# A facultative mutualism between aphids and an invasive ant increases plant reproduction

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**Abstract.** 1. The consequences to plants of ant–aphid mutualisms, particularly those involving invasive ants, are poorly studied. Ant–aphid mutualisms may increase or decrease plant fitness depending on the relative cost of herbivory by ant-tended aphids versus the relative benefit of increased ant suppression of other (non-aphid) herbivores.

2. We conducted field and greenhouse experiments in which we manipulated the presence and absence of cotton aphids (*Aphis gossypii*) on cotton plants to test the hypothesis that a mutualism between cotton aphids and an invasive ant, the red imported fire ant (*Solenopsis invicta*), benefits cotton plants by increasing fire ant suppression of caterpillars. We also manipulated caterpillar abundance to test whether the benefit of the mutualism varied with caterpillar density.

3. We found that more fire ants foraged on plants with cotton aphids than on plants without cotton aphids, which resulted in a significant reduction in caterpillar survival and caterpillar herbivory of leaves, flower buds, and bolls on plants with aphids. Consequently, cotton aphids indirectly increased cotton reproduction: plants with cotton aphids produced 16% more bolls, 25% more seeds, and 10% greater seedcotton mass than plants without aphids. The indirect benefit of cotton aphids, however, varied with caterpillar density: the number of bolls per plant at harvest was 32% greater on plants with aphids than on plants without aphids at high caterpillar density, versus just 3% greater at low caterpillar density.

4. Our results highlight the potential benefit to plants that host ant–hemipteran mutualisms and provide the first experimental evidence that the consequences to plants of an ant–aphid mutualism vary at different densities of non-aphid herbivores.

**Key words.** Ant–aphid interaction, *Aphis gossypii*, biological control, biological invasions, herbivory, indirect effects, plant defence, *Solenopsis invicta*.

## Introduction

Due to high local abundance, extreme aggression, and broad omnivory, invasive ants can significantly disrupt native communities and are, thus, considered some of the most ecologically damaging invasive species (Moller, 1996; Holway *et al.*, 2002). Potentially facilitating their ability to establish populations in non-native habitats, invasive ants commonly engage in mutualistic interactions with honeydew-producing hemipteran insects, including aphids, scales, and mealybugs (Holway *et al.*, 2002; Ness & Bronstein, 2004). Like native ants, invasive ants are attracted to honeydew as a predictable, renewable food resource and subsequently tend honeydew-producing hemipterans, protecting them from predators and parasitoids (Way, 1963; Buckley, 1987; Hölldobler & Wilson, 1990; Holway *et al.*, 2002).

The community-level consequences of ant–hemipteran mutualisms in general, but particularly those involving invasive ants, have been little studied and are poorly understood (Styrsky & Eubanks, 2007). This oversight is potentially important in terms of our understanding of the role of mutualisms in biological invasions, as well as the effects of mutualisms on complex trophic interactions and community structure. Ant–hemipteran mutualisms are very common, occurring on plants in most plant families from temperate to tropical latitudes in both managed (e.g. agricultural) and natural systems (Buckley, 1987; Way & Khoo, 1992; Blüthgen *et al.*, 2000). Further, because of their active and catholic foraging

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behaviour, their efficiency in recruiting to and collectively subduing prey, and their numerical dominance, omnivorous ants have strong direct and indirect effects in terrestrial food webs (Hölldobler & Wilson, 1990). These effects are probably strengthened in the presence of honeydew-producing hemipterans, because hemipteran-tending ants often become more aggressive towards other arthropods they encounter on plants (Way, 1963) and, in the case of typically ground-foraging ants, become more numerous on hemipteran host plants (Way & Khoo, 1992; Kaplan & Eubanks, 2005). The facilitation of ants by honeydew-producing hemipterans, therefore, may amplify and diversify the effects of both native and invasive ants in plant-based arthropod food webs (Holway *et al.*, 2002; O'Dowd *et al.*, 2003).

Considering the ubiquity of ant–hemipteran mutualisms in terrestrial communities, surprisingly few studies have investigated their consequences to host plants (Styrsky & Eubanks, 2007). Increased densities of honeydew-producing hemipterans in the presence of tending ants may result in decreased plant growth and reproduction, because hemipterans consume plant sap and can vector bacterial and viral pathogens (Buckley, 1987; Delabie, 2001). The increased aggressiveness by hemipteran-tending ants, however, potentially affords plants protection from other, more damaging herbivores (e.g. Nickerson *et al.*, 1977). The net effect of an ant–hemipteran mutualism on its host plant, therefore, could be negative or positive depending on the ratio of the direct cost of feeding by honeydew-producing hemipterans to the indirect benefit of increased ant suppression of other (non-honeydew-producing) herbivores (Carroll & Janzen, 1973; Buckley, 1987; Lach, 2003).

Due to their comparatively greater abundance and aggressiveness in invaded habitats, invasive ants may have much stronger indirect effects on plants, either positive or negative, relative to native ants. Invasive ants are potentially costly to plants because they are often associated with local hemipteran outbreaks (Holway *et al.*, 2002; O'Dowd *et al.*, 2003; Ness & Bronstein, 2004), but they may also more effectively suppress other (non-honeydew producing) herbivores (Way *et al.*, 1999; Lach, 2003; Altfeld & Stiling, 2009). Studies that investigate the effects on plants of ant–hemipteran mutualisms involving invasive ants, therefore, are needed to better predict and manage the ecological consequences of invasive ants in both natural and managed systems.

We present here the results of field and greenhouse experiments designed to investigate the consequences of a facultative mutualism between an invasive ant species, the red imported fire ant (*Solenopsis invicta* Buren) (Formicidae), and cotton aphids (*Aphis gossypii* Glover) (Aphididae) to an annual host plant of agricultural importance, cotton (*Gossypium hirsutum* Linnaeus). We asked two questions: (i) Can the fire ant–cotton aphid mutualism indirectly benefit cotton reproduction as a consequence of increased fire ant predation of an important cotton herbivore, the beet armyworm caterpillar [*Spodoptera exigua* (Hübner)] (Noctuidae)?, and (ii) Does the influence of the fire ant–cotton aphid mutualism on cotton reproduction vary with caterpillar density? We predicted that fire ant suppression of caterpillars would be greater on plants

hosting cotton aphids than on plants without aphids, resulting in decreased caterpillar damage and increased production of cotton bolls (cotton fruit) on plants with aphids. We further predicted that these effects would vary with caterpillar density. Fire ant predation of caterpillars is more likely to outweigh the direct cost of cotton aphids when caterpillar density (hence, potential caterpillar damage to plants) is high. Thus, we predicted that plants with aphids would produce more bolls than plants without aphids at high caterpillar density, but not at low caterpillar density.

