures model with individual 1-df treatment contrasts (SAS Institute, 2002). Fixed effects in the fitted model included treatment effect and day (time) effects and their interaction day × treatment effect. Random effects in the model included an autoregressive error structure associated to the set of repeated measures within each plant, with correlation among two observations within the same plant defined as a function of the distance in time (days difference) between the pair of observations. Residual covariance structure was modeled separately for each treatment when necessary to attain homogeneity of variances. Denominator degrees of freedom for F-tests of fixed effects and calculation of standard error for the least squares mean was calculated using the Satterwaite correction available in PROC MIXED. Ant numbers were square-root transformed and proportional change in aphid number was log transformed for normality. The ratio of parasitized *A. gossypii* exposed to *A. colemani* was arcsine square-root transformed and *A. gossypii* populations exposed to *C. carnea* larvae for two hours were analyzed in SAS 9.1.3 using PROC MIXED with treatments as fixed effects, and residual variances estimated separately for each treatment, with a Satterwaite correction for calculation of denominator degrees of freedom on F-test of fixed effects and standard error of least squares means. One-degree of freedom contrasts were used for treatment least squares mean comparisons, at a 0.05 alpha level (SAS Institute, 2002). Ant worker mortality was analyzed using an ANOVA model under PROC GLM with 1-df treatment contrasts (SAS Institute, 2002).

3. Results

When pooled across all laboratory experiments, worker mortality of *L. humile* (18.9 ± 1.06% SE) and *T. sessile* (19.4 ± 1.23% SE) was not different ($F_{1,198} = 0.09$, $p = 0.76$). Also, 98% of *L. humile* and 97% of *T. sessile* queens were recovered in all laboratory replicates. In

the field experiment, *L. humile* worker mortality (72.7 ± 7.51% SE) did not differ from *T. sessile* worker mortality (65.4 ± 8.53% SE, $F_{1,28} = 0.42$, $p = 0.52$) and 93% of *L. humile* and 87% of *T. sessile* queens were recovered at the end of the experiment. Though not counted, brood of both ant species was present at the completion of all experiments.

3.1. Aphid predation by *Chrysoperla carnea*

Qualitative observations of interactions between *C. carnea* and either ant species revealed that upon contact *C. carnea* larvae walked quickly away from a foraging ant. The ant, alerted to the predator's presence, would pursue, but did not seem able to follow the exact trajectory of the predator's escape. *Chrysoperla carnea* remained stationary until probed again by the ant, and after a few minutes would either successfully evade the ant or be driven from the plant temporarily. No physical conflict was seen between both ant species and *C. carnea*.

3.1.1. Plants with 100 *A. gossypii*

Over twice as many *L. humile* than *T. sessile* were counted on the plants irrespective of whether *C. carnea* were present ($F_{1,26.2} = 93.72$, $p < 0.0001$, Fig 1A), and protection by *L. humile* was different than *T. sessile* ($F_{1,18} = 9.30$, $p = 0.0069$, Fig 1A). There was no change in recruitment in response to *C. carnea* by *T. sessile* ($F_{1,17.8} = 0.90$, $p = 0.36$, Fig 1A), however *L. humile* recruitment increased ($F_{1,42.7} = 11.50$, $p = 0.0015$, Fig 1A). When *C. carnea* were added to plants without ants nearly all aphids were consumed within 2 h (Fig 1A). *Aphis gossypii* survival was higher when *L. humile* ($F_{1,10} = 39.48$, $p < 0.0001$) or *T. sessile* ($F_{1,9.93} = 103.08$, $p < 0.0001$) foraged on plants with *C. carnea* than when ants were absent (Fig 1). Ant protection, however was not absolute, as *A. gossypii* numbers declined when both ants and *C. carnea* were present (*L. humile*: $F_{1,18} = 76.18$, $p < 0.0001$, *T. sessile*: $F_{1,18} = 25.02$, $p < 0.0001$).

