

CONSEQUENCES OF MUTUALISMS BETWEEN APHIDS AND  
AN INVASIVE ANT TO ARTHROPOD COMMUNITIES  
AND THEIR HOST PLANTS

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CONSEQUENCES OF MUTUALISMS BETWEEN APHIDS AND  
AN INVASIVE ANT TO ARTHROPOD COMMUNITIES  
AND THEIR HOST PLANTS

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CONSEQUENCES OF MUTUALISMS BETWEEN APHIDS AND  
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DISSERTATION ABSTRACT  
CONSEQUENCES OF MUTUALISMS BETWEEN APHIDS AND  
AN INVASIVE ANT TO ARTHROPOD COMMUNITIES  
AND THEIR HOST PLANTS

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Mutualisms between ants and honeydew-producing Hemipteran insects (e.g., aphids) are abundant and widespread in arthropod food webs, yet their ecological consequences are very poorly known. Previous work in several agricultural systems in Alabama show that the community-level effects of red imported fire ants (*Solenopsis invicta*) vary among crops and that this variation may be correlated with the presence and absence of aphids. Fire ants have stronger and more pervasive effects in cotton, for example, where they are attracted onto plants by cotton aphids (*Aphis gossypii*) than they do in soybean, which has not historically hosted aphids. Alternatively, fire ants may be hindered from foraging on soybean plants by dense trichomes on soybean stems and

leaves. In chapter one, I present field and greenhouse experiments in which I manipulated fire ant density in plots of three soybean isolines varying in trichome density to test this hypothesis. Trichomes did not inhibit fire ants from foraging on plants in the field or in the greenhouse, and fire ant predation of herbivores in the field was actually greater on pubescent plants relative to glabrous plants. In chapter two, I review several studies that investigate explicitly the consequences of ant-Hemipteran mutualisms to plants. In most cases, plants benefit from these mutualisms as a consequence of increased predation or harassment of more damaging herbivores by Hemipteran-tending ants, resulting in decreased plant damage and increased plant reproduction. In chapter three, I describe field and greenhouse experiments in which I manipulated the presence and absence of cotton aphids on cotton plants to test the hypothesis that a mutualism between cotton aphids and red imported fire ants benefits cotton plants by increasing fire ant suppression of beet armyworm caterpillars (*Spodoptera exigua*). Greater numbers of fire ants foraged on plants with cotton aphids than on plants without cotton aphids, resulting in a significant reduction in caterpillar survival and caterpillar herbivory of leaves, squares, and bolls on plants with aphids. In chapter four, I describe field and greenhouse experiments to test the hypothesis that the range expansion of the soybean aphid (*Aphis glycines*), will cause a dramatic increase in the effects of fire ants as predators in soybean. In field experiments in which I misted soybean plants with artificial honeydew as a surrogate for soybean aphid honeydew, fire ants were significantly more abundant on honeydew-misted plants, resulting in decreased beet armyworm caterpillar abundance and caterpillar damage to plants. Results from greenhouse experiments with soybean aphids were consistent with the field experiments.

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**CHAPTER ONE:**  
**PLANT TRICHOMES INDIRECTLY ENHANCE TRITROPHIC**  
**INTERACTIONS INVOLVING A GENERALIST PREDATOR,**  
**THE RED IMPORTED FIRE ANT**

**Abstract**

Morphological defense traits of plants such as trichomes potentially compromise biological control in agroecosystems because they may hinder predation by natural enemies. To investigate whether plant trichomes hinder red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), as biological control agents in soybean, field and greenhouse experiments were conducted in which I manipulated fire ant density in plots of three soybean isolines varying in trichome density. Resulting treatment effects on the abundance of herbivores, other natural enemies, plant herbivory, and yield were assessed. Trichomes did not inhibit fire ants from foraging on plants in the field or in the greenhouse, and fire ant predation of herbivores in the field was actually greater on pubescent plants relative to glabrous plants. Consequently, fire ants more strongly reduced plant damage by herbivores on pubescent plants. This effect, however, did not translate into greater yield from pubescent plants at high fire ant densities. Intraguild predation by fire ants, in contrast, was weak, inconsistent, and did not vary with trichome density. Rather than hindering fire ant predation, therefore,

soybean trichomes instead increased fire ant predation of herbivores resulting in enhanced tritrophic effects of fire ants on pubescent plants. This effect was likely the result of a functional response by fire ants to the greater abundance of caterpillar prey on pubescent plants. Given the ubiquity of lepidopteran herbivores and the functional response to prey shown by many generalist arthropod predators, a positive indirect effect of trichomes on predation by natural enemies might be far more common than is currently appreciated.

## **1. Introduction**

Plants employ a battery of physical and chemical defenses against insect herbivores including structural traits such as surface waxes, pubescence, and spines, and secondary metabolites that act as repellents, toxins, and digestibility-reducers (Marquis, 1992; Panda and Khush, 1995). Parasitoids and predators that use herbivores as hosts or prey provide an additional, indirect line of plant defense (Price et al., 1980). Plant resistance and parasitism/predation by natural enemies are not necessarily compatible means of plant defense, however, because plant resistance traits may directly or indirectly inhibit natural enemies (Price et al., 1980; Bottrell et al., 1998; Cortesero et al., 2000; Kennedy, 2003). Surface waxes on leaves, for example, can reduce the searching efficiency of natural enemies by decreasing their ability to grip the plant (Eigenbrode and Espelie, 1995), and plant allelochemicals ingested by herbivores may reduce their quality as hosts or prey (Turlings and Benrey, 1998). The potential antagonism between host plant resistance traits and natural enemies is particularly important in agriculture because



resistance bred into crop plants may preclude biological control (Bottrell et al., 1998; Cortesero et al., 2000).

Trichomes are hair-like structural elements of the epidermis of plants that may confer physical resistance to insect herbivores by impeding locomotion, feeding, and oviposition (Levin, 1973). Glandular trichomes may confer chemical resistance as well by exuding noxious secondary plant metabolites (Levin, 1973). Besides deterring herbivores, however, trichomes may also inhibit natural enemies, principally by restricting their mobility on plants (Belcher and Thurston, 1982, Treacy et al., 1986, 1987; Sutterlin and van Lenteren, 1997; Krips et al., 1999; Lovinger et al., 2000). As a result, trichomes decrease herbivore suppression by both parasitoids (Treacy et al., 1986; Kaufman and Kennedy, 1989; McAuslane et al., 1995) and predators (Treacy et al., 1985, 1987; Barbour et al., 1993; Krips et al., 1999). In lab experiments with green lacewings, *Chrysopa carnea* Stephens (Neuroptera: Chrysopidae), for example, trichomes slowed the walking speed of lacewing larvae on tomato plants (*Lycopersicon* spp.), thereby reducing the rate at which the larvae captured herbivorous mites (Fordyce and Agrawal, 2001). Similarly, trichomes on poinsettia plants (*Euphorbia pulcherrima*) reduced twelve-spotted lady beetle, *Coleomegilla maculata* Timberlake (Coleoptera: Coccinellidae), predation of whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), eggs by physically obstructing 4<sup>th</sup>-instar larvae and adults (Lucas et al., 2004).

Despite consistent support for the hypothesis that trichomes hinder generalist predators in several lab and greenhouse experiments, consonant evidence under field conditions is limited to one of only two field studies. Treacy et al. (1985) reported an

inverse relationship between trichome density and predation of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) eggs by the lacewing predator *Chrysopa rufilabris* (Burmeister) in cotton. In contrast, Obrycki et al. (1983) found that aphid predation by Coccinellid and Chrysopid predators was unrelated to trichome density in potato (*Solanum tuberosum*). Clearly, a need exists for additional studies that investigate the effects of trichomes on predation by natural enemies in the field. To make such studies more useful to biological control, however, they should also consider the tritrophic consequences of interactions between trichomes and natural enemies to plant damage and plant reproduction.

In this study, I ask whether trichomes hinder red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), as predators of herbivores and other natural enemies in soybean (*Glycine max*). Red imported fire ants ('fire ants' from here forward) were unintentionally introduced into Alabama approximately 75 years ago and have spread throughout the Southeastern United States (Vinson, 1997). Because they are broadly omnivorous, extremely aggressive, and often superabundant, fire ants negatively impact invertebrate communities in both natural and managed ecosystems (Vinson, 1997; Wojcik et al., 2001; Holway et al., 2002). Although fire ants are reportedly significant predators of insect pests in several crops (reviewed in Taber, 2000; Holway et al., 2002), the utility of fire ants as biological control agents has not been well established because they may attack or disrupt other predators and parasitoids (Vinson and Scarborough, 1989, 1991; Tedders et al., 1990; Eubanks et al., 2002; Kaplan and Eubanks, 2002). Further, recent work has shown that the magnitude of the effects of fire ants as predators varies among crops. In a comparison of the effects of fire ants on herbivores and natural

enemies in cotton and soybean, for example, Eubanks (2001) reported that fire ants negatively affected a greater number of taxa in cotton despite very similar arthropod communities and roughly equivalent densities of fire ants in both crops.

One hypothesis to account for the disparity in the effects of fire ants between cotton and soybean is that dense trichomes on soybean stems and leaves hinder the ability of fire ants to forage on soybean plants. Here, I present the results of field and greenhouse experiments in which I manipulated fire ant density in plots of three soybean isolines that differed in trichome density to test the hypothesis that trichomes hinder fire ant predation of herbivores and intraguild predation of other natural enemies in soybean. I predicted that fire ants would be more abundant on glabrous plants relative to moderately and densely pubescent plants, resulting in greater suppression of herbivores and other natural enemies on glabrous plants at high fire ant densities. Consequently, I predicted that glabrous plants would be less damaged by herbivores at high fire ant densities, therefore producing a higher yield than pubescent plants.

## **2. Methods**

### *2.1. Field experiment*

I conducted the field experiment at the E. V. Smith Research Center of the Auburn University Agricultural Experiment Station in Macon County, Alabama, U.S.A. from May through October 2002. In this experiment, fire ant density (low and high) was manipulated in field plots of three soybean isolines varying in trichome density using a 2 x 3 split-plot, randomized block design. Seeds from three isolines of 'Lee' soybeans (provided by the USDA Crop Genetics and Production Research Unit, Stoneville,

Mississippi, U.S.A.) were planted in 76-cm row spacings at 30 seeds / m. The three isolines were isogenic except for trichome density, which ranged from glabrous to moderately pubescent to densely pubescent. Four 6.1-m rows of each isoline were planted in four 6.1 x 9.1-m plots arranged as quarters of a larger, 15.2 x 21.3-m field. Six fields were planted in this configuration to result in 24 replicate plots including each of the three isolines. The location of each isoline within each plot was determined at random. Three-m alleys separated adjacent plots within each field, and all six fields were separated by at least 150 m.