## Materials and methods

### Study organisms

Red imported fire ants ('fire ants' from here forward) were unintentionally introduced into the United States through the port of Mobile, Alabama approximately 75 years ago and have since spread throughout the southeast (Vinson, 1997). Fire ants are notorious for their pervasive negative effects on invertebrate and vertebrate fauna in invaded habitats, effects typically attributed to their broad omnivory, extreme aggression, and superabundance (Vinson, 1997; Wojcik *et al.*, 2001; Holway *et al.*, 2002). Fire ants are important predators in agricultural systems in particular where they can exert effective biological control of several herbivore pests, but also attack or disrupt other beneficial predators (Vinson, 1997; Eubanks, 2001; Eubanks *et al.*, 2002). Fire ants forage primarily on the ground but will readily climb plants to tend honeydew-producing hemipterans (Ness & Bronstein, 2004), including cotton aphids on cotton plants (Kaplan & Eubanks, 2002; Diaz *et al.*, 2004).

Cotton aphids injure cotton plants by continuously feeding from plant phloem, which can result in stunted growth, loss of leaf surface area, premature leaf abscission, lower boll retention, and reduced cotton lint mass (Fuchs & Minzenmayer, 1995; Rosenheim *et al.*, 1997). Cotton aphids are protected from other predators by tending fire ants and can reach high densities as a result (Kaplan & Eubanks, 2002). Thus, the presence of fire ants on plants potentially exacerbates the negative effects of cotton aphids on cotton plant growth and reproduction. Cotton plants are tolerant of cotton aphid herbivory, however, at low to moderate aphid densities (Rosenheim *et al.*, 1997) and could indirectly benefit from aphid-tending fire ants if fire ants significantly reduce the abundance of more damaging, non-aphid herbivores including caterpillars and plant bugs (Diaz *et al.*, 2004; Kaplan & Eubanks, 2005). The non-aphid herbivore used in this study, the beet armyworm caterpillar, is considered a secondary pest of cotton, but can decrease plant reproduction by defoliating plants and feeding on developing cotton squares (flower buds), flowers, and bolls (Mascarenhas *et al.*, 1999). Cotton aphids and beet armyworm caterpillars are found together on cotton plants throughout the growing season.

### Field experiment

We conducted a field experiment in 2004 at the E. V. Smith Research Center of the Auburn University Agricultural Experiment Station in Macon and Elmore Counties,

Alabama, U.S.A. The field experiment consisted of a $2 \times 2$ factorial, randomised block design in which we manipulated cotton aphid density (aphids present or excluded) and beet armyworm caterpillar density (low or high) on cotton plants in $1.8 \times 1.8 \times 1.8$-m field cages. The field cages were constructed of $32 \times 32$ mesh Lumite screening (opening size 530 μm; Bio-quip, Gardena, California) that prevented aphid movement in or out of the cages. We randomly assigned each treatment combination to one of four cages in each of nine blocks ($n = 36$ cages total) in a 4-ha field of conventional cotton (single variety) in Elmore County. Densities of fire ants were high along field margins in both years and additional colonies established naturally within the fields over the course of the growing season. The blocks were arranged linearly so that each cage was 30–40 m from a field edge.

We erected each field cage over two rows of cotton seedlings before they were colonised by arthropods. We removed all the seedlings from one of the rows and thinned the seedlings to five evenly spaced plants in the remaining row to facilitate sampling individual plants. The bottom edges of the cages were buried to block movement of all arthropods except fire ants, which readily excavated tunnels under the cage walls.

We established the experimental treatments in late June, when cotton aphids appeared naturally in the field. We manipulated cotton aphid density by transferring approximately 300 aphids collected from the surrounding field (aphid-inclusion treatment) to each plant in half of the cages. In the absence of predators, aphid colonies were quickly established on caged plants. No aphids were added to plants in the remaining cages (aphid-exclusion treatment). Over the course of the field experiment (see Fig. 1), cotton aphid densities in the aphid-inclusion treatment were well within the range that we have observed naturally in the field (M. D. Eubanks, unpublished; see also Kaplan & Eubanks, 2005) and followed the typical seasonal pattern of high densities early, declining densities over the middle of the growing season, and then increasing densities late (Kaplan & Eubanks, 2002).

We manipulated caterpillar density by transferring to plants caterpillars that were reared in the laboratory from commercially purchased eggs (Benzon Research, Carlisle, Pennsylvania). Cages in the low-density and high-density treatments received 10 and 30 neonate caterpillars per plant per week, respectively. We chose these densities based on preliminary experiments to determine how many caterpillars would need to be applied to plants so that a certain number survived under typical field conditions. The densities observed over the course of the field experiment (see Fig. 1) are within the range of natural caterpillar densities in cotton and are consistent with densities observed in beet armyworm caterpillar outbreaks. Beet armyworm caterpillars have become a more serious pest in recent years and breed throughout the cotton growing season (Ruberson *et al.*, 1994).