3.1.2. Plants with higher initial numbers of *A. gossypii*

Again, more *L. humile* than *T. sessile* workers were on the plants prior to *C. carnea* introduction ($F_{1,29.5} = 76.23$, $p < 0.0001$, Fig 1B); however ant numbers for either species did not change after *C. carnea* was introduced (*L. humile*: $F_{1,37.7} = 0.08$, $p = 0.77$, *T. sessile*: $F_{1,54.7} = 0.02$, $p = 0.89$, Fig 1B). Compared with treatments without *C. carnea* (Fig 1B), *C. carnea* reduced *A. gossypii* numbers without ants ($F_{1,14.4} = 111.75$, $p < 0.0001$) with *L. humile* ($F_{1,20.2} = 67.22$, $p < 0.0001$) or with *T. sessile* ($F_{1,16.1} = 102.48$, $p < 0.0001$, Fig 1B). Despite the drop in aphid number, *A. gossypii* declines were less when *L. humile* were present ($F_{1,15.8} = 38.88$, $p < 0.0001$), but not when *T. sessile* were present ($F_{1,18.1} = 0.26$, $p = 0.61$, Fig 1B). A direct comparison of ant-tended *A. gossypii* reveals a difference between the two ants ($F_{1,14.3} = 31.89$, $p < 0.0001$, Fig 1B). *C. carnea* larvae remained in the treatments until pupation, approximately five days after initial release.

3.2. Aphid predation by *Hippodamia convergens*

We observed adult *H. convergens* dropping or flying from the plant in response to contact with ant workers.

3.2.1. Plants with 100 *A. gossypii*

More *L. humile* than *T. sessile* were counted on the plants irrespective of whether *H. convergens* were present ($F_{1,22.7} = 36.85$, $p < 0.0001$, Fig 2A), yet both ants affected *H. convergens* predation equally ($F_{1,14} = 0.04$, $p = 0.85$). Again, there was no change in recruitment in response to *H. convergens* by either ant species (*L. humile*: $F_{1,20.8} = 0.80$, $p = 0.38$, *T. sessile*: $F_{1,23.1} = 0.38$, $p = 0.55$). After *H. convergens* were placed on plants without ants nearly all aphids were consumed within 3 days (Fig 2A). *A. gossypii* survival was

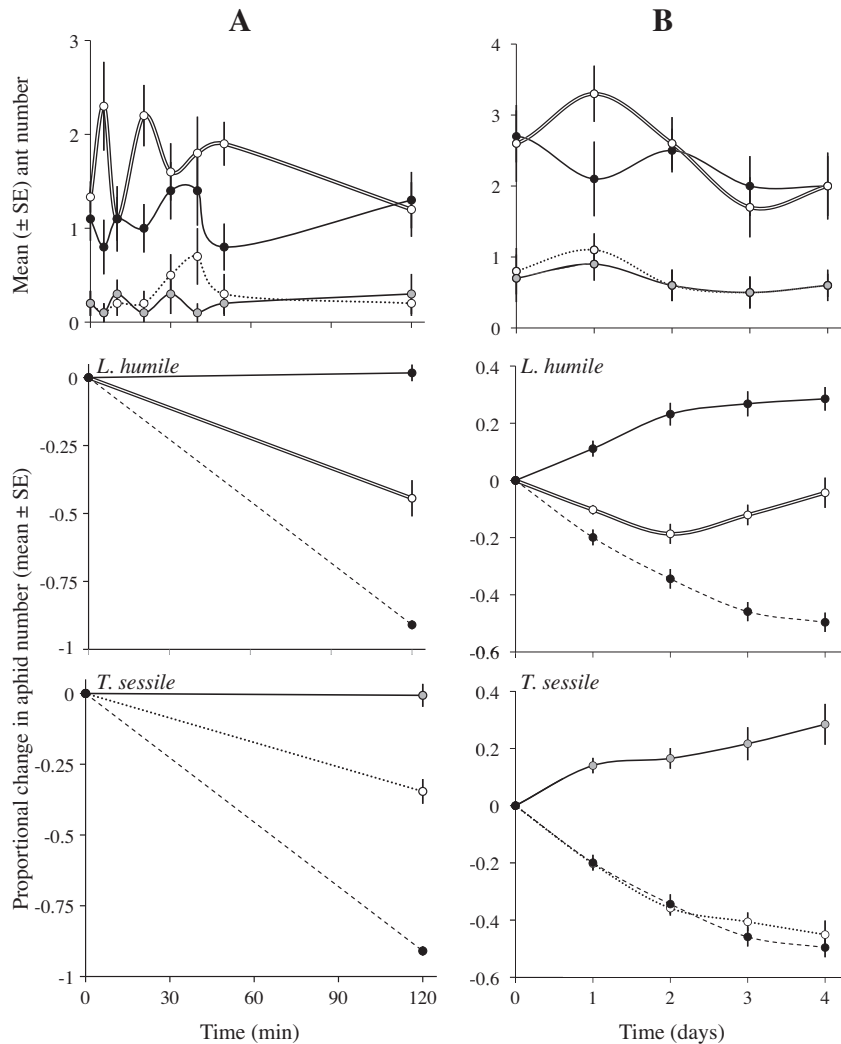


Fig. 1. Mean (\pm SE) proportional change in aphid number and ant number during exposure to *C. carnea* larvae. Column "A" represents ants and ca. 100 *A. gossypii* exposed to *C. carnea* larvae. Column "B" represents ants and high population levels of *A. gossypii* exposed to *C. carnea*. \circ — \circ —*T. sessile* and *C. carnea* present, \square — \square —*T. sessile* present, \bullet — \bullet —*L. humile* present.