Fire ant densities were naturally high at the study area; therefore, I suppressed fire ant densities in three of the six study fields by broadcasting approximately 40 g of Amdro<sup>®</sup> per field every other week throughout the growing season. Amdro is an ant-specific bait consisting of corn grit impregnated with a soybean oil attractant and hydramethylnon, a toxicant. Amdro is particularly effective at reducing fire ant densities because it can eradicate entire colonies by killing queens. Because fire ants can forage several tens of meters from their colonies, however, the smallest area in which fire ant densities could be manipulated using Amdro was at the level of individual fields.

I sampled soybean plants for arthropods using a beat cloth (Kogan and Pitrie, 1980) approximately once weekly from beginning bloom in mid-July through beginning seed maturity in late September. A 1-m<sup>2</sup> cloth was spread between two adjacent rows of plants that were then beat vigorously to dislodge canopy arthropods. All arthropods that fell on the cloth were quickly identified and counted. I then measured the height of one haphazardly chosen plant at that same location and quantified leaf damage by herbivores on that plant (sampling dates 1, 3 and 6 only) by summing estimates of the percentage

leaf area consumed on ten haphazardly chosen leaves from throughout the canopy and dividing by 10 (Kogan and Turnipseed, 1980). Each isoline in each plot was randomly sampled twice on each sampling date ( $n = 7$  dates). Means of the two samples were log ( $n + 1$ )-transformed for statistical analysis. Soybeans were mechanically harvested in late October to determine yield ( $\text{g} / \text{m}^2$ ) for each isoline in each plot.

Because Amdro was applied to entire fields, the experimental unit for the fire ant treatment was the field ( $n = 3$  replications). Accordingly, I tested for effects of the Amdro treatment and trichome density (isoline) on fire ant abundance on plants using a split-plot, repeated-measures ANCOVA in which Amdro treatment was the whole-plot factor and trichome density was the sub-plot factor (SAS proc mixed; SAS Institute, Inc, 2001). The same model was used to test for the effects of fire ant abundance (Amdro treatment) and trichome density on herbivore abundance, natural enemy abundance, and leaf damage by herbivores. Because plant height varied among plots and fields, plant height was included as a covariate in these models to control for associated variation in arthropod abundance. I tested for the effects of fire ant abundance and trichome density on soybean yield using a split-plot ANCOVA with plant height as a covariate.

Experiment-wise error rate was controlled in post-hoc pairwise comparisons of treatment means using the Tukey-Kramer adjustment. Means  $\pm 1$  SE are presented in the text and  $P$ -values  $\leq 0.05$  are considered significant in all analyses. Based on my predictions, I was most interested in statistical interactions between the effects of fire ant abundance and trichome density. For brevity and clarity, therefore, only the results of relevant  $F$ -tests in the analyses of treatment effects are presented in the text.

To verify differences in trichome densities among the three isolines, I haphazardly collected six individual plants of each isoline from the field plots in early August. Trichome density on each plant was estimated by counting the total number of trichomes on a 1-cm length of stem and petiole, and on a 1-cm<sup>2</sup> section of leaf using a dissecting microscope with an ocular micrometer. Trichome density (number / cm<sup>2</sup>) on stems and petioles was calculated using the formula for the surface area of a cylinder ( $\pi \times \text{diameter} \times \text{length}$ ). Stems were sampled between the 6<sup>th</sup> and 7<sup>th</sup> node, and trichomes were counted on the petiole and underside of one fully expanded leaf haphazardly chosen from the upper half of the plant. I tested for differences in trichome densities among the three isolines using a MANOVA (proc glm; SAS Institute, Inc., 2001). Means  $\pm$  1 SE are presented in the text.

## 2.2. Greenhouse experiment

I conducted the greenhouse experiment on the campus of Auburn University, Auburn, Alabama, U.S.A. in July and August 2002. In this experiment, the presence and absence of both fire ants and big-eyed bug nymphs, *Geocoris punctipes* Say (Hemiptera: Geocoridae), was manipulated on plants of three soybean isolines that varied in trichome density using a 2 x 2 x 3 randomized complete block design. The experiment was designed to test 1) whether trichomes disrupt fire ant predation of an important soybean herbivore, the velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), 2) whether trichomes disrupt big-eyed bug predation of velvetbean caterpillars, and 3) whether trichomes disrupt fire ant intraguild predation of big-eyed bugs. I used big-eyed bugs in this experiment because they are one of the most abundant

generalist predators in soybean and they readily attack small caterpillars (personal observation).

Seeds from the three 'Lee' isolines described above were sown in 20.3-cm pots and the seedlings ( $n = 40$  of each isoline) were allowed to grow until they developed their fourth set of true leaves. Individual potted plants were placed in cages constructed of a PVC pipe frame (30 x 33 x 75 cm) that sat within 37-L plastic tubs filled to the rim of the pot with potting soil. Mosquito netting covered each frame and was fastened around the outside rim of each tub with an elastic band. Tubs with plants that were randomly assigned to the 'fire ants present' treatment were connected by a short section of 5-cm diameter plastic tubing to plastic pans (53 x 43 x 13 cm) containing a small colony of approximately 2000 fire ants. The plastic pans were lined with Fluon® to prevent fire ants from escaping. Fire ants were allowed to access the cages for 24 h prior to the initiation of the experiment in order to give them time to discover the plants.

At the initiation of the experiment, seven 2<sup>nd</sup>-instar velvetbean caterpillars were placed on each plant in each treatment. At the same time, three 4<sup>th</sup>- or 5<sup>th</sup>-instar big-eyed bug nymphs were placed in those cages with plants randomly assigned to the 'big-eyed bugs present' treatment. Velvetbean caterpillars were lab-reared on artificial diet (Product #F9219B, Bio-Serv, Frenchtown, NJ) from commercially purchased eggs. Big-eyed bug nymphs were collected from agricultural fields on the Auburn University campus, transferred to an environmental chamber, and provided only water for 48 h prior to the experiment. Densities of fire ants, big-eyed bugs, and velvetbean caterpillars used in the experiment are all within the range of densities commonly encountered in Alabama soybean fields (Eubanks, 2001).

I attempted to replicate each treatment combination 10 times but fire ants failed to forage in three cages. These cages, therefore, were reassigned to the ‘fire ants absent’ treatment. Twenty-four hours after initiating the experiment, I recorded the number of fire ants, big-eyed bug nymphs, and caterpillars on the plants. Missing big-eyed bug nymphs and caterpillars were considered depredated (Eubanks et al., 2002). I analyzed the effects of fire ants, big-eyed bugs, and trichome density (isoline) on caterpillar survival ( $n$  surviving / 7) using a three-way ANOVA (SAS proc glm; SAS Institute, Inc., 2001). I analyzed the effects of fire ants and trichome density on big-eyed bug survival ( $n$  surviving / 3) using a two-way ANOVA. Raw survival data were used in both analyses because residuals were normally distributed and homoscedastic. Error probabilities estimated from type-III sums of squares were used to account for the unbalanced design. Experiment-wise error rate was controlled in post-hoc pairwise comparisons of treatment means using the Tukey-Kramer adjustment. Means  $\pm$  1 SE are presented in the text and  $P$ -values  $\leq$  0.05 were considered significant in both analyses.

### **3. Results**

#### *3.1. Field experiment*

##### *3.1.1. Trichome density*

Trichome density varied significantly among the three soybean isolines (Table 1). Trichome density was significantly greater in both the moderately pubescent and densely pubescent isoline compared to the glabrous isoline (which had no trichomes), and was significantly greater in the densely pubescent isoline (stem =  $1370 \pm 128$  trichomes / cm<sup>2</sup>; petiole =  $663 \pm 70$ ; leaves =  $823 \pm 128$ ) than in the moderately pubescent isoline



(stem =  $477 \pm 55$ ; petiole =  $335 \pm 40$ ; leaves =  $350 \pm 35$ ) (Table 1). Standardized canonical coefficients indicate that the differences among the isolines were due primarily to differences in the density of trichomes on the stems, followed by the petioles, and then the leaves (Table 1).

### 3.1.2. Fire ants

The effects of the Amdro treatment and trichome density interacted to influence the abundance of fire ants on plants ( $F_{2,457} = 3.74$ ,  $P = 0.03$ ). Averaged across all sampling dates, fire ant abundance was statistically indistinguishable from zero in each soybean isolate in Amdro-treated ('low-fire-ant') plots (Fig. 1). Although more fire ants were sampled in untreated ('high-fire-ant') plots, fire ant abundance on plants was still low, averaging between two and four fire ants per two row-meters (i.e., per beat-cloth sample) (Fig. 1). In high-fire-ant plots, fire ant abundance on plants varied with trichome density such that fire ants were slightly more abundant on moderately pubescent plants than on densely pubescent plants ( $P = 0.01$ , Fig. 1). Mean fire ant abundance was no greater, however, on glabrous plants than on pubescent plants in high-fire-ant plots (Fig. 1).

### 3.1.3. Herbivores

The three most commonly sampled herbivore taxa (accounting for 94% of the total number of individuals sampled) were Noctuid larvae (including velvetbean, soybean looper [*Pseudoplusia includens* (Walker)], green cloverworm [*Plathypena scabra* (Fabricius)], and armyworm [*Spodoptera* sp.] caterpillars), leafhoppers (Hemiptera:

Cicadellidae; including potato leafhoppers [*Empoasca fabae* (Harris)], and three-cornered alfalfa hoppers, *Spissistilus festinus* (Say) (Hemiptera: Membracidae). The effects of fire ant abundance and trichome density interacted to influence the abundance of caterpillars on plants. Caterpillar abundance differed significantly among the three isolines over the season (isoline x date interaction, Table 2) such that caterpillars were more abundant on pubescent plants than on glabrous plants (Fig. 2). The effect of fire ant abundance varied among isolines, however, such that fire ants reduced caterpillar abundance more in the two pubescent isolines than in the glabrous isoline as the season progressed (fire ant x isoline x date interaction, Table 2; Fig. 2). Averaged over the final three sampling dates (when caterpillar abundance peaked), fire ants reduced caterpillar abundance by 34.5%, 27.5%, and 3.1% on moderately pubescent, densely pubescent, and glabrous plants, respectively.

Fire ant abundance and trichome density also interacted to affect leafhopper abundance on plants (fire ant x isoline interaction, Table 2). Unlike caterpillars, leafhoppers were significantly more abundant on glabrous plants than on pubescent plants. Similar to their effect on caterpillars, however, fire ants more greatly reduced leafhopper abundance in the two pubescent isolines (Fig. 3a). Fire ants reduced mean leafhopper abundance by 75.1%, 75.7%, and 5.1% on moderately pubescent, densely pubescent, and glabrous plants, respectively.