The cotton aphid and caterpillar density treatments were applied for 9 weeks (late June to late August). This period of time coincided with the reproductive period of the plants, when cotton yield is most threatened by cotton aphid and caterpillar herbivory (Rosenheim *et al.*, 1997; Mascarenhas *et al.*, 1999). We sampled all five individual plants weekly for 7 weeks (early
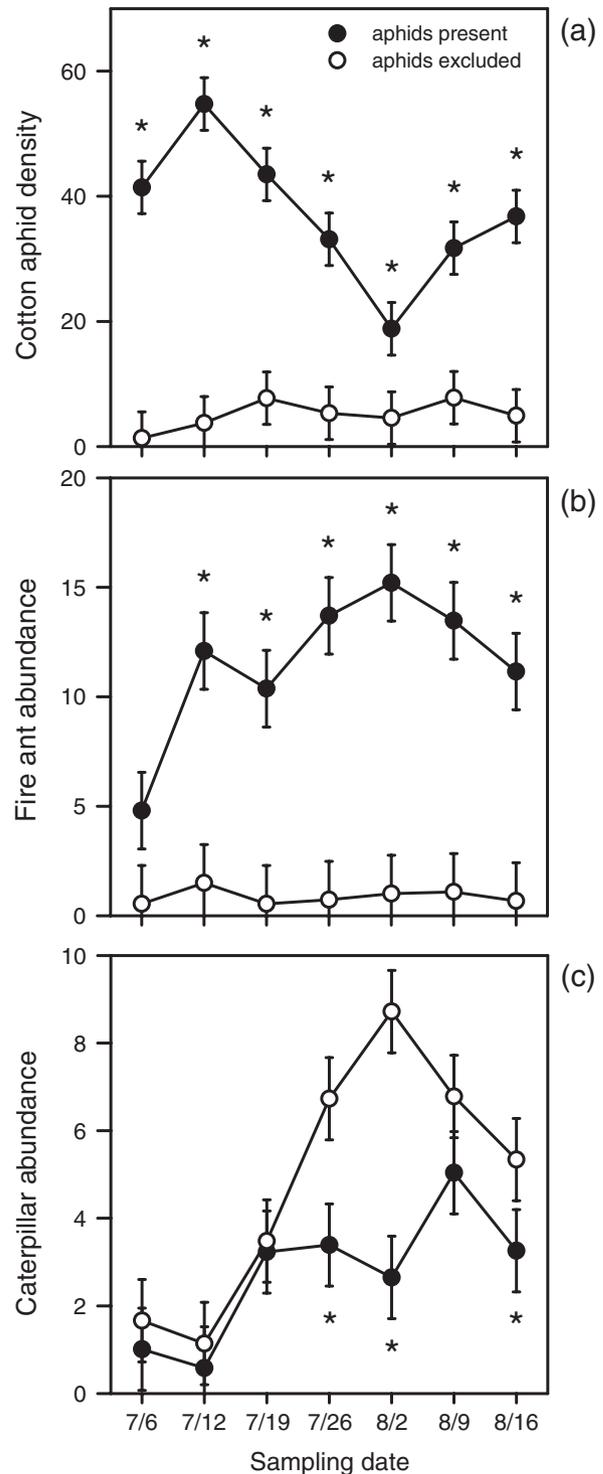


**Fig. 1.** Effect of the cotton aphid manipulation (aphids included or excluded from cotton plants in field cages) on mean cotton aphid density (mean number per leaf) (a), mean fire ant abundance per plant (b), and mean beet armyworm caterpillar abundance per plant (c) on seven sampling dates in the field experiment. Error bars represent 1 SE. Asterisks indicate significant differences between the two aphid treatments.

July to late August). Each week, we estimated cotton aphid density (number of aphids per leaf) and recorded the number of fire ants and caterpillars on each plant. We estimated cotton aphid density by averaging the number of aphids counted on six leaves, including the terminal leaf on branches 4, 8, and 12 (from the top of the plant), and the mainstem leaf growing from the node giving rise to each of these branches. We also quantified caterpillar damage to plant foliage each week by summing estimates of the per cent leaf area consumed for each mainstem leaf, and dividing by the total number of leaves sampled. Per cent leaf area consumed was estimated visually in 10% increments. Finally, we quantified plant reproduction each week by recording the number of squares (flower buds), flowers, and bolls on each plant, noting whether the bolls were damaged or undamaged by caterpillars.

Developing bolls were allowed to mature and open before collecting them on 20 September, 4 weeks after the final weekly sampling date. We dried the bolls at 60 °C for 3 days and then extracted and weighed the seedcotton (cotton seeds plus lint) to the nearest 0.01 g to determine total seedcotton yield for each plant. Additionally, we counted the total number of seeds produced by each plant.

### Greenhouse experiment

As we did not manipulate the presence and absence of fire ants independently of cotton aphid density in the field experiment (see Discussion for explanation), we implicitly assume that any reduction in caterpillar abundance and caterpillar damage to plants colonised by cotton aphids is the consequence of increased fire ant predation of caterpillars. This assumption is not unreasonable, because fire ant abundance and predation of herbivores on cotton plants is almost solely determined by the presence and abundance of cotton aphids (Kaplan & Eubanks, 2002, 2005). It is possible, however, that cotton aphids or their honeydew disrupt or impede foraging caterpillars, or that aphid herbivory alters cotton plant quality such that the plant becomes less suitable nutritionally for caterpillar growth and survival. Cotton aphids, thus, could decrease caterpillar abundance and caterpillar damage to plants independently of fire ant predation, potentially reducing or even nullifying the beneficial effect of fire ants. On the other hand, aphid damage to plants has been shown to benefit caterpillar growth and survival in other study systems (e.g. tomato: Stout *et al.*, 1998; Rodriguez-Saona *et al.*, 2005).

We tested for an effect (negative or positive) of cotton aphids on beet armyworm caterpillars in a greenhouse experiment, in which we monitored caterpillar survival in the absence of fire ants on cotton plants with and without cotton aphids. Seeds from cotton of the same variety used in the field experiment were sown in 20.3-cm pots in a greenhouse on the Auburn University campus in April 2005. Seedlings were allowed to grow until they developed their fourth set of true leaves, at which time we transferred approximately 100 cotton aphids from a laboratory colony to each of 16 plants. Two weeks later, we transferred five neonate beet armyworm caterpillars to each of the aphid-colonised plants (mean ± 1 SD aphid

density = 249 ± 163 aphids per leaf) and to 15 plants without aphids. We counted the number of caterpillars on each plant every 24 h for 10 days. After 10 days, we collected the remaining caterpillars and weighed them individually to the nearest 0.01 g. We quantified caterpillar damage to plants by summing visually-based estimates of the per cent leaf area consumed on each leaf, and dividing by the total number of leaves on the plant.