higher when *L. humile* ($F_{1,23.3} = 229.73$, $p < 0.0001$) or *T. sessile* ($F_{1,18.1} = 271.51$, $p < 0.0001$) foraged on plants with *H. convergens* (Fig 2A). There was no effect of *H. convergens* on tended *A. gossypii* populations of either ant (*L. humile*: $F_{1,11.1} = 2.84$, $p = 0.12$, *T. sessile*: $F_{1,14.2} = 1.22$, $p = 0.29$, Fig 2A).

3.2.2. Plants with higher initial numbers of *A. gossypii*

Again, more *L. humile* than *T. sessile* were counted on the plants regardless of whether *H. convergens* were present ($F_{1,23.3} = 66.63$, $p < 0.0001$, Fig 2B). Compared to treatments without *H. convergens*, *H. convergens* reduced *A. gossypii* numbers without ants present ($F_{1,16.8} = 89.58$, $p < 0.0001$, Fig 2B) or with *T. sessile* ($F_{1,17.7} = 6.06$, $p = 0.024$, Fig 2B) but not with *L. humile* ($F_{1,20.5} = 1.35$, $p = 0.26$, Fig 2B). The impact of *L. humile* on *H. convergens* predation was marginally greater than *T. sessile* ($F_{1,16.1} = 4.27$, $p = 0.055$, Fig 2B), but both had a positive impact when compared to control *A. gossypii* populations with *H. convergens* present (*L. humile*: $F_{1,16.7} = 90.11$, $p < 0.0001$, *T. sessile*: $F_{1,17.6} = 78.90$, $p < 0.0001$, Fig 2B).

3.3. Parasitism by *Aphidius colemani*

We observed *A. colemani* remaining apart from ants until the ants were absent from a patch of *A. gossypii* then fly to the aphids

and oviposit. Oviposition was completed within ~ 2 s. There were almost always ants present nearby, which interfered with many *A. colemani* oviposition attempts.

Over 50% of *A. gossypii* were parasitized by *A. colemani* when ants were absent (Fig 3). Parasitism was reduced similarly by both ant species (*L. humile*: $F_{1,11.1} = 16.03$, $p = 0.0020$, *T. sessile*: $F_{1,12.1} = 11.57$, $p = 0.0052$, Fig 3), but was not different between the two ant species ($F_{1,17.3} = 0.99$, $p = 0.33$, Fig 3).

3.4. Field assessment of aphid protection by ants

There were marginally more *L. humile* than *T. sessile* on plants during the field trial ($F_{1,74.5} = 3.43$, $p = 0.068$, Fig 4A). Mesh-covered plants had higher aphid numbers than all exposed plants ($F_{1,89} = 559.38$, $p < 0.0001$), suggesting that *A. gossypii* were affected by natural enemies in our field plot (Fig 4B). The supplemental diet with Tanglefoot[®] barrier did not affect predator abundance and *A. gossypii* populations were similar to those in exposed treatments with aphids and no ants ($F_{1,49.7} = 0.34$, $p = 0.56$, Fig 4B). While there were ~ 5 –10% more *A. gossypii* numbers on plants tended by *L. humile* this increase was not different from *A. gossypii* tended by *T. sessile* ($F_{1,34} = 0.77$, $p = 0.39$, Fig 4B) with both ants providing protection to *A. gossypii* compared with the non-ant treatment (*L. humile*: $F_{1,47.2} = 27.26$, $p < 0.0001$, *T. sessile*:

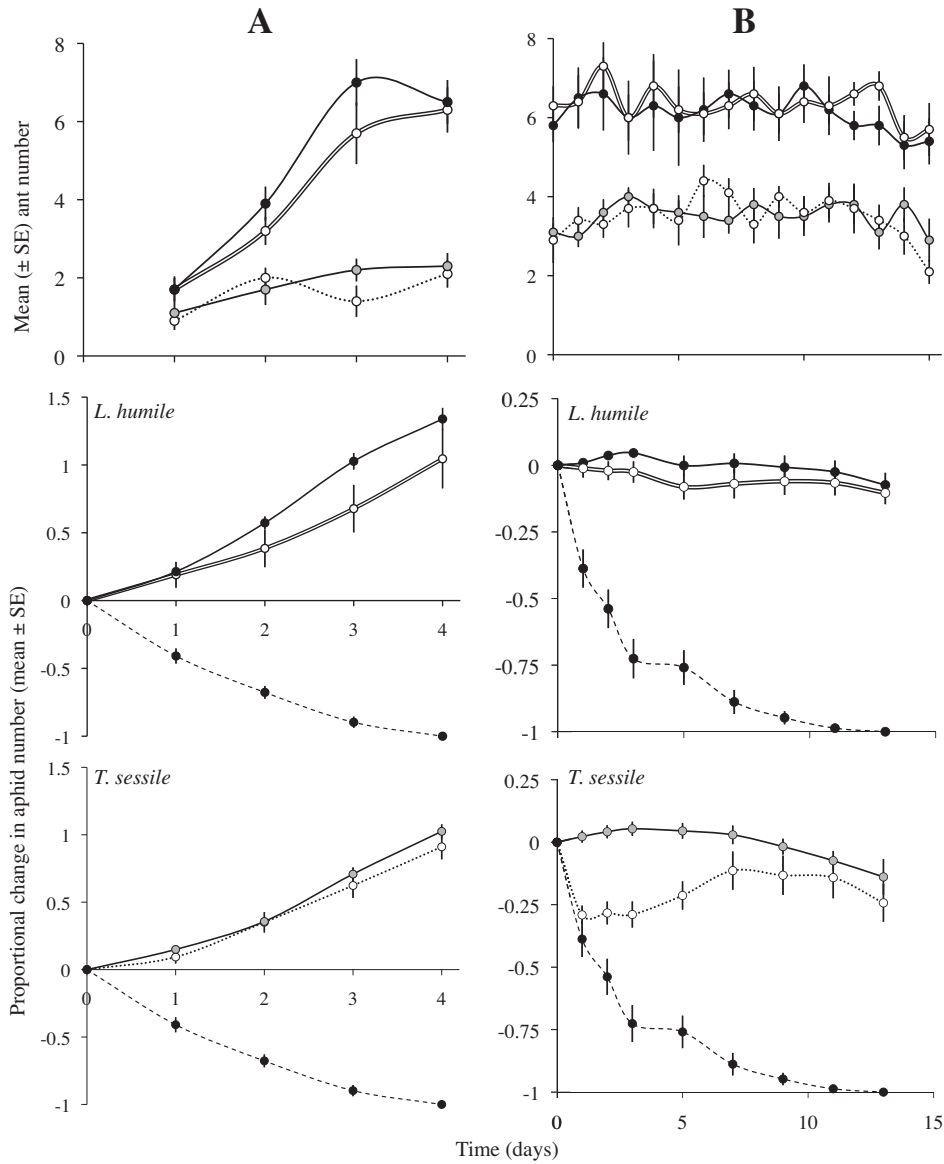


Fig. 2. Mean (\pm SE) proportional change in aphid number and ant number during exposure to *H. convergens*. Column “A” represents ants and ca. 100 *A. gossypii* exposed to *H. convergens* adults. Column “B” represents ants and high population levels of *A. gossypii* exposed to *H. convergens* adults. \circ — \circ —*T. sessile* and *H. convergens* present, \bullet — \bullet —*T. sessile* present, \square — \square —*H. convergens* present, \triangle — \triangle —*L. humile* and *H. convergens* present, \blacktriangle — \blacktriangle —*L. humile* present.

$F_{1,39.2} = 12.07$, $p = 0.0013$, Fig 4B). Predators observed most frequently on the plants were adult Coccinellidae, (including *H. convergens*, *Harmonia axyridis* (Pallas), and *Coccinella sanguinea* var. *polita*. (L.)). The same number of these beetles was recorded across all treatments ($F_{1,56} = 0.63$, $p = 0.60$) except those plants covered with mesh. Other predators observed included *C. carnea*, but there were too few and not enough of any other natural enemy group or guild observed to analyze the impact of ants on their abundance.