Fire ants had the opposite effect on three-cornered alfalfa hoppers. Three-cornered alfalfa hoppers were significantly more abundant over the season in high-fire-ant plots than in low-fire-ant plots (fire ant x date interaction, Table 2). The beneficial effect of fire ants on *S. festinus* abundance varied with trichome density (fire ant x isoline

interaction, Table 2), however, such that alfalfa hoppers were more abundant on moderately pubescent plants than on densely pubescent plants at high fire ant densities ( $P < 0.0001$ ; Fig. 3b). Alfalfa hopper abundance was no greater on glabrous plants relative to pubescent plants (Fig. 3b).

#### 3.1.4. *Natural enemies*

The three most commonly sampled natural enemy taxa (accounting for 92% of the total number of individuals sampled) were big-eyed bugs (Geocoridae), damsel bugs (Nabidae), and spiders (Araneae). Fire ants negatively affected big-eyed bugs and damsel bugs, but did so independently of trichome density. Significantly fewer big-eyed bugs (Fig. 4a) and damsel bugs (Fig. 4b) were found on plants in high-fire-ant plots than in low-fire-ant plots, but only on single sampling dates (fire ant x date interactions, Table 3). Trichome density also affected big-eyed bug and damsel bug abundances on plants (isoline effects, Table 3). Averaged over all sampling dates, big-eyed bugs were more abundant on moderately pubescent ( $3.8 \pm 0.3$  bugs/sample) and densely pubescent plants ( $3.0 \pm 0.3$ ) than on glabrous plants ( $1.9 \pm 0.3$ ;  $P < 0.0001$  for both comparisons). In contrast, damsel bug abundance did not differ between pubescent plants and glabrous plants ( $1.5 \pm 0.5$  bugs/sample); however, damsel bugs were more abundant on moderately pubescent plants ( $1.7 \pm 0.5$ ) relative to densely pubescent plants ( $1.3 \pm 0.5$ ;  $P = 0.003$ ). In contrast to big-eyed bugs and damsel bugs, neither fire ant abundance nor trichome density affected spider abundance on plants (Table 3).

### 3.1.5. Plant damage and yield

Caterpillars were likely responsible for the majority of leaf herbivory as they were the most abundant chewing herbivores sampled. As a consequence of the effects of fire ant abundance and trichome density on caterpillar abundance, the effects of fire ant abundance and trichome density interacted to influence the level of caterpillar damage to plants. Percent leaf damage differed significantly among the three isolines over the season (isoline x date interaction:  $F_{4,193} = 23.81$ ,  $P < 0.0001$ ) such that pubescent plants were more damaged by caterpillars than were glabrous plants (Fig. 5). The effect of fire ants varied among isolines, however, such that pubescent plants rather than glabrous plants were significantly less damaged by caterpillars over the season in high-fire-ant plots relative to low-fire-ant plots (fire ant x isoline x date interaction:  $F_{4,193} = 2.83$ ,  $P = 0.03$ ; Fig. 5). On the final date that plants were scored for damage, caterpillar herbivory of moderately pubescent and densely pubescent plants was 13% and 10% lower, respectively, in high-fire-ant plots compared to low-fire-ant plots (Fig. 5). Conversely, caterpillar herbivory of glabrous plants was 4% higher in high-fire-ant plots compared to low-fire-ant plots (Fig. 5).

Although the effects of fire ant abundance and trichome density interacted to influence caterpillar herbivory, the effect of the fire ant x isoline interaction on yield was not statistically significant ( $F_{2,62} = 0.21$ ,  $P = 0.81$ ). Soybean yield did vary significantly among isolines ( $F_{2,62} = 3.67$ ,  $P = 0.03$ ); however, despite the fact that glabrous plants were less damaged by herbivores, yield from glabrous plants ( $5.9 \pm 0.4$  g/m<sup>2</sup>) was no greater than that of either pubescent isoline. Yield differed statistically only between moderately pubescent plants ( $5.7 \pm 0.3$  g/m<sup>2</sup>) and densely pubescent plants

( $4.8 \pm 0.3 \text{ g/m}^2$ ;  $P = 0.03$ ). In contrast, fire ant abundance on plants did not significantly affect yield ( $F_{1,4} = 1.05$ ,  $P = 0.36$ ), despite a 27% increase in yield in high-fire-ant plots ( $6.1 \pm 0.9 \text{ g/m}^2$ ) relative to low-fire-ant plots ( $4.8 \pm 0.9 \text{ g/m}^2$ ).

### 3.2. Greenhouse experiment

In cages with fire ants, trichome density did not affect the number of fire ants foraging on plants (glabrous:  $1.1 \pm 0.3$  ants/plant; moderately pubescent:  $0.9 \pm 0.3$ ; densely pubescent:  $1.2 \pm 0.3$ ; one-way ANOVA:  $F_{2,54} = 0.31$ ,  $P = 0.74$ ). Likewise, trichome density did not affect the ability of fire ants to depredate velvetbean caterpillars (fire ant x isoline interaction:  $F_{2,105} = 0.31$ ,  $P = 0.73$ ). Significantly fewer caterpillars survived for 24 h in the presence of fire ants ( $68.7 \pm 2.3\%$ ) than in the absence of fire ants ( $93.5 \pm 2.2\%$ ), regardless of trichome density ( $F_{1,105} = 63.95$ ,  $P < 0.0001$ ). In contrast, caterpillar survival was not affected by either big-eyed bug nymphs ( $F_{1,105} = 1.79$ ,  $P = 0.18$ ) or trichome density ( $F_{2,105} = 0.34$ ,  $P = 0.71$ ).

Although fire ants reduced caterpillar survival, fire ants had no effect on the survival of big-eyed bug nymphs ( $F_{1,50} = 1.89$ ,  $P = 0.18$ ). Trichome density, however, did have a marginally significant effect on nymph survival ( $F_{2,50} = 3.09$ ,  $P = 0.054$ ). Nymph survival was greater on moderately pubescent plants ( $64.7 \pm 7.0\%$ ) relative to glabrous plants ( $41.3 \pm 7.0\%$ ;  $P = 0.05$ ). Nymph survival did not differ between densely pubescent plants ( $59.3 \pm 7.1\%$ ) and either glabrous or moderately pubescent plants.

## 4. Discussion

### 4.1. *Soybean trichomes enhance fire ant predation of herbivores*

Plant trichomes are generally thought to impede arthropod predator-prey interactions by disrupting predator mobility and decreasing searching efficiency (Bottrell et al., 1998; Cortesero et al., 2000). Despite supporting evidence from several lab and greenhouse experiments, however, little corroborating evidence exists that trichomes have the same effect in the field. In addition to a general lack of field data, no previous studies have shown whether the effects of trichomes on predation of herbivores have any consequence to plant damage or plant reproduction. Finally, no studies to date have investigated the effects of trichomes on ants, a very common and important group of generalist predators that may prey on other natural enemies as well as herbivores (Hölldobler and Wilson, 1990; Holway et al., 2002).

In this study, I conducted field and greenhouse experiments to test the hypothesis that trichomes hinder predation by red imported fire ants in soybean resulting in increased plant damage by herbivores and decreased yield. My results suggest that rather than hindering fire ant predation, soybean trichomes instead increased fire ant predation of herbivores resulting in decreased plant damage. Firstly, fire ants were no more abundant on glabrous plants than on moderately or densely pubescent plants either in the field or in the greenhouse. In fact, fire ants were slightly more numerous on pubescent plants in the field. Secondly, increased trichome density either had no effect or even a positive effect on fire ant predation of arthropods. Fire ant predation of caterpillars did not vary with trichome density in the greenhouse, whereas fire ant predation of herbivores including several caterpillar species in the field was generally greater on

pubescent plants than on glabrous plants. Further, intraguild predation by fire ants, though inconsistent and weak in the field, was no greater on glabrous plants than on pubescent plants. Thirdly, fire ants more strongly suppressed caterpillar herbivory on pubescent plants than on glabrous plants in the field. This effect did not result in greater yield from pubescent plants because plant damage was relatively greater on pubescent plants, but, averaged over all three isolines, yield was almost 30% greater in high-fire-ant plots. Combined, these results demonstrate a trophic cascade whereby the direct effect of a natural enemy on an herbivore population indirectly benefits plant fitness, at least in terms of reduced plant damage (Schmitz et al., 2000). In this particular tritrophic interaction, however, the cascade is apparently contingent upon the expression of a morphological resistance trait by the host plant. I am not aware of any previous report in the literature of such a phenomenon.

Our results are inconsistent with most previous studies investigating the effect of trichomes on generalist predators other than ants. Trichomes reportedly decrease the foraging efficiency of coccinellid larvae and adults (Belcher and Thurston, 1982; Obryicki and Tauber, 1984; Lucas et al., 2004), lacewing larvae and adults (Treacy et al., 1985, 1987; Fordyce and Agrawal, 2001), an Anthocorid bug (Coll et al., 1997), a predaceous stink bug (De Clercq et al., 2000), and a predaceous mite (Krips et al., 1999) by impeding predator movement and prey encounter rate. The effect of trichomes on intraguild predation has been far less studied, but in one laboratory experiment, Lucas and Brodeur (1999) found that dense pubescence on potato plants reduced the susceptibility of the eggs of a predatory midge to predation by the lady beetle *C. maculata*. All but one of these studies (Treacy et al., 1985) was conducted under

controlled conditions in labs and greenhouses, and none focused on ants. In the comparison of a greenhouse and a field study of aphid predation, Obrycki and Tauber (1984) suggested that coccinellid and chrysopid predators were less hindered by glandular trichomes on potato plants in the field because the effects of dust, wind, and rain decreased trichome adhesiveness. Soybean trichomes are predominantly non-glandular, however, and do not produce sticky exudates. Perhaps trichomes simply do not compromise the mobility of ants on plants as they do for other generalist predators. I did not compare fire ant prey encounter rates among isolines, but the fact that fire ants suppressed caterpillars more on pubescent plants than on glabrous plants in the field without a concomitant increase in ant abundance on pubescent plants argues against the possibility that trichomes hindered fire ant searching efficiency.

I hypothesize that trichomes increased fire ant predation of soybean herbivores as an indirect consequence of their effect on the abundance of herbivore prey. Soybean trichomes provide strong resistance to leafhoppers (Turnipseed, 1977, this study [Fig. 3a]) but reportedly facilitate oviposition by lepidopteran herbivores such as the corn earworm (Lambert and Kilen, 1989), velvetbean caterpillar (Gregory, 1989), and soybean looper (Beach and Todd, 1988). Such an oviposition preference by gravid moths may explain why caterpillars were more abundant on pubescent plants in both high-fire-ant and low-fire-ant plots (Fig. 2). Increased fire ant predation of caterpillars on pubescent plants relative to glabrous plants (Fig. 2), therefore, may represent a functional response by fire ants to the greater abundance of caterpillar prey on pubescent plants. Indeed, density-dependent predation is a common attribute of generalist predators including ants (Risch and Carroll, 1982; Way and Khoo, 1992; Harvey and Eubanks, 2004). The fact



that trichomes did not increase fire ant predation of caterpillars in the greenhouse experiment supports this interpretation. In contrast to the field experiment, caterpillar abundance was standardized across the three soybean isolines in the greenhouse experiment, thus precluding a functional response by fire ants.