### Statistical analyses

We tested for treatment effects on cotton aphid density, fire ant abundance on plants, caterpillar abundance, per cent caterpillar damage to leaves and bolls, and the number of flowers and undamaged bolls per plant using repeated-measures ANOVA (SAS proc mixed; SAS Institute, Inc., 2001). We analysed the number of undamaged bolls because plants quickly aborted damaged bolls. We tested for treatment effects on number of bolls per plant at harvest, number of seeds, and seedcotton mass using two-way ANOVA (SAS proc glm). Cage means were used in all analyses. Values for aphid density, fire ant abundance, caterpillar abundance, per cent caterpillar damage to leaves and bolls, and number of flowers and bolls were $\log(n + 1)$-transformed to meet statistical assumptions. Values for number of bolls and number of seeds were log-transformed. Experiment-wise error rate was controlled in *post hoc* pairwise comparisons of treatment means within specific dates using sequential Bonferroni corrections.

In the greenhouse experiment, we tested for an effect of cotton aphids on mean caterpillar survival ($n$ surviving/5) using repeated-measures ANOVA. Survival data were arcsine square root-transformed prior to analysis. We used two-sample $t$-tests (SAS proc ttest) to compare mean caterpillar mass and mean per cent caterpillar damage to plants with and without cotton aphids.

Means ± 1 SE are presented in the text and figures, and $P$-values ≤ 0.05 are considered significant in all analyses.

## Results

### Field experiment

A few cotton aphids colonised plants in some of the 'aphid exclusion' cages, but mean aphid density was statistically indistinguishable from zero on every sampling date. Mean aphid density in the 'aphids present' cages fluctuated somewhat over the seven sampling dates, but was significantly greater than aphid density in the 'aphids excluded' cages on all dates (aphid density × date interaction: $F_{6,192} = 3.68$, $P = 0.002$; Fig. 1a). Averaged over all sampling dates, mean aphid density in the 'aphids present' cages was 37.1 ± 2.2 aphids per leaf, and in the 'aphids excluded' cages was 5.1 ± 2.2 aphids per leaf ($F_{1,24} = 133.47$, $P < 0.0001$).

Fire ants tended cotton aphids in the 'aphids present' cages within a week of establishing the experimental treatments. Fire ant abundance was significantly greater on plants with aphids than on plants without aphids (i.e. plants in 'aphid exclusion'

cages) on all but the first sampling date (aphid density × date interaction, Fig. 1b). Averaged over all sampling dates, mean fire ant abundance on plants with cotton aphids was $11.5 \pm 1.1$ ants per plant, and on plants without aphids was $0.9 \pm 1.1$ ants per plant (aphid density effect, Table 1).

Both the abundance of beet armyworm caterpillars on plants and per cent leaf damage by caterpillars increased over the seven sampling dates (date effects, Table 1). Averaged across all sampling dates, however, there were significantly fewer caterpillars on plants with cotton aphids ($2.7 \pm 0.5$ caterpillars per plant) than on plants without aphids ($4.8 \pm 0.5$ caterpillars per plant; aphid density effect, Table 1), but this effect varied with sampling date (aphid density × date interaction, Fig. 1c). Further, averaged across all sampling dates, caterpillars caused significantly less damage to cotton leaves on plants with aphids ($3.2 \pm 1.1\%$ leaf damage) than on plants without aphids ($7.7 \pm 1.1\%$; aphid density effect, Table 1).

Effects of cotton aphid density on caterpillar abundance and caterpillar damage were not influenced by the caterpillar density treatment (i.e. no aphid × caterpillar interactions; Table 1). In contrast, caterpillar density did influence the effect of cotton aphid density on caterpillar damage to bolls (Table 1). Caterpillar damage to bolls did not differ between plants with aphids ($0.8 \pm 1.7\%$ boll damage) and without aphids ($1.9 \pm 1.7\%$) at low caterpillar density ($P = 0.66$). At high caterpillar density, however, caterpillars damaged a significantly smaller proportion of bolls on plants with aphids ($0.3 \pm 1.7\%$) than on plants without aphids ($9.2 \pm 1.7\%$; $P = 0.01$).

Averaged over all sampling dates, cotton squares, flowers, and undamaged bolls were significantly more abundant on plants with cotton aphids (squares: $22.4 \pm 0.6$; flowers: $2.6 \pm 0.1$; bolls: $9.4 \pm 0.3$) than on plants without aphids (squares: $18.9 \pm 0.6$; flowers: $2.0 \pm 0.1$; bolls: $7.8 \pm 0.3$; aphid density effects, Table 2). The effect of cotton aphid density on number of squares varied with caterpillar density (aphid × caterpillar interaction, Table 2), however, such that mean number of squares was 17% greater on plants with aphids than on plants without aphids at high caterpillar density ($P = 0.002$) versus just 4% greater at low caterpillar density ($P = 0.35$) (Fig. 2a). Similarly, the effect of cotton aphid density on number of undamaged bolls varied with caterpillar density (aphid × caterpillar × date interaction, Table 3) such that the mean number of undamaged bolls was 76% greater on plants with aphids than on plants without aphids at high caterpillar density ($P < 0.0001$) versus just 11% greater at low caterpillar density ($P = 0.41$) in the final week of sampling (Fig. 2b).

**Table 1.** Results of repeated measures ANOVA of the effects of cotton aphid density and caterpillar density treatments on the mean abundance of fire ants and beet armyworm caterpillars on cotton plants, and mean per cent leaf damage and boll damage by caterpillars in the 2004 field experiment.

| | | Fire ant abundance | | Caterpillar abundance | | Per cent leaf damage | | Per cent boll damage† | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | d.f. | F | P | F | P | F | P | F | P |
| Block | 8.24 | 2.22 | 0.06 | 0.63 | 0.74 | 0.34 | 0.94 | 1.22 | 0.33 |
| Aphid density | 1.24 | 63.24 | **<0.0001** | 11.87 | **0.002** | 14.21 | **0.0009** | 8.70 | **0.007** |
| Caterpillar density | 1.24 | 0.43 | 0.52 | 20.09 | **0.0002** | 6.87 | **0.01** | 4.09 | **0.05** |
| Sampling date | 6.192 | 3.61 | **0.002** | 21.84 | **<0.0001** | 10.15 | **<0.0001** | 1.96 | 0.15 |
| Aphid × caterpillar | 1.24 | 0.04 | 0.85 | 0.29 | 0.60 | 0.52 | 0.48 | 5.40 | **0.03** |
| Aphid × date | 6.192 | 2.62 | **0.02** | 2.66 | **0.02** | 1.36 | 0.23 | 1.13 | 0.33 |
| Caterpillar × date | 6.192 | 1.61 | 0.15 | 2.30 | **0.04** | 0.13 | 0.99 | 0.44 | 0.65 |
| Aphid × Caterpillar × date | 6.192 | 0.71 | 0.64 | 0.52 | 0.80 | 0.31 | 0.93 | 0.67 | 0.52 |

†Analysis includes data from the final three sampling dates only (interaction terms d.f. = 2.64) because boll damage by caterpillars prior to these dates was negligible.
P-values for significant treatment effects appear in bold.