4. Discussion

Both ant species provided protection to *A. gossypii* against aphid natural enemies employing different methods of prey/host detection, attack and escape from ants. Yet, though smaller *A. gossypii* aggregations tended by either *L. humile* or *T. sessile* were similarly impacted by predation by *H. convergens*, smaller groups of aphids exposed to *C. carnea* and all larger aphid aggregations tended by *L. humile* were less affected by the predators in our laboratory

experiments. More *L. humile* than *T. sessile* were on aphid-infested plants at any one time and consequently *L. humile* workers may have encountered and disturbed these predators more frequently. We did not record ant species-specific interactions with the natural enemies and thus cannot determine whether greater *L. humile* aggression and/or higher worker foraging tempo or perhaps other factors (including those related to possible co-evolution of *C. carnea* and *H. convergens* with *T. sessile*, but not *L. humile*) accounted for the different responses to aphid aggregation sizes. Both ant species similarly reduced *A. colemani* parasitization of *A. gossypii*, which is consistent with a brief description by Bartlett (1961) of an equivalent response of some hymenopteran parasites of *Saissetia oleae* Bernard (Hemiptera: Coccidae) to both *L. humile* and *T. sessile*. Our field experiment revealed that ants improved *A. gossypii* survival; however, there was only a marginal difference between the impact of *L. humile* and that of *T. sessile*. Some of the natural enemies we observed on our field plants were different from those in our laboratory experiments, plus we assume that there were other natural enemies attacking *A. gossypii* that we were unable

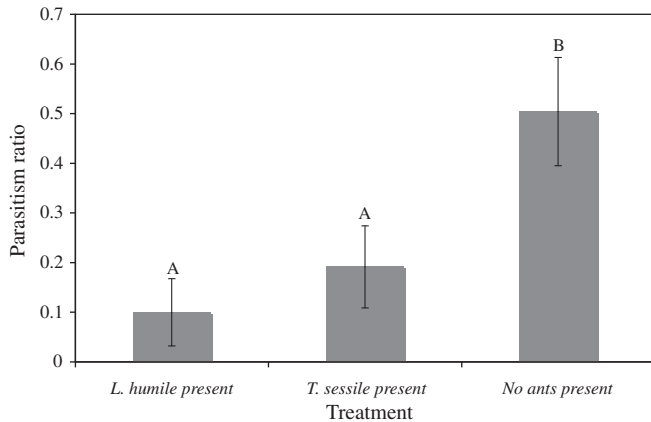


Fig. 3. Effect of *L. humile* and *T. sessile* on parasitism of *A. gossypii* by *A. colemani*. Bars represent the mean (\pm SE) parasitism ratio (number of aphids parasitized/initial aphid number). Different letters indicate significant differences at 0.05 alpha level.

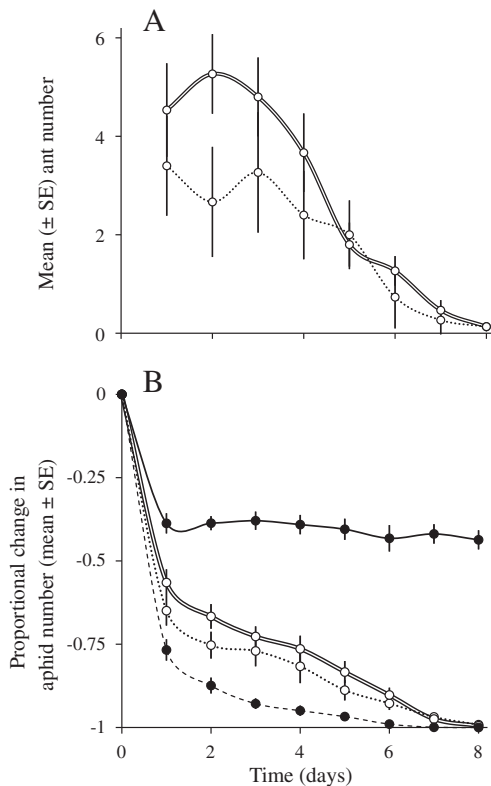


Fig. 4. Effect of *L. humile* and *T. sessile* on *A. gossypii* field populations. ○—○— *T. sessile* present, ◻—◻— *L. humile* present, ●—●— No ants present, ◼—◼— No ants present, with viral mesh enclosure around all replicates. (A) Mean (\pm SE) ant numbers per plant over time (B) Mean (\pm SE) *A. gossypii* populations per plant over time.