#### *4.2. Fire ants as biological control agents in soybean*

Previous work has shown that fire ants can be both useful and disruptive as predators of cotton arthropods (Eubanks 2001, Eubanks et al. 2002, Kaplan and Eubanks 2002, 2005). Fire ants can reduce the abundance of important cotton pests such as *Lygus* plant bugs (Hemiptera: Miridae) and caterpillars, for example, but can also increase the abundance of another important cotton pest, the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), by attacking natural enemies of the aphid. Given that trichomes increased fire ant predation of an important group of soybean herbivores but did not appreciably increase intraguild predation in this study, my results highlight the role of fire ants as important beneficial predators in soybean. Fire ants can reach extremely high densities in soybean fields in the southeastern United States, with mound densities ranging from 22.2 to 207.5 active mounds per ha (Banks et al., 1990). Although fire ants may be the most abundant natural enemies in soybean fields, few studies have investigated their effects as predators of herbivores and no studies have investigated intraguild predation by fire ants in the soybean canopy. Fire ants reportedly prey on velvetbean caterpillar eggs (Buschman et al., 1977), larvae (Elvin et al., 1983), and pupae (Lee et al., 1990) in soybean, as well as on the eggs of the southern green stink bug,

*Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae), an important pod-feeding pest (Krispyn and Todd, 1982). My results provide even broader evidence that fire ants can significantly reduce the abundance of herbivore pests in soybean and that fire ant suppression of defoliating herbivores potentially benefits soybean yield.

Unfortunately, the very traits that make fire ants potentially useful in biological control, namely their abundance, aggression, and omnivorous feeding habit, may also make them a threat to yield. Fire ants may reduce soybean yield directly, for example, by feeding on germinating seeds, seedlings, and the root nodules of growing soybean plants (Shatters and Vander Meer, 2000). Further, fire ants may indirectly harm soybean plants by tending *S. festinus* nymphs for their honeydew, thereby protecting them from other predators. *Spissitilus festinus* nymphs and adults can cause extensive yield losses by feeding on petioles and racemes during the pod-setting and pod-filling stages (Kogan and Turnipseed, 1987). I found that *S. festinus* nymphs and adults were more abundant over the season on plants in high-fire-ant plots than in low-fire-ant plots, suggesting that fire ants disrupted biological control of this herbivore. Whether fire ants directly or indirectly harmed plants in this study was apparently of little consequence, however, because yield did not differ statistically between low-fire-ant and high-fire-ant plots.

#### 4.3. Conclusion

In summary, results from field and greenhouse experiments show that soybean trichomes do not inhibit fire ants from foraging on plants but rather increase fire ant predation of herbivores, possibly as an indirect consequence of greater caterpillar abundance on pubescent plants. I suggest that this phenomenon might actually be quite

common in nature because lepidopteran larvae are ubiquitous herbivores, many generalist arthropod predators including ants show density-dependent predation responses to prey density, and ants are often abundant predators in both natural and managed systems (Hölldober and Wilson, 1990; Way and Khoo, 1992; Holway et al., 2002). I emphasize the need for additional field studies of the effects of trichomes on generalist predators and their consequences to biological control. As demonstrated in many lab and greenhouse experiments, trichomes may reduce the mobility and prey encounter rate of some predator species. Numerical or functional responses to prey density by these same predators in the field, however, may compensate for reduced mobility resulting in increased plant protection. In contrast to previous studies, therefore, my results suggest that trichomes, though providing resistance to some herbivores (e.g., leafhoppers) but not to others (e.g., lepidopteran herbivores) in soybean, are compatible with predation by a generalist predator as means of direct and indirect plant defense.

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**Table 1.** Results of a MANOVA of trichome density on stems, petioles, and leaves of three isolines of soybeans: glabrous, moderately pubescent, and densely pubescent.

Main effect	Wilks' $\lambda$	F	d.f.	P	Standardized canon. coefficient <sup>a</sup>		
					Stem	Petiole	Leaf
Isoline	0.0419	16.84	6, 26	<0.0001	2.527	1.238	0.713
Contrasts							
Glabrous vs. Moderate	0.2319	14.36	3, 13	0.0002	2.171	1.706	0.520
Glabrous vs. Dense	0.0485	85.07	3, 13	<0.0001	2.511	1.263	0.704
Moderate vs. Dense	0.1237	30.69	3, 13	<0.0001	2.696	0.935	0.816

<sup>a</sup> Values represent estimates of the total amount of variation in trichome density accounted for by each variable alone. The largest value within a specific test indicates the variable that most differed in trichome density between isolines.

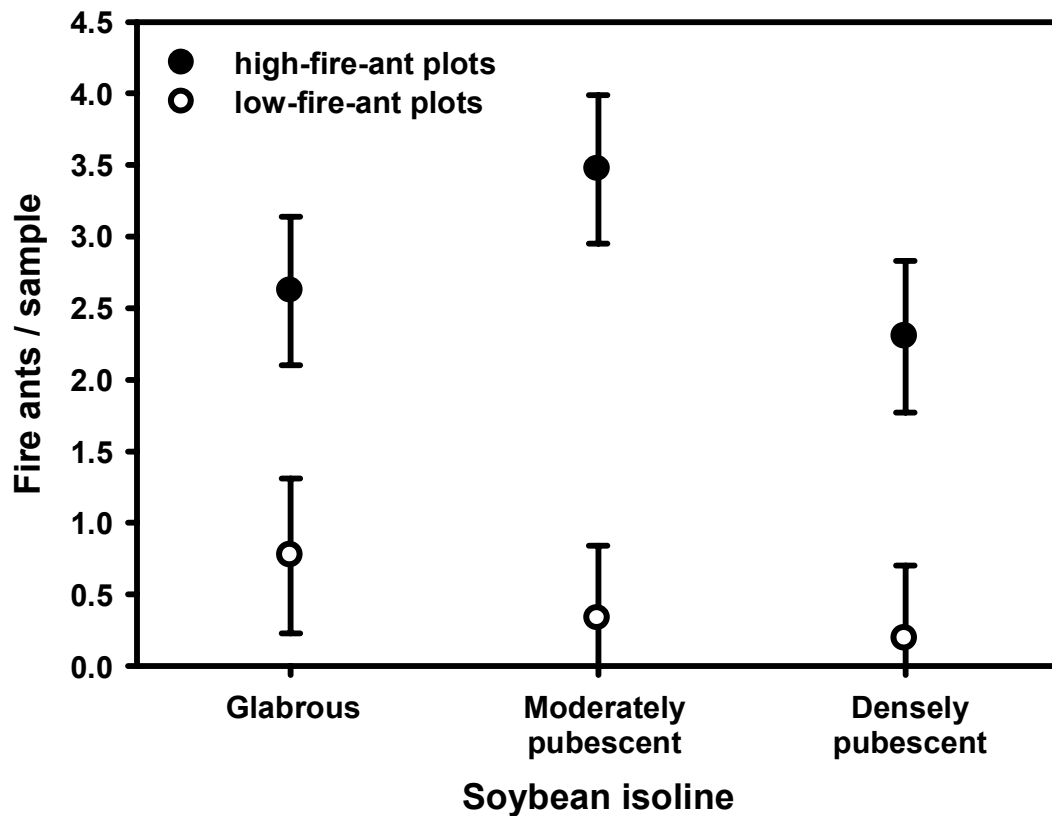
**Table 2.** Results of split-plot, repeated measures ANCOVA of the effects of fire ant density (Amdro treatment) and trichome density (soybean isoline) on the abundances of three groups of herbivores on soybean plants.

Factor	d.f.	Caterpillars		Leafhoppers		Alfalfa hoppers	
		F	P	F	P	F	P
Fire ants	1,4	1.90	0.24	4.87	0.09	1.88	0.24
Isoline	2,457	5.22	0.006	115.19	<0.0001	13.83	<0.0001
Plant height	1,457	71.27	<0.0001	3.98	0.05	3.70	0.06
Sampling date	6,457	243.87	<0.0001	51.70	<0.0001	6.87	<0.0001
Fire ants x Date	6,457	3.69	0.001	3.20	0.004	6.79	0.0001
Isoline x Date	12,457	5.30	<0.0001	4.13	<0.0001	1.50	0.12
Fire ants x Isoline	2,457	0.18	0.83	4.96	0.007	3.32	0.04
Fire ants x Isoline x Date	12,457	3.51	<0.0001	0.64	0.81	0.58	0.86

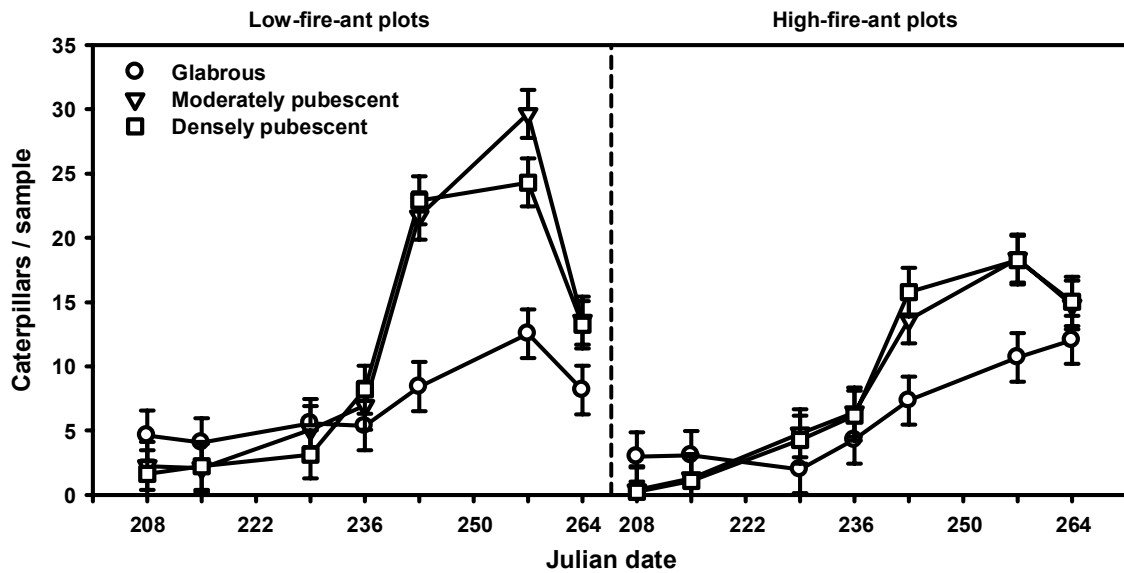
**Table 3.** Results of split-plot, repeated measures ANCOVA of the effects of fire ant density (Amdro treatment) and trichome density (soybean isoline) on the abundances of three groups of natural enemies on soybean plants.