**Table 2.** Results of repeated measures ANOVA of the effects of cotton aphid density and caterpillar density treatments on the mean number of squares, flowers, bolls, and nodes on cotton plants in the 2004 field experiment.

| | | No. of squares | | No. of flowers | | No. of undamaged bolls | | No. of nodes | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | d.f. | F | P | F | P | F | P | F | P |
| Block | 8.24 | 5.98 | 0.0003 | 1.71 | 0.15 | 1.17 | 0.36 | 6.80 | 0.0001 |
| Aphid density | 1.24 | 13.85 | **0.0011** | 11.50 | **0.002** | 7.73 | **0.01** | 21.71 | **<0.0001** |
| Caterpillar density | 1.24 | 1.22 | 0.28 | 3.40 | 0.08 | 5.02 | **0.03** | 0.08 | 0.77 |
| Sampling date | 6.192 | 18.49 | **<0.0001** | 152.23 | **<0.0001** | 762.94 | **<0.0001** | 184.38 | **<0.0001** |
| Aphid × caterpillar | 1.24 | 5.69 | **0.03** | 0.10 | 0.76 | 7.71 | **0.01** | 0.02 | 0.89 |
| Aphid × date | 6.192 | 0.41 | 0.87 | 3.80 | **0.001** | 2.80 | **0.01** | 0.73 | 0.63 |
| Caterpillar × date | 6.192 | 0.04 | 0.99 | 0.56 | 0.76 | 0.82 | 0.56 | 0.53 | 0.79 |
| Aphid × caterpillar × date | 6.192 | 0.15 | 0.99 | 1.35 | 0.24 | 3.11 | **0.006** | 0.56 | 0.76 |

P-values for significant treatment effects appear in bold.
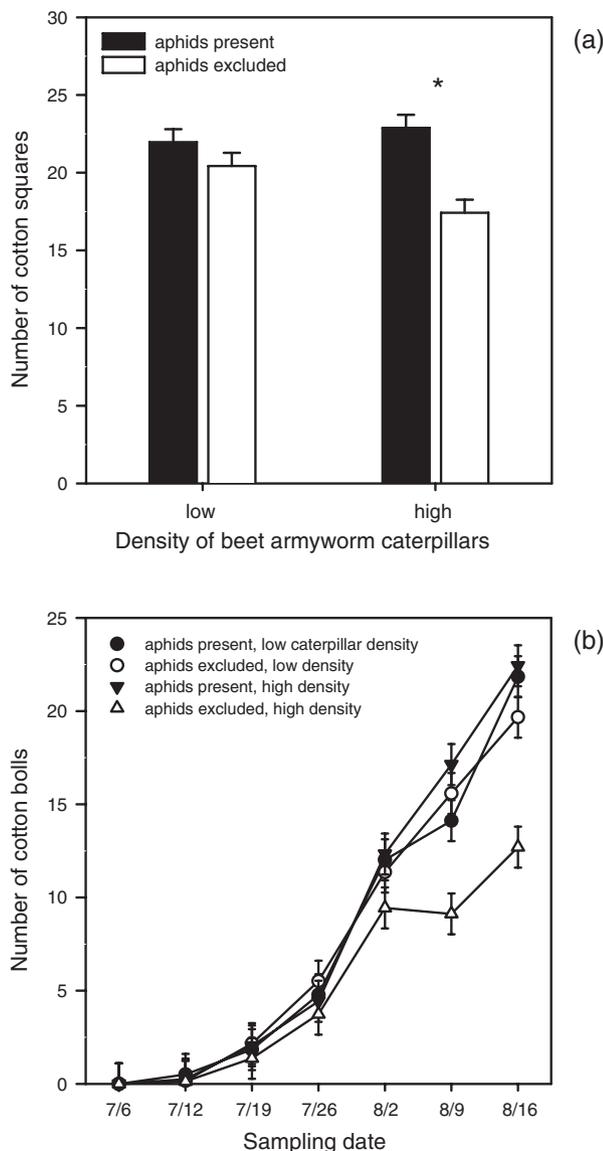
**Fig. 2.** Effect of cotton aphid density (aphids included or excluded from cotton plants in field cages) on mean number of cotton squares (flower buds) per plant averaged over seven sampling dates at low and high beet armyworm caterpillar density (a), and effect of cotton aphid density and caterpillar density on mean number of cotton bolls per plant undamaged by caterpillars on seven sampling dates (b) in the field experiment. Error bars represent 1 SE. Asterisk in (a) indicates a significant difference between the two aphid treatments.

At harvest, plants with aphids had significantly more cotton bolls ($22.0 \pm 0.8$ bolls per plant) than did plants without aphids ($19.0 \pm 0.8$ bolls per plant) (Table 3). The effect of cotton aphids varied with caterpillar density (Table 3), however, such that the mean number of bolls at harvest was 32% greater on plants with aphids than on plants without aphids at high caterpillar density ($P = 0.002$) versus just 3% greater at low caterpillar density ($P = 0.80$) (Fig. 3a). Plants with aphids also had 20% more seeds (Fig. 3b) and 12% greater seedcotton

mass (Fig. 3c) than did plants without aphids (significant aphid effects; Table 3). In contrast to boll number, however, the effects of cotton aphids on seed number and seedcotton mass did not vary with caterpillar density (no aphid × caterpillar interaction; Table 3). Plants produced significantly more seeds at low caterpillar density ($597.3 \pm 19.4$ seeds) than at high caterpillar density ($535.9 \pm 19.4$ seeds), but caterpillar density had no effect on seedcotton mass (Table 3).