to observe. Therefore, we were not surprised that the difference between ant species was minimal. We compared equal numbers of *L. humile* and *T. sessile* workers in our experiments. Field populations of *L. humile* can reach extremely high levels (Markin, 1970) and it is at these levels that the impact of *L. humile* on pest hemipterans and their natural enemies is generally assessed (e.g. Daane et al. 2007). How *L. humile* attains these high damaging levels from much smaller introduced propagules is unclear, although diminished intraspecific aggression provides a possible explanation (Holway et al., 1998; Tsutsui et al., 2000). Though individual *L. humile* workers may not be more aggressive than *T. sessile* towards the

natural enemies in our study and perhaps other natural enemies of honeydew-producing hemipterans, domination of local native ant species (Holway, 1999) may reduce or eliminate competition for resources. Thereafter, hemipteran honeydew may fuel rapid Argentine ant colony growth whose numerous workers may more effectively interfere with the natural enemies of Hemiptera.

Predators and parasitoids of ant-tended hemipterans employ various strategies to avoid ant attack, including reduced activity (Eubanks, 2001) oviposition away from ant-controlled territories (Oliver et al. 2008), moving and waiting until ant foraging lessens (Hubner and Völkl, 1996) and rapid host handling time (Barzman and Daane, 2001). Yet, we found that both *L. humile* and *T. sessile* deterred *C. carnea*, *H. convergens* and *A. colemani* on *A. gossypii*; however, *C. carnea*, but not *H. convergens*, removed ant-tended aphids, particularly at high aphid densities. Harmon and Andow (2007) reported that other Coccinellidae species preyed on aphids when ant (*Lasius neoniger* (Emery)) density was low, but were deterred from aphid aggregations at high ant density. Also, benefits to *A. varians* on fireweed from *Formica cinerea* attendance diminished as aphid density increased (Breton and Addicott, 1992). However, when ant number is limiting in ant-hemipteran interactions benefits from ants are directly proportional to hemipteran population size (Cushman and Whitman, 1989). Since *L. humile* can become particularly numerous, we suggest that high ant density is largely responsible for disruption of biological control. Interference with biological control is not a certain outcome when *L. humile* or other ants invade a new habitat, as Kaplan and Eubanks (2005) suggested that *S. invicta* could also enhance natural biological control in cotton.

Though we report an impact of both *L. humile* and *T. sessile* specifically on *A. gossypii* aggregations exposed to three polyphagous natural enemies, we would also expect a similar outcome with other facultative mutualists of these ants that are attacked by the same biological control agents. However, the influence of host plant and prey/host behavior may affect the ant-natural enemy interaction, and thus further experiments are needed before extending our findings to other unique multitrophic systems.

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References

- Abbott, K.L., Green, P.T., 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. *Oikos* 116, 1238–1246.
- Addicott, J.F., 1979. A multispecies aphid-ant association: density dependence and species-specific effects. *Canadian Journal of Zoology* 56, 2093–2096.
- Barbani L.E., 2003. Foraging activity and food preferences of the odorous house ant (*Tapinoma sessile* Say) (Hymenoptera: Formicidae). MS Thesis, Virginia Polytechnic Institute and State University.
- Bartlett, B.R., 1961. The influence of ants upon parasites, predators and scale insects. *Annals of the Entomological Society of America* 54, 543–551.
- Barzman, M.S., Daane, K.M., 2001. Host-handling behaviors in parasitoids of the black scale: A case for ant-mediated evolution. *Journal of Animal Ecology* 70, 237–247.
- Beattie, A., 1985. *The Evolutionary Ecology of Ant-Plant Mutualisms*. Cambridge University Press, Cambridge.
- Bhatkar, A., Whitcomb, W.H., 1970. Artificial diet for rearing various species of ants. *Florida Entomologist: Journal* 53, 229–232.
- Breton, L.M., Addicott, J.F., 1992. Density-dependent mutualism in an aphid-ant interaction. *Ecology* 73, 2175–2180.
- Bristow, C.M., 1984. Differential benefits from ant attendance to two species of Homoptera on New York ironweed. *Journal of Animal Ecology* 53, 715–726.
- Buckley, R.C., Gullan, P.J., 1991. More aggressive ant species (Hymenoptera: Formicidae) provide better protection for soft scales and mealybugs (Homoptera: Coccidae, Pseudococcidae). *Biotropica* 23, 282–286.