Factor	d.f.	Big-eyed bugs		Damsel bugs		Spiders	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Fire ants	1,4	0.17	0.70	0.23	0.65	0.72	0.44
Isoiline	2,457	35.72	<0.0001	5.37	0.005	2.47	0.09
Plant height	1,457	4.77	0.03	0.46	0.50	9.58	0.002
Sampling date	6,457	38.02	<0.0001	51.22	<0.0001	6.59	<0.0001
Fire ants x Date	6,457	3.05	0.006	5.22	<0.0001	0.76	0.60
Isoiline x Date	12,457	2.46	0.004	1.32	0.20	0.79	0.66
Fire ants x Isoiline	2,457	0.73	0.48	0.88	0.41	0.18	0.84
Fire ants x Isoiline x Date	12,457	0.71	0.75	0.92	0.53	1.23	0.26

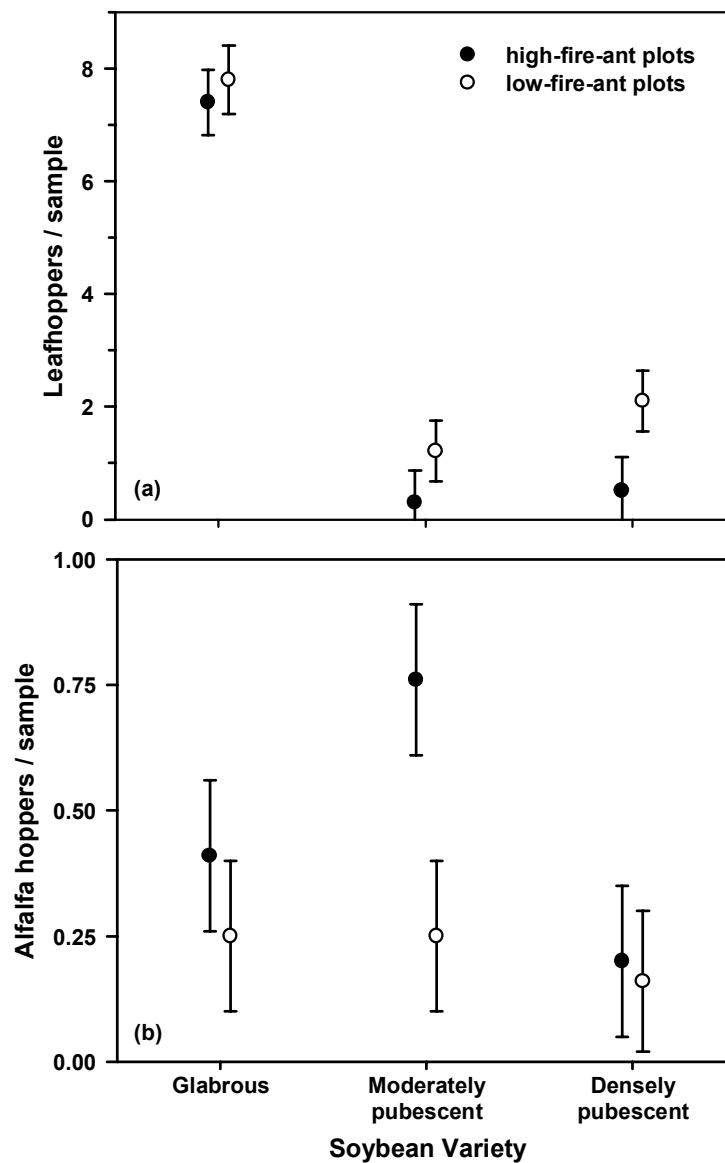
**Fig. 1.** Mean ( $\pm 1$  SE) number of fire ants per beat-cloth sample of soybean foliage in high-fire-ant (untreated) and low-fire-ant (Amdro-treated) plots of three soybean isolines varying in trichome density in 2002. The application of Amdro effectively reduced the abundance of fire ants on plants and fire ant abundance varied only slightly among isolines in high-fire-ant plots.



**Fig. 2.** Mean ( $\pm 1$  SE) number of caterpillars per beat-cloth sample of soybean foliage over the 2002 growing season in high-fire-ant and low-fire-ant plots of three soybean isolines varying in trichome density. Caterpillars were least abundant on glabrous plants later in the season but fire ants most reduced caterpillar abundance on pubescent plants.

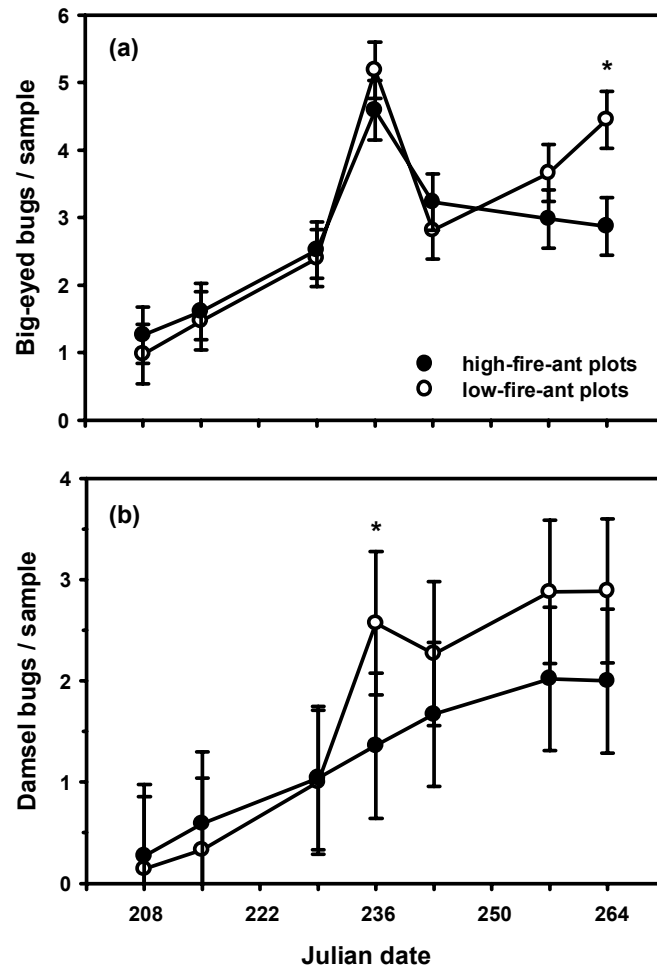


**Fig. 3.** Mean ( $\pm 1$  SE) number of (a) leafhoppers and (b) three-cornered alfalfa hoppers per beat-cloth sample of soybean foliage in high-fire-ant and low-fire ant plots of three soybean isolines varying in trichome density in 2002. Fire ants most reduced leafhopper abundance on pubescent plants. Fire ants positively affected alfalfa hopper abundance only in the moderately pubescent isolate.

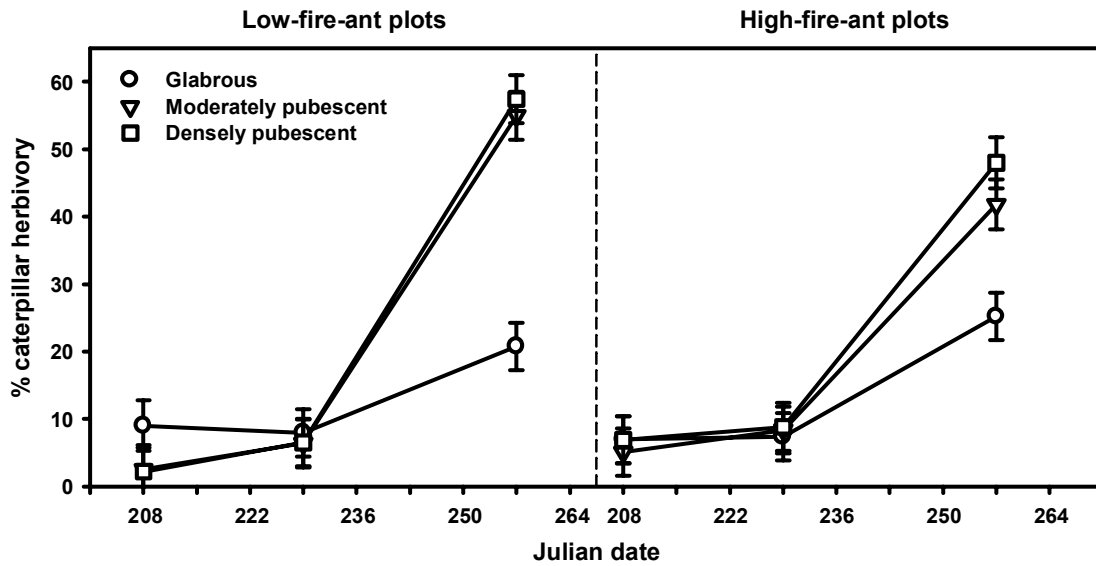




**Fig. 4.** Mean ( $\pm 1$  SE) number of (a) big-eyed bugs and (b) damsel bugs per beat-cloth sample of soybean foliage over the 2002 growing season in high-fire-ant and low-fire-ant plots of soybean plants. Natural enemies were significantly more abundant in the low-fire-ant plots on the dates indicated with asterisks.



**Fig. 5.** Mean ( $\pm 1$  SE) percent leaf damage by caterpillars over the 2002 growing season in high-fire-ant and low-fire-ant plots of three soybean isolines varying in trichome density. Fire ants suppressed caterpillar herbivory only on pubescent plants.



**CHAPTER TWO:**  
**ECOLOGICAL CONSEQUENCES OF ANT-HEMIPTERAN MUTUALISMS**

Mutualisms between ants and honeydew-producing Hemipterans are abundant and widespread in arthropod food webs, yet their ecological consequences are very poorly known. Ant-Hemipteran mutualisms have potentially broad ecological effects because the presence of honeydew-producing Hemipterans dramatically alters the abundance and predatory behavior of ants on plants. I review several studies that investigate explicitly the consequences of ant-Hemipteran mutualisms as ‘keystone interactions’ to arthropod communities and their host plants. Ant-Hemipteran mutualisms have mostly negative effects on the local abundance and species richness of several guilds of herbivores and predators. In contrast, of 25 studies that document the effects of ant-Hemipteran mutualisms on plants, the majority (68%) show that plants actually benefit from these mutualisms. In these cases, increased predation or harassment of other, more damaging herbivores by Hemipteran-tending ants resulted in decreased plant damage by other herbivores. Both the community-level and host plant effects of ant-Hemipteran mutualisms are likely conditional, however, depending on specific ecological contexts. The ecological consequences of mutualisms between Hemipterans and invasive ants relative to native ants have rarely been studied but may be particularly important because of the greater abundance, aggressiveness, and extreme omnivory of invasive ants. I argue

that ant-Hemipteran mutualisms are largely overlooked and underappreciated interspecific interactions that have strong and pervasive effects on the communities in which they are embedded.