*Greenhouse experiment*

Mean caterpillar survival decreased over the 10-day sampling period in both the presence and absence of aphids (day: $F_{9,261} = 56.20$, $P < 0.0001$), but significantly fewer caterpillars survived on plants without aphids (treatment × day interaction: $F_{9,261} = 6.80$, $P < 0.0001$). Consequently, a greater proportion of caterpillars survived on plants with aphids by the final 3 days of sampling (Fig. 4). As a result, per cent caterpillar damage to leaves was significantly greater on plants with aphids ($10.0 \pm 1.4\%$) than on plants without aphids ($6.1 \pm 0.7\%$; unequal variance *t*-test: $t_{22.5} = 2.42$, $P = 0.02$). Finally, although mean caterpillar mass was 16% less on plants with aphids ($45.5 \pm 3.34$ g) than on plants without aphids ($54.1 \pm 4.69$ g), it did not differ statistically between the treatments (equal variance *t*-test: $t_{26} = 1.52$, $P = 0.14$).

**Discussion**

Invasive ants are notorious for their strong, negative effects on the abundance and distribution of native terrestrial arthropods, and are often associated with local hemipteran outbreaks, which are generally assumed to harm plants (Wojcik *et al.*, 2001; Helms & Vinson, 2002; Holway *et al.*, 2002). Our results show, however, that a much maligned invasive ant species, the red imported fire ant, can actually benefit plants when tending aphids as a consequence of the increased effects of fire ants as predators in the arthropod community associated with the plant canopy. In the field experiment, cotton aphids attracted fire ants onto plants, resulting in fire ant predation of beet armyworm caterpillars and suppression of caterpillar herbivory. Decreased caterpillar damage to leaves and developing bolls on plants hosting the fire ant–cotton aphid mutualism, resulted in increased cotton reproduction in terms of the mean number of bolls and seeds produced per plant, and mean seedcotton mass. Results from the greenhouse experiment suggest that neither physical interference by cotton aphids nor their effect on plant quality likely contributed to the reduction in caterpillar abundance and damage to plants in the field. Combined, these results demonstrate that the indirect benefit to cotton plants of suppression of caterpillar herbivory by aphid-tending fire ants outweighed any direct cost of herbivory by cotton aphids themselves.

Although we believe the results clearly show that the fire ant–cotton aphid mutualism can benefit cotton plants, our interpretation of the results could be questioned because we did not manipulate the presence and absence of fire ants in the field cages in addition to manipulating the presence and

**Table 3.** Results of ANOVA of the effects of cotton aphid density and caterpillar density treatments on the mean number of bolls and seeds, and mean seedcotton mass harvested from cotton plants in the 2004 field experiment.

| Factor | d.f. | No. of bolls | | No. of seeds | | Seedcotton mass | |
|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P |
| Block | 8.24 | 5.96 | 0.0003 | 5.51 | 0.0005 | 7.25 | <0.0001 |
| Aphid density | 1.24 | 6.72 | **0.02** | 12.21 | **0.002** | 3.55 | **0.04** |
| Caterpillar density | 1.24 | 4.66 | **0.04** | 4.58 | **0.04** | 2.02 | 0.17 |
| Aphid × caterpillar | 1.24 | 4.93 | **0.04** | 0.88 | 0.36 | 0.14 | 0.71 |

*P*-values for significant treatment effects appear in bold.

absence of cotton aphids. Arguably, a fully crossed design is ideal when testing for an effect of an ant–aphid mutualism on a host plant, because it allows isolation of the effects of ants alone, aphids alone, and the ant–aphid mutualism itself (i.e. the interaction term) (Styrsky & Eubanks, 2007). Not manipulating the presence and absence of fire ants in our experiment would be a problem if fire ants normally foraged on cotton plants without cotton aphids, because we would be unable to distinguish between the effects of fire ants alone and the effects of fire ants that tend aphids. Fire ants do not, however, typically forage on cotton plants unless cotton aphids are present (Kaplan & Eubanks, 2002, 2005; present study). In the field experiment, fire ants were occasionally observed on cotton plants without cotton aphids, but mean fire ant abundance was statistically indistinguishable from zero on those plants on all sampling dates (Fig. 1b). Further, when fire ants were observed on cotton plants without aphids, they were recorded only from the main stem within 15 cm of the ground, and not on leaves, flower buds, or bolls where they would be far more likely to encounter beet armyworm caterpillars. As we did not expect fire ants to forage on cotton plants unless cotton aphids were present, and given that a limited number of cages were available, we did not separately manipulate the presence and absence of fire ants. We argue that manipulating the presence and absence of cotton aphids is considerably more important, because it is the aphids to which the fire ants respond, thereby allowing us to quantify the effects of the fire ant–cotton aphid mutualism (see Styrsky & Eubanks, 2007).

Results similar to ours have been reported in two of only three other studies of the consequences to plants of ant–hemipteran mutualisms involving invasive ants. Way *et al.* (1999) observed that defoliation of the pine *Pinus pinaster* Aiton by the larvae of the pine processionary moth [*Thaumetopoea pityocampa* (Denis and Schiffermuller)] was less severe in trees patrolled by the invasive Argentine ant [*Linepithema humile* (Mayr)] relative to trees patrolled by native ants. The authors suggested that aggregations of honeydew-producing aphids facilitated protection of pines by Argentine ants, but they did not specifically test whether ant predation was any greater in the presence of aphids than in their absence (i.e. Argentine ants foraged in trees whether or not aphids were present). In an experimental field study of aphid-tending Argentine ants, Altfeld and Stiling (2009) compared survivorship of the coastal shrub *Baccharis halimifolia* (Asteraceae) in the presence and absence of ants. Although the presence of Argentine ants increased aphid

abundance on plants by over 150%, significantly more of these plants survived relative to plants from which ants were experimentally excluded, because the aphid-tending ants protected the plants from the stemborer *Oidaematophorus balanotes* Meyrick. In contrast to these two studies, Lach (2007) reported a potentially detrimental effect of a facultative mutualism between a native membracid (*Beaufortiana* sp.) and invasive Argentine ants on host plant *Protea nitida* Miller in South Africa. Membracid presence on plants substantially increased the activity of Argentine ants on inflorescences relative to several native ant species, which significantly reduced the diversity of inflorescence-associated arthropods as well as pollinator abundance. Increased pollinator deterrence by membracid-tending Argentine ants apparently had no measurable effect on plant fitness, however, because plant seed set was no greater when ants were experimentally excluded from plants.