- Buckley, R.C., 1987. Interactions involving plants, homoptera, and ants. *Annual Review of Ecology and Systematics* 18, 111–135.
- Buczowski, G., Bennett, G.W., 2006. Dispersed central-place foraging in the polydomous odorous house ant, *Tapinoma sessile* as revealed by a protein marker. *Insectes Sociaux* 53, 282–290.
- Buczowski, G., Bennett, G.W., 2008. Aggressive interactions between the introduced Argentine ant, *Linepithema humile* and the native odorous house ant, *Tapinoma sessile*. *Biological Invasions* 10, 1001–1011.
- Cushman, J.H., Whitman, T.G., 1989. Conditional mutualism in a membracid-ant association: Temporal, age-specific, and density-dependent effects. *Ecology* 70, 1040–1047.
- Daane, K.M., Sime, K.R., Fallon, J., Cooper, M.L., 2007. Impact of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards. *Ecological Entomology* 32, 583–596.
- Diaz, R., Knutson, A., Bernal, J.S., 2004. Effect of the red imported fire ant on cotton aphid population density and predation of bollworm and beet armyworm eggs. *Journal of Economic Entomology* 97, 222–229.
- Ebert, T.A., Cartwright, B., 1997. Biology and ecology of *Aphis gossypii* (Homoptera: Aphididae). *Southwest. Entomology* 22, 116–153.
- Eubanks, M.D., 2001. Estimates of the direct and indirect effects of red imported fire ants on biological control in field crops. *Biological Control* 21, 35–43.
- Fellers, J.H., 1987. Interference and exploitation in a guild of woodland ants. *Ecology* 68, 1466–1478.
- González-Hernández, H., Johnson, M.W., Reimer, N.J., 1999. Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the Biological Control of *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). *Biological Control* 15, 145–152.
- Grover, C.D., Dayton, K.C., Menke, S.B., Holway, D.A., 2008. Effects of aphids on foliar foraging by Argentine ants and the resulting effects on other arthropods. *Ecological Entomology* 33, 101–106.
- Hagen, K.S., 1962. Biology and ecology of predacious Coccinellidae. *Annual Review of Entomology* 7, 289–326.
- Harmon, J.P., Andow, D.A., 2007. Behavioral mechanisms underlying ants' density-dependent deterrence of aphid-eating predators. *Oikos* 116, 1030–1036.
- Henry, C.S., Brooks, S.J., Duelli, P., Johnson, J.B., 2002. Discovering the true *Chrysoperla carnea* (Insecta: Neuroptera: Chrysopidae) using song analysis, morphology, and ecology. *Annals of the Entomological Society of America* 95, 172–191.
- Holway, D.A., 1998. Effect of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. *Oecologia* 116, 252–258.
- Holway, D.A., Suarez, A.V., Case, T.J., 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science* 282, 949–952.
- Holway, D.A., 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. *Ecology* 80, 238–251.
- Holway, D.A., Lach, L., Suarez, A.V., Tsutsui, N.D., Case, T.J., 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* 33, 181–233.
- Hubner, G., Völkl, W., 1996. Behavioral strategies of aphid hyperparasitoids to escape aggression by honeydew-collecting ants. *Journal of Insect Behavior* 9, 143–157.
- Human, K.G., Gordon, D.M., 1996. Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecologia* 105, 405–412.
- Human, K.G., Gordon, D.M., 1999. Behavioral interactions of the invasive Argentine ant with native ant species. *Insectes Sociaux* 46, 159–163.
- James, D.G., Stevens, M.M., O'Malley, K.J., Faulder, R.J., 1999. Ant foraging reduces the abundance of beneficial and incidental arthropods in citrus canopies. *Biological Control* 14, 121–126.
- Kaneko, S., 2003. Different impacts of two species of aphid-attending ants with different aggressiveness on the number of emerging adults of the aphid's primary parasitoid and hyperparasitoids. *Ecological Research* 18, 199–212.
- Kaplan, I., Eubanks, M.D., 2002. Disruption of cotton aphid (Homoptera: Aphididae) natural enemy dynamics by red imported fire ants (Hymenoptera: Formicidae). *Environmental Entomology* 31, 1175–1183.
- Kaplan, I., Eubanks, M.D., 2005. Aphids alter the community-wide impact of fire ants. *Ecology* 86, 1640–1649.
- Lach, L., 2005. Interference and exploitation competition of three nectar-thieving invasive ant species. *Insectes Sociaux* 52, 257–262.
- Liepert, C., Dettner, K., 1993. Recognition of aphid parasitoids by honeydew-collecting ants: The role of cuticular lipids in a chemical mimicry system. *Journal of Chemical Ecology* 19, 2143–2153.