## **1. Introduction**

Mutualism, defined as a reciprocally beneficial interaction between individuals of two species, is increasingly recognized as a common and important ecological interaction (Boucher et al. 1982, Bronstein 1994a, Stachowicz 2001). Perhaps one of the most familiar examples is the food-for-protection mutualism between ants (Hymenoptera: Formicidae) and honeydew-producing Hemipteran insects. Many ant species ‘tend’ Hemipteran insects in the suborders Sternorrhyncha (specifically aphids, whiteflies, scales, and mealybugs) and Auchenorrhyncha (specifically treehoppers and leafhoppers) for their honeydew (Hölldobler & Wilson 1990). Honeydew is a sugary excretion of carbohydrates, amino acids, and water derived from plant phloem upon which Hemipterans feed (Way 1963). Ants are attracted to honeydew as a predictable, renewable food resource and consequently protect Hemipterans from predators and parasitoids (Way 1963, Buckley 1987).

Ant-Hemipteran mutualisms are very common, if not well-appreciated, interactions in terrestrial communities from temperate to tropical latitudes (Buckley 1987, Hölldobler & Wilson 1990, Delabie 2001). Ants tend honeydew-producing Hemipterans on an extremely wide range of plants, including grasses (e.g., Moya-Ragoza & Nault 2000), forbs (e.g., Messina 1981, Renault et al. 2005), and vines, shrubs, and trees (e.g., Floate & Whitham 1994, Rico-Gray & Castro 1996, Way et al. 1999). In a study of the

ant community in the Amazonian rainforest canopy, Blüthgen et al. (2000) recorded ant-Hemipteran associations on 20 of 24 tree genera and 41 of 66 individual trees surveyed. Mutualistic interactions between ants and Hemipterans are also very common in managed (e.g., agricultural) habitats in addition to being widespread in natural habitats from grasslands to forests (Buckley 1987, Way & Khoo 1992).

The vast majority of studies of mutualisms between ants and honeydew-producing Hemipterans have focused historically on the costs and benefits to both partners. In contrast, ant-Hemipteran mutualisms have received comparatively little scrutiny from a broader, community-level perspective, particularly their potential effects on trophic interactions in food webs, arthropod community structure, and host plant fitness. Predatory species of ants are important constituents of arthropod food webs because of their active and catholic feeding habits, efficiency in recruiting to and subduing prey, and sheer abundance (Hölldobler & Wilson 1990). Mutualistic interactions between ants and honeydew-producing Hemipterans may dramatically increase the effects of ants as predators, however, because the presence of Hemipterans alters ant behavior such that ants become more aggressive and attack insects that they might otherwise ignore, including other predators as well as other herbivores (Way 1963) (Figure 1). Further, Hemipteran honeydew supports entire colonies of arboreal ants, allowing them to maintain much greater densities than could be supported by scavenging and predation alone (Davidson 1997, Blüthgen et al. 2000, Davidson et al. 2003), and attracts ground-dwelling ants onto plant foliage, increasing the likelihood that they encounter arthropods in plant-based food webs (Way & Khoo 1992, Del-Claro & Oliveira 1999, Kenne et al. 2003, Kaplan & Eubanks 2005).

Because the strength of interactions between ants and other species in the local arthropod community may be enhanced in the presence of honeydew-producing Hemipterans, Hemipteran-tending ants may also indirectly affect plant fitness. Increased ant predation of other herbivores may benefit plants if those herbivores impose a greater cost to plant growth and reproduction than do Hemipterans (Buckley 1987, Way & Khoo 1992, Delabie 2001). In contrast, by protecting Hemipterans from predators and parasitoids, and by stimulating Hemipteran feeding rate, fecundity, and dispersal, tending ants may exacerbate the negative effects of Hemipterans on plants, including stunted growth, reduced leaf area, and the introduction of plant pathogens, all of which can decrease plant fitness (Way 1963, Beattie 1985, Buckley 1987, Delabie 2001). The consequences of ant-Hemipteran mutualisms to plant fitness, thus, should represent a trade-off between the indirect benefit of increased ant suppression of other herbivores and the direct cost of herbivory by Hemipterans (Carroll & Janzen 1973, Buckley 1987, Lach 2003) (Figure 1).

Here, I review the published literature on the ecological consequences of ant-Hemipteran mutualisms. My goal is to highlight mutualistic interactions between ants and honeydew-producing Hemipterans as 'keystone interactions' that have broad yet underappreciated effects on the abundance and distribution of species in arthropod communities and on host plant fitness in both natural and managed systems. Further, I discuss evidence that the consequences of ant-Hemipteran mutualisms vary in different ecological contexts. I also discuss the extreme paucity of information regarding the ecological consequences of mutualistic interactions between Hemipterans and invasive ants. I conclude by considering several reasons why ecologists have overlooked the

ecological consequences of ant-Hemipteran mutualisms and I argue that these mutualisms are important components of terrestrial food webs that may dramatically influence community structure and trophic interactions.

## **2. Effects on community structure and species diversity**

The vast majority of previous studies of the effects of ant-Hemipteran mutualisms on other arthropods have focused primarily on ant predation or harassment of predators and parasitoids of Hemipterans. That Hemipteran-tending ants reduce the survival, abundance, and alter the spatial distribution of the natural enemies of Hemipterans on plants is extensively documented (e.g., Bradley 1973, Cushman and Whitham 1989, Tedders et al. 1990, Stechmann et al. 1996, Del-Claro & Oliveira 2000, Kaplan & Eubanks 2002, Renault et al. 2005). Fewer studies have focused on the effects of ant-Hemipteran mutualisms on other herbivores sharing the same host plant, but in most cases Hemipteran-tending ants significantly reduce their survival and abundance (e.g., Nickerson et al. 1977, Messina 1981, Skinner & Whittaker 1981, Ito & Higashi 1991, Floate & Whitham 1994, Moya-Raygoza & Nault 2000, Sipura 2002, Suzuki et al. 2004, Kaplan & Eubanks 2005).

Only three studies have investigated the effects of ant-Hemipteran mutualisms on the broader community (multiple species of predators and herbivores) in which they are embedded. These studies have shown that mutualistic interactions between ants and Hemipterans alter the abundance and distribution of specialist and generalist predators and parasitoids, and multiple species of herbivores in several feeding guilds, resulting in changes to local species diversity. In an experimental study of the effects of the ant

*Formica lugubris* on the guild structure of herbivorous insects in birch (*Betula pubescens*), Fowler and MacGarvin (1985) found that the abundance of the ant-tended aphid, *Symydobius oblongus*, increased by 8200% on branches with ants compared to branches from which ants had been excluded. In contrast, species richness of non-tended Hemipterans was reduced by 28%, species richness of leaf-chewing caterpillars was reduced by 69%, and total herbivore species richness was reduced by 28% on branches with ants. The results of a survey of herbivores on birches at ant and non-ant sites were similar and also showed that species richness of beetles (Coleoptera) was 61% lower on trees with aphid-tending ants. In contrast, species richness of leaf-mining caterpillars, a guild of herbivores that were not vulnerable to ant predation, was actually 44% greater on trees with aphid-tending ants, presumably because the ants indirectly protected the concealed caterpillars from other predators (Fowler & MacGarvin 1985).

Wimp and Whitham (2001) more directly tested for community-level effects of an ant-Hemipteran mutualism by manipulating the presence and absence of the ant-tended aphid *Chaitophorus populicola* in a cottonwood (*Populus* sp.) system. On trees from which aphids were removed, tending *Formica propinqua* abandoned the trees resulting in a 76% increase in herbivore abundance and a 76% increase in the abundance of generalist predators. Moreover, total arthropod abundance increased by 80% and total species richness increased by 57% on trees from which aphids were removed. Likewise, a mutualistic interaction between cotton aphids (*Aphis gossypii*) and red imported fire ants (*Solenopsis invicta*) strongly affected the abundance and distribution of other arthropods in cotton (*Gossypium hirsutum*) (Eubanks 2001, Eubanks et al. 2002, Kaplan and Eubanks 2002, 2005). In large-scale field manipulations of fire ant densities, the



mutualism between *S. invicta* and *A. gossypii* negatively affected the abundance of 27-33% of herbivore taxa and 40-47% of predator taxa (Kaplan & Eubanks 2005). Similar to Wimp and Whitham (2001), the percentage of taxa that was negatively affected by the ant-aphid mutualism did not differ between herbivores and predators (although there was a trend towards a stronger effect on predators).

These three studies provide compelling evidence that ant-Hemipteran mutualisms can act as 'keystone interactions' that dramatically alter the structure of arthropod communities on plants. In the presence of honeydew-producing Hemipterans, tending ants disproportionately affected complex food webs by directly or indirectly altering the abundance of many interacting species. Predation or harassment by tending ants typically reduced the abundance of other herbivores, generalist predators, and specialist predators and parasitoids. Further, ant-Hemipteran mutualisms typically reduced local species diversity. In some cases, however, ant-Hemipteran mutualisms may actually benefit specific herbivore species or herbivore guilds that are not as vulnerable to ant predation (e.g., leaf-mining caterpillars) by reducing the abundance of their natural enemies (Fowler & MacGarvin 1985; see also Fritz 1983). Ant-Hemipteran mutualisms may even increase arthropod diversity within a plant community if the mutualisms occur only on specific plant species (or genotypes) and certain arthropod species (e.g., specialist natural enemies of Hemipterans) are found only on those plants hosting the mutualism (Wimp & Whitham 2001).

### 3. Effects on host plant damage and fitness

Given both the ecological and economic significance of Hemipterans as plant pests, and the widespread distribution and abundance of both Hemipterans and ants in terrestrial communities, surprisingly few studies have quantified the consequences of ant-Hemipteran mutualisms to plant fitness. Table 1 lists 25 studies that have explicitly tested for an effect of the interaction between ants and honeydew-producing Hemipterans on plants by either 1) comparing plants on which Hemipterans and/or tending ants are naturally present or absent, or 2) by comparing plants on which the densities of Hemipterans and/or tending ants has been manipulated.