The studies by Way *et al.* (1999) and Lach (2007) are notable because they specifically compare the consequences to plants of ant–hemipteran mutualisms involving native versus invasive ant species. We did not compare effects between aphid-tending fire ants and native aphid-tending ants, but we suspect that fire ants provide relatively stronger indirect benefits to cotton plants. Native pyramid ants [*Dorymyrmex bureni* (Trager)] are common along field margins in our study area and will tend cotton aphids, but they are not as aggressive, do not show the same numerical response to cotton aphids, and are not as abundant in the interior of cotton fields as fire ants (J. Styrsky, pers. obs.). Further, disruption of pollination by fire ants is unlikely because aphid-tending fire ants are rarely observed on cotton inflorescences (J. Styrsky, pers. obs.). Given the potential for invasive ants to displace native hemipteran-tending ants and the pervasive ecological impact of invasive ants in both natural and managed habitats (Holway *et al.*, 2002; Lach, 2003; Ness & Bronstein, 2004), additional studies comparing the consequences to plants of interactions between honeydew-producing hemipterans and native versus invasive ants are urgently needed.

The consequences to plants of ant–hemipteran mutualisms involving native ants are relatively better studied and are generally positive, in terms of both reduced plant damage by non-honeydew-producing herbivores (e.g. Khoo & Ho, 1992; Floate & Whitham, 1994; Karhu, 1998) and increased plant growth and reproduction (e.g. Messina, 1981; Ito & Higashi, 1991). In fact, in a recent literature review, Styrsky and Eubanks (2007) found that 20 out of 30 studies of
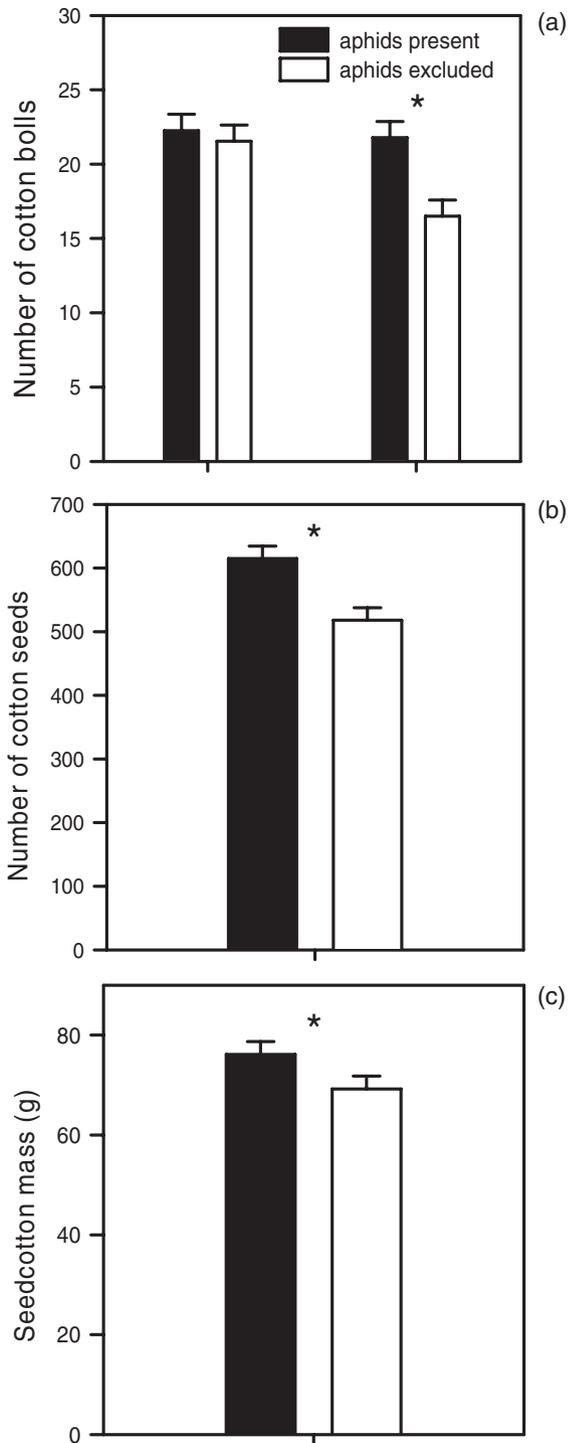
**Fig. 3.** Effect of cotton aphid density (aphids included or excluded from cotton plants in field cages) on mean number of cotton bolls per plant at harvest at low and high beet armyworm caterpillar density (a), and on mean number of cotton seeds per plant (b) and mean seedcotton mass per plant (c) at harvest averaged across the two caterpillar density treatments in the field experiment. Error bars represent 1 SE. Asterisks indicate significant differences between the two aphid treatments.
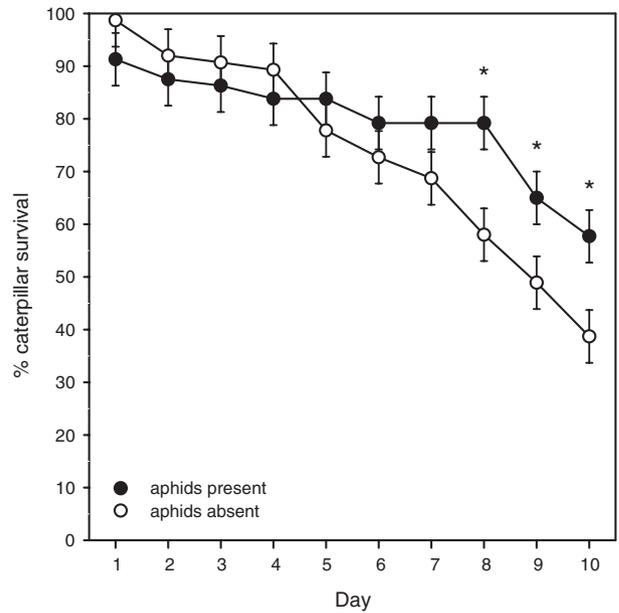


**Fig. 4.** Mean (± 1 SE) per cent beet armyworm caterpillar survival over 10 days on cotton plants with and without cotton aphids in the greenhouse experiment. Asterisks indicate significant differences in caterpillar survival between the two aphid treatments.

the consequences to plants of ant−hemipteran mutualisms involving native ants documented net positive effects on plants, despite extensive variation in geographical locations, habitats, and plant, ant, and hemipteran species involved. Our results further add to the growing body of evidence that mutualistic interactions between ants and honeydew-producing hemipterans generate important ecological effects in terrestrial communities, a phenomenon that we argue has been generally overlooked (Styrsky & Eubanks, 2007).