- Markin, G.P., 1970. The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae) in southern California. *Annals of the Entomological Society of America* 63, 1238–1242.
- Messing, R.H., Rabasse, J.M., 1995. Oviposition behavior of the polyphagous aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphididae). *Agriculture, Ecosystems & Environment* 52, 13–17.
- Mgocheki, N., Addison, P., 2009. Interference of ants (Hymenoptera: Formicidae) with biological control of the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). *Biological Control* 49, 180–185.
- Mondor, E.B., Rosenheim, J.A., Addicott, J.F., 2008. Mutualist-induced transgenerational polyphenisms in cotton aphid populations. *Functional Ecology* 22, 157–162.
- Ness, J.H., Bronstein, J.L., 2004. The effects of invasive ants on prospective ant mutualists. *Biological Invasions* 6, 445–461.
- O'Dowd, D.J., Green, P.T., Lake, P.S., 2003. Invasional 'meltdown' on an oceanic island. *Ecological Letters* 6, 812–817.
- Oliver, T.H., Jones, I.A.N., Cook, J.M., Leather, S.R., 2008. Avoidance responses of an aphidophagous ladybird, *Adalia bipunctata*, to aphid-tending ants. *Ecological Entomology* 33, 523–528.
- Passera, L., 1994. Characteristics of tramp species, Exotic Ants. *Biology, Impact, and Control of Introduced Species*. Westview Press, Oxford. pp. 23–43.
- Powell B.E., Silverman J. In press. Population growth of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) in the presence of *Linepithema humile* and *Tapinoma sessile* (Hymenoptera: Formicidae). *Environmental Entomology*
- Reimer, N.J., Cope, M.L., Yasuda, G., 1993. Interference of *Pheidole megacephala* (Hymenoptera: Formicidae) with biological control of *Coccus viridis* (Homoptera: Coccidae) in coffee. *Environmental Entomology* 22, 483–488.
- Institute, S.A.S., 2002. SAS/STAT Guide for Personal Computers, Version 8.1. SAS Institute, Cary, NC.
- Silverman, J., Brightwell, R.J., 2008. The Argentine ant: Challenges in managing an invasive unicolonial pest. *Annual Review of Entomology* 53, 231–252.
- Smith, D.A., 1928. The biology of *Tapinoma sessile* Say, an important house infesting ant. *Annals of the Entomological Society of America* 21, 307–330.
- de Souza, A.L.B., Delabie, J.H.C., Fowler, H.G., 1998. *Wasmannia* spp (Hymenoptera: Formicidae) and insect damages to cocoa in Brazilian farms. *Journal of Applied Entomology* 122, 339–341.
- Styrsky, J.D., Eubanks, M.D., 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of Royal Society of London B* 274, 151–164.
- Tsutsui, N.D., Suarez, A.V., Holway, D.A., Case, T.J., 2000. Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences of the United States of America* 97, 5948–5953.
- Vinson, S.B., Scarborough, T.A., 1989. Impact of the imported fire ant on laboratory populations of cotton aphid (*Aphis gossypii*) predators. *Florida Entomologist: Journal* 72, 107–111.
- Völkl, W., Mackauer, M., 1993. Interactions between ants attending *Aphis fabae* ssp. *Cirsiiacanthoidis* on thistles and foraging parasitoid. *Journal of Insect Behaviour* 6, 301–312.
- Völkl, W., Kroupa, A.S., 1997. Effects of adult mortality risks on parasitoid foraging tactics. *Animal Behaviour* 54, 349–359.
- Ward, D.F., Harris, R.J., 2005. Invasibility of native habitats by Argentine ants, *Linepithema humile*, in New Zealand. *New Zealand Journal of Ecology* 29, 215–219.
- Ward, P.S., 1987. Distribution of the introduced Argentine ant (*Iridomyrmex humilis*) in natural habitats of the lower Sacramento Valley and its effects on the indigenous ant fauna. *Hilgardia* 55, 1–16.
- Wetterer, J.W., Wild, A.W., Suarez, A.S., Roura-Pascual, N.R.P., Espadaler, X.E., 2009. Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). *Myrmecology News* 12, 187–194.
- Zamani, A., Talebi, A., Fathipour, Y., Baniamiri, V., 2007. Effect of temperature on life history of *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: Braconidae), two parasitoids of *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae). *Physiological Ecology* 36, 263–271.
- Zarpas, K.D., Margaritopoulos, J.T., Tsitsipis, J.A., 2007. Life histories of generalist predatory species, control agents of the cotton aphid *Aphis gossypii* (Hemiptera: Aphididae). *Entomologia Generalis* 30, 85–101.