I note that of the 18 studies that were experimental rather than correlative, 14 employed ant-exclusion manipulations (Table 1). Although perhaps easier than manipulating Hemipteran densities, a major limitation of ant-exclusion experiments is that they do not specifically test the net effect of the interaction *per se* between ants and Hemipterans on plants (i.e., whether ants have stronger effects as predators in the presence of Hemipterans). In many systems, ants forage on plants even in the absence of Hemipterans. Experiments in which Hemipteran density is manipulated (Hemipterans either excluded from plants or added to plants where there were previously none) ( $n = 2$  studies; Table 1) provide a more realistic test of this hypothesis because ant behavior changes in response to the presence of honeydew-producing Hemipterans rather than vice versa (see Floate & Whitham 1994). Better still are experiments that manipulate both ants and Hemipterans in a crossed design ( $n = 2$  studies; Table 1) because they allow comparison of the effects of ants alone and the effects of Hemipterans alone with the effects of the interaction between ants and Hemipterans specifically.

**(a) net negative effects**

Despite abundant evidence that tending ants can cause outbreaks of honeydew-producing Hemipterans (Beattie 1985, Buckley 1987, Holway et al. 2002) and that Hemipteran insects can impose tremendous costs on plant fitness via phloem consumption and transmission of plant pathogens (Carter 1962, Buckley 1987, Delabie 2001), negative effects of mutualistic interactions between ants and Hemipterans on plant fitness have rarely been demonstrated ( $n = 7$  studies; Table 1). Whether negative effects of ant-Hemipteran mutualisms are truly uncommon in nature or whether they have simply been assumed and, thus, not quantified is unclear. In the only published study of a negative effect in a managed system, Banks & Macaulay (1967) reported that the aphid *Aphis fabae* was 30-50% more numerous on faba bean (*Vicia faba*) when tended by the ant *Lasius niger*, resulting in a 26% reduction in pods per plant, a 53% reduction in seeds per plant, and a 38% reduction in seed mass relative to plants with *A. fabae* that were not tended by ants. Similarly, a facultative mutualism between the ant *Camponotus planatus* and an unidentified aphid species hosted by the woody vine *Paullinia fuscecens* reduced the proportion of inflorescences with seeds by 7-23% and reduced seed number per inflorescence by 46-60% compared to plants with untended aphids (Rico-Gray & Castro 1996). In a study of the effects of an ant-aphid mutualism on the fitness of the forb *Bidens pilosa*, Renault et al. (2005) reported that the aphid *Aphis coreopsidis* was 34% more abundant in the presence of tending *Camponotus* ants than in their absence. Consequently, plants hosting ant-tended aphids produced significantly fewer viable seeds than did plants from which ants were excluded. Finally, in the only study of which I am

aware that documents an effect of an ant-Hemipteran mutualism on the transmission of a plant pathogen, the presence of *S. invicta* caused a 240% increase in aphid abundance on tomato (*Lycopersicon esculentum*), resulting in a 65% increase in the level of infection of plants by the aphid-vectored *Cucumber Mosaic Virus* (Cooper 2005).

**(b) no net effect**

Two published studies failed to find any net effect, negative or positive, of ant-Hemipteran mutualisms on plants (Table 1). The ant *Formica subsericea* protected the treehopper *Vanduzca arquata* from its natural enemies on black locust (*Robinia pseudoacacia*), but increased treehopper densities in the presence of ants did not harm plants, apparently because the plants were tolerant of treehopper herbivory (Fritz 1983). The tending ants also attacked the adults and eggs of a leaf-mining beetle but indirectly protected beetle larvae by excluding a damsel bug predator, resulting in no net gain or loss in plant growth. Similarly, Moya-Raygoza & Nault (2000) found that although ants protected the leafhopper *Dalbulus quinquenotatus* from predation by spiders, populations of the leafhopper on its gamagrass host plants (*Tripsacum pilosum* and *T. dactyloides*) never reached damaging levels in the field, apparently as a result of incidental predation of leafhoppers by the tending ants. Further, even though tending ants reduced the abundance of two nonmyrmecophilous leafhopper species, the ant-leafhopper mutualism provided no net benefit to the plant.

**(c) net positive effects**

Studies documenting net positive effects of ant-Hemipteran mutualisms on plants are more numerous ( $n = 17$ ; Table 1). In these studies, ant-Hemipteran mutualisms benefited plants as a consequence of increased ant predation or harassment of other, more damaging insects, resulting in decreased plant damage by those insects and/or increased plant fitness. Treehopper-tending ants on *Solidago altissima*, for example, deterred feeding by the larvae of two species of leaf beetles (Messina 1981). Increased plant protection by ants in the presence of treehoppers resulted in increased plant growth and seed production relative to neighboring plants on which treehoppers were naturally absent. Similarly, oaks (*Quercus dentata*) that hosted the aphid *Tuberculatus quercicola* had a significantly lower percentage of acorns infested by acorn-boring Lepidopteran larvae in areas where the aphid-tending ant *Formica yessensis* was abundant relative to areas where the ants were rare (Ito & Higashi 1991).

Skinner and Whittaker (1981) showed that leaf damage by Lepidopteran larvae was significantly reduced on sycamore trees (*Acer pseudoplatanus*) on which the ant *Formica rufa* tended the aphid *Periphyllus testudinaceus* compared to trees from which ants were experimentally excluded. In later ant-exclusion experiments in the same system, Whittaker and Warrington (1985) showed that the radial growth of sycamore trees was 2-3 times greater in the presence of the ant-aphid mutualism, presumably as a consequence of herbivore suppression by the aphid-tending ants. Floate and Whitham (1994) manipulated aphid densities rather than ant densities to test whether a facultative mutualism between the aphid *Chaitophorus populicola* and a *Formica* ant species indirectly protected cottonwood (*Populus fremontii* and hybrids) foliage from beetle

herbivory. The addition of aphids to trees dramatically increased ant abundance, which translated into a 95% reduction in the abundance of beetle larvae and a 50% reduction in leaf damage.

Six of the studies that documented a net positive effect were conducted in managed systems (Table 1), demonstrating that ant-Hemipteran mutualisms may be exploited for biological control of more damaging pests of agriculturally important plants. In fact, arboreal ants attracted to Hemipteran honeydew have long been utilized to control non-Hemipteran herbivore pests in several tree crops (Way and Khoo 1992). Growers manipulate colonies of *Dolichoderus* ants in cocoa (*Theobroma cacao*) and sapodilla (*Manilkara zapota*) plantations in Southeast Asia, for example, because the mealybug-tending ants protect cocoa pods and sapodilla fruits from damage by Mirids (Heteroptera) (Khoo and Ho 1992) and Pyralid larvae (Lepidoptera) (Van Mele and Cuc 2001) without any direct yield loss caused by the mealybugs. Far less is known, however, about the net effects of ant-Hemipteran mutualisms on herbaceous crop plants in more ephemeral systems such as row and vegetable crops. Protection by tending ants can lead to Hemipteran outbreaks in agricultural monocultures of herbaceous plants resulting in significantly reduced yield caused by loss of plant sap (e.g., Banks & Macaulay 1967) and transmission of plant pathogens (e.g., Cooper 2005). If particular Hemipteran species do not vector plant pathogens to certain herbaceous crop plants and if those plants show high tolerance to Hemipteran herbivory, ant-Hemipteran mutualisms may still benefit plant yield. In cotton, for example, I have shown experimentally that *A. gossypii* attract ground-foraging *S. invicta* onto plants, resulting in increased ant predation of an important cotton herbivore, the beet armyworm caterpillar (*Spodoptera*

*exigua*). Cotton plants hosting the mutualism suffered less caterpillar damage to leaves and bolls (cotton fruit) and, consequently, produced 16% more bolls and 10% more seedcotton relative to plants from which *A. gossypii* were excluded (Chapter 3). I encourage additional studies of the effects of ant-Hemipteran mutualisms in other annual cropping systems to further evaluate their utility for increased biological control of non-Hemipteran herbivores.

#### **4. Conditionality in the effects of ant-Hemipteran mutualisms on plants**

Conditionality in the outcome of mutualistic interactions between ants and Hemipterans and between ants and plants has been widely noted (Bronstein 1994b). Because the net effects of ant-Hemipteran mutualisms on plants represent a trade-off between the direct cost of feeding by Hemipterans and the indirect benefit of ant suppression of other herbivores, any biotic or abiotic factor that influences the magnitude of the cost or benefit may alter the consequence of the interaction to the plant (Lach 2003). Such conditionality has been often suggested but rarely tested. Factors that could mediate the effects of ant-Hemipteran mutualisms on plant fitness include: 1) the proximity of ant colonies to host plants (because distance may affect the numerical response of ants to honeydew-producing Hemipterans; Karhu 1998, Wimp & Whitham 2001), 2) the proximity and abundance of other plants hosting honeydew-producing Hemipterans that may distract tending ants (Cushman & Whitham 1991), 3) the abundance and efficacy of other arthropod predators, and the effects of tending ants on those predators (Fritz 1983), 4) the abundance and relative cost to plant fitness of insects in other herbivore guilds (Messina 1981, Fritz 1983, Chapter 3), 5) indirect interactions

between Hemipteran and non-Hemipteran herbivores mediated by host plants (Stout et al. 1998, Rodriguez-Saona et al. 2005), 6) host plant tolerance of and resistance to other herbivores (Sipura 2002; see also Vrieling et al. 1991), 7) variation in plant vulnerability to Hemipteran and non-Hemipteran herbivory associated with plant phenology (Rosenheim et al. 1997), 8) the availability and quality of plant extrafloral nectar (Buckley 1983), 9) the effects of plant genotype on plant quality (Floate & Whitham 1994), and 10) the effects of other biotic (e.g., pathogen infection) and abiotic factors (e.g., nitrogen availability, shade) on host plant quality (Cushman 1991).

As suggested by Messina (1981) and Fritz (1983), the consequences of an ant-Hemipteran mutualism to a particular host plant may vary simply with the intensity of herbivory by other herbivores on the plant (Figure 2). I tested this hypothesis in an experiment in which I manipulated the presence and absence of the aphid *A. gossypii*, and densities of *S. exigua* caterpillars (low and high) on cotton plants in a field that was naturally infested by *S. invicta*. As described earlier, *S. invicta* workers were more abundant on plants with *A. gossypii*, resulting in increased predation of caterpillars. The fitness benefit of reduced caterpillar damage to plants varied with caterpillar density, however, such that plant protection by aphid-tending *S. invicta* resulted in a 32% increase in bolls (relative to plants without aphids) at high caterpillar density versus just a 3% increase in bolls at low caterpillar density (Chapter 3). Thus, the indirect benefit of ant suppression of caterpillars substantially outweighed the direct cost of *A. gossypii* only when caterpillars were abundant.