Plants may even be genetically invested in hosting ant−hemipteran mutualisms if the consequences of those mutualisms are typically beneficial. In a recent study of interactions between aphids, native aphid-tending ants, and milkweed plants (*Asclepias syriaca* Linnaeus), Mooney and Agrawal (2008) suggest that natural selection may favour plant genotypes that facilitate ant−hemipteran mutualisms via genotype effects on honeydew quality or quantity. This in turn affects the rate of ant recruitment to honeydew-producing hemipterans. Effects of plant genotype on ant−hemipteran mutualisms, therefore, may partially drive the evolution of plants, in which case hemipteran-tending ants may represent a genetically-based indirect defence against non-honeydew-producing herbivores (Mooney & Agrawal, 2008). Given the numerical dominance, extreme aggressiveness, and strong attraction to honeydew of invasive ants, displacement of native ants by invasive ants would likely only increase the efficacy of such an indirect mechanism of plant defence.

Our study builds on previous work by providing experimental evidence of conditionality in the outcome of a specific ant−hemipteran mutualism to its host plant. Messina (1981)

and Fritz (1983) previously recognised this possibility, arguing specifically that the cost-to-benefit ratio of ant–hemipteran mutualisms to plants may vary simply with the density of non-honeydew-producing herbivores on the plant, relative to the density of honeydew-producing hemipterans, as we show in this study. Beet armyworm caterpillar damage to developing bolls at high caterpillar density was on average almost 8% greater on cotton plants without cotton aphids than on plants with aphids. In contrast, caterpillar damage to developing bolls averaged only 1% greater on plants without aphids than on plants with aphids at low caterpillar density. As a consequence, the mean number of bolls per plant at harvest was 32% greater on plants with aphids than on plants without aphids at high caterpillar density, but was no different between plants with and without aphids at low caterpillar density (Fig. 2a). This conditionality in the benefit of cotton aphids to boll production was not reflected in the mean number of seeds produced per plant or mean seedcotton mass. Both number of seeds and seedcotton mass were significantly greater on plants with aphids, but equally so at both levels of caterpillar density. Plants at high caterpillar density may have compensated for the greater loss of bolls in the absence of aphids than in the presence of aphids by investing more resources in each boll. Mean boll mass was 0.35 g (10%) greater on plants without aphids than on plants with aphids at high caterpillar density versus just 0.08 g (2%) greater at low caterpillar density. Statistically, however, this increase in mean boll mass on plants with aphids did not differ between the two levels of caterpillar density ($F_{1,24} = 2.07$, $P = 0.16$).

Indirect interactions between honeydew-producing hemipterans and other herbivores mediated by plants could also influence the effects of ant–hemipteran mutualisms on plants. The results of our greenhouse experiment suggest a positive indirect interaction: beet armyworm caterpillar survival over time was significantly higher on plants with cotton aphids relative to plants without cotton aphids. Consistent with these results, Rodriguez-Saona *et al.* (2005) found that aphid damage to tomato plants increased consumption of leaves by and survival of *S. exigua* caterpillars. Similarly, Stout *et al.* (1998) reported that *S. exigua* caterpillars grew 10–50% faster when fed aphid-damaged tomato leaves than when fed leaves not damaged by aphids. If aphid herbivory improves host-plant quality for leaf-chewing herbivores, aphid-tending ants that are not very aggressive towards other herbivores may actually make plants more vulnerable to herbivory, thus increasing the net cost of aphids to plants. The results of our field experiment, however, show that fire ant predation of caterpillars outweighed any plant-mediated benefit of cotton aphids on beet armyworm caterpillars. Other factors that could mediate the outcome to the host plant of ant–hemipteran mutualisms include the proximity of ant colonies to host plants (Karhu, 1998), the abundance and efficacy of other arthropod predators (Fritz, 1983), variation in plant vulnerability to herbivory associated with plant phenology (Rosenheim *et al.*, 1997), availability and quality of extrafloral nectar (Buckley, 1983; Lach, 2008), and the effects of plant genotype (Johnson, 2008) and other biotic (e.g.

pathogen infection) and abiotic factors (e.g. nitrogen availability, shade) on host-plant quality (Cushman, 1991). As so many factors may influence the effects of ant–hemipteran mutualisms on host plants, additional studies considering the variation in associated costs and benefits are needed to provide greater insight into the range of possible outcomes and the ecological dynamics of these very common interspecific interactions.

Invasive ants represent a global threat to local biodiversity because of their tendency to severely disrupt native communities (Holway *et al.*, 2002; O'Dowd *et al.*, 2003). Like native ants, invasive ants are strongly attracted to honeydew produced by hemipteran insects feeding on plants, but the consequences to plants of ant–hemipteran interactions, particularly those involving invasive ants, are poorly known. Our results show that a facultative mutualism between an invasive ant, the red imported fire ant, and cotton aphids can indirectly benefit cotton plant reproduction as a consequence of increased fire ant predation of leaf-chewing herbivores. Negative effects of ant–hemipteran mutualisms involving invasive ants, however, such as pollinator disruption (Lach, 2007) and increased transmission of plant pathogens (Cooper, 2005), have been documented in other systems. We encourage additional studies of the effects on plants of ant–hemipteran mutualisms involving invasive ants to better understand and predict the consequences of ant invasions. Investigating mutualisms to predict the outcome of complex trophic interactions is relatively novel, because of a historical focus on negative species interactions as factors regulating the distribution and abundance of organisms (Bronstein, 1994; Stachowicz, 2001). Our results, however, add to a growing body of evidence (e.g. Bronstein, 1994; Stachowicz, 2001; Bruno *et al.*, 2003) that positive species interactions can dramatically affect the structure and dynamics of ecological communities.

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