Only one other study has investigated conditionality in the ecological consequences of ant-Hemipteran mutualisms to plants. Sipura (2002) manipulated the



presence and absence of *Formica aquilonia* on shoots of two willow species with and without aggregations of the aphid *Pterocomma salicis* to test the effect of the ant-aphid mutualism on willow herbivory and growth. The two willow species differed in salicylate concentration in the foliage, a trait that confers resistance to leaf-chewing herbivores (Sipura 1999). Leaf damage was significantly reduced and shoot length and radial growth were significantly enhanced in the presence of the mutualism only in the willow species with a low concentration of salicylate (*Salix phylicifolia*) and, thus, low resistance to herbivores. In contrast, the ant-aphid mutualism negatively affected growth in the willow species with high resistance (*S. myrsinifolia*), presumably because the cost of aphid herbivory outweighed the very limited benefit of ant suppression of leaf-chewing insects.

##### **5. Ecological consequences of ant-Hemipteran mutualisms involving native ants versus invasive ants**

The degree of protection provided by tending ants to Hemipterans varies among native ant species, primarily because of differences among species in aggressiveness and territoriality (Buckley & Gullan 1991, Kaneko 2003, Novgorodova 2005). Given that more aggressive ant species with more strongly defended territories provide greater protection to Hemipterans, these ant species may be expected to have stronger direct and indirect effects on community structure and host plant fitness. I am unaware of any studies that have compared the effects on arthropod community structure of ant-Hemipteran mutualisms involving different native ant species, but a few studies suggest that more aggressive ant species have stronger indirect effects on plants. Sipura (2002),

for example, compared the effects of mutualisms between aphids and two native ant species on the herbivory and growth of two willow species and found that only the more aggressive ant species had measurable effects (both positive and negative, depending on the willow species [see section 4]) on plant growth.

Invasive ants, that subset of unintentionally introduced ant species that establish populations in natural areas undisturbed by human activity, are particularly notorious for their extreme abundance and aggressiveness, two traits at least partly responsible for their pervasive negative ecological effects in invaded habitats (Holway et al. 2002). Invasive ants are also very attracted to Hemipteran honeydew and rapidly recruit in large numbers to Hemipteran aggregations from which they may exclude native tending ants (Holway et al. 2002, Lach 2003, Ness & Bronstein 2004). Indeed, exploitation of Hemipteran honeydew and plant extrafloral nectar is hypothesized to contribute to the ecological dominance of invasive ants (Holway et al. 2002). Although invasive ants can cause local declines in arthropod abundance and diversity (Holway et al. 2002), no studies have investigated the direct and indirect effects of ant-Hemipteran mutualisms involving invasive ant species on interspecific interactions within arthropod food webs.

Few data exist as well to evaluate the consequences to plants of ant-Hemipteran mutualisms involving invasive ants (Table 1). Invasive ants are exceptional tenders and frequently cause local Hemipteran outbreaks (Holway et al. 2002, Ness & Bronstein 2004). Because of their abundance, aggression, and territoriality, however, invasive ants may provide comparatively greater protection to plants from other herbivores than do native ants (Holway et al. 2002, Lach 2003; but see Rapp & Salum 1995). Way et al. (1999), for example, observed that defoliation of the pine *Pinus pinaster* by the larvae of

the pine processionary moth (*Thaumetopoea pityocampa*) was slight to negligible in trees patrolled by the invasive Argentine ant (*Linepithema humile*), but was moderate to severe in trees patrolled by several native ant species. The authors suggested that aggregations of honeydew-producing aphids facilitated protection of pines by *L. humile*, but they did not specifically test whether the ants had the same effect in the absence of aphids.

Facultative mutualisms between the invasive ant *S. invicta* and aphids in tomato can harm plants by increasing the severity of infection by a plant virus (Cooper 2005), but can benefit plant reproduction in cotton by increasing ant suppression of more damaging herbivores (Chapter 3). Whether the effects of *S. invicta* in these systems differ from those by native aphid-tending ants is unknown.

## **6. Why so little information on such a ubiquitous and potentially important interaction?**

Despite their ubiquity in terrestrial communities, mutualistic interactions between ants and honeydew-producing Hemipterans have received relatively little attention in terms of their effects on food web dynamics and tritrophic interactions involving their host plants. This dearth of information regarding the ecological consequences of ant-Hemipteran mutualisms potentially represents a major void in our understanding of the dynamics of complex trophic interactions in arthropod communities. My review of the literature shows that ant-Hemipteran mutualisms can broadly affect the local abundance and distribution of specialist and generalist predators and parasitoids, as well as herbivores in several feeding guilds. In addition, though some evidence shows that ant-Hemipteran mutualisms result in a net decrease in the fitness of their host plants, a

majority of studies document an indirect positive effect in which Hemipteran-tending ants protect host plants from other, more damaging herbivores. Because the broad ecological effects of Hemipteran-tending ants as predators are precipitated by their interaction with Hemipterans (i.e., ant effects would not be as strong in the absence of Hemipterans), ant-Hemipteran mutualisms may, thus, represent ‘keystone interactions’ in many communities.

Why, then, have the ecological consequences of ant-Hemipteran mutualisms been so little studied? I suggest that in many systems the effects of mutualistic interactions between ants and honeydew-producing Hemipterans, either positive or negative, are often assumed without ever being quantified. A mutualism between the invasive ant *Pheidole megacephala* and the gray pineapple mealybug (*Dysmicoccus neobrevipes*), for instance, is thought to severely damage pineapple trees in Hawaii because the mealybug vectors a devastating phytopathogen (Beardsley et al. 1982). Whether the interaction *per se* actually reduces plant fitness (i.e., whether ant-tending exacerbates the negative effects of the mealybugs), however, has not been tested. Similarly, Way et al. (1999) surmise that the protection of pine trees by *L. humile* is partly a consequence of ant attendance of aphids, but they never specifically test this hypothesis. In observational studies such as these (of which there are many), the assumed consequences of the ant-Hemipteran mutualisms may very well be real, but the lack of empirical data precludes any definitive conclusions or generalizations.

I also argue that the ecological effects of ants and their interactions with other arthropods are simply ignored. Ants may be noted as abundant predators, particularly in annual agricultural systems, but the consequences of their interactions with other

arthropods are often not quantified. Several ant species, for example, tend the recently introduced aphid *Aphis glycines* in soybean (*Glycine max*) in North America (Ragsdale et al. 2004), but no effort has been made to determine the consequences of these mutualisms to the abundance and distribution of other natural enemies and herbivores, or to soybean yield. Ecological consequences of ant-Hemipteran mutualisms are also often dismissed because they are assumed to be too local or too ephemeral to affect community structure or plant fitness. Wimp and Whitham (2001) showed, however, that the local effects of an ant-aphid mutualism on the abundance and distribution of arthropods on individual trees also influenced arthropod abundance and diversity throughout the surrounding plant community. Further, populations of some honeydew-producing Hemipteran taxa (e.g., aphids) may indeed be ephemeral, but even short-term ant-Hemipteran associations can have dramatic community-level consequences as demonstrated by Floate and Whitham (1994), and Kaplan and Eubanks (2005) (see above). Ephemeral ant-Hemipteran associations may even induce long-term changes in host plant quality that affect other herbivores and their interactions with natural enemies later in the season (cf Van Zandt & Agrawal 2004).

## **7. Conclusions**

Whether because they are assumed, ignored, dismissed, or simply overlooked, the ecological consequences of ant-Hemipteran mutualisms and their importance in arthropod communities remain poorly understood. Mutualisms between ants and honeydew-producing Hemipterans are abundant and widespread in arthropod food webs and have potentially broad ecological effects because the presence of honeydew-

producing Hemipterans dramatically alters the abundance and predatory behavior of ants on plants. Future studies should focus on the ecological factors that influence the consequences of ant-Hemipteran mutualisms in order to provide greater insight into the role of positive species interactions in food web dynamics and greater predictability of the direct and indirect effects of herbivores and natural enemies on host plants. Such predictability could substantially benefit biological control in agricultural systems with ants and Hemipterans. Finally, I encourage a more concerted effort to study the consequences to arthropod communities and host plants of ant-Hemipteran mutualisms involving invasive ants, and to make comparisons with native ants. Invasive ants have the potential to wreak ecological havoc in invaded habitats; thus, understanding the consequences of their interactions with Hemipterans could help predict and even manage their effects.

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Table 1. Summary of 25 studies that explicitly test for effects on plants of mutualistic interactions between ants and honeydew-producing Hemipterans. Negative effects indicate increased plant damage and/or decreased plant growth and reproduction. Positive effects indicate decreased plant damage and/or increased plant growth and reproduction.

Ant Species (status <sup>a</sup> )	Hemipteran Species	System	Host plant	Method <sup>b</sup>	Plant trait measured	Citation
<b>Negative effect</b>						
<i>Lasius niger</i> (N)	<i>Aphis fabae</i>	Managed: field crop	Faba bean <i>Vicia faba</i>	Exp(1)	# viable seeds	1
<i>Iridomyrmex</i> sp. (N)	<i>Sextius virescens</i>	Natural: woodland	Acacia <i>Acacia decurrens</i>	Exp(3)	Plant growth, # seeds	2
<i>Crematogaster brevispinosa</i> (N)	<i>Planococcus citri</i>	Natural: coastal dune	Orchid <i>Schomburgkia tibicinis</i>	Exp(1)	Plant damage, # flowers, # fruits	3
<i>Camponotus planatus</i> (N)	Unidentified aphid sp.	Natural: coastal dune	Liana <i>Paullinia fusceceus</i>	Exp(1)	# flowers with seeds, # seeds per flower	4
<i>Formica aquilonia</i> (N)	<i>Pterocomma salicis</i>	Natural: woodland	Willow	Exp(3)	Leaf damage, plant growth	5
<i>Myrmica rubra</i> (N)			<i>Salix myrsinifolia</i>			
<i>Camponotus</i> sp. (N)	<i>Aphis coreopsidis</i>	Natural: grassland	Aster <i>Bidens pilosa</i>	Exp(1)	# seeds	6

<i>Solenopsis invicta</i> (I)	Unidentified aphid spp.	Managed: vegetable crop	Tomato	Exp(1)	Level of infection by plant virus	7
<b>No effect</b>						
<i>Formica subsericea</i> (N)	<i>Vanduzeca arquata</i>	Natural: woodland	Black locust	Corr	Leaf damage,	8
<i>Brachymyrmex obscurior</i> (N)	<i>Dalbulus quinquevittatus</i>	Natural: grassland	<i>Robinia pseudoacacia</i> Gamagrass Two <i>Tripsacum</i> species	Exp(1)	plant growth # leaves, plant growth	9
<b>Positive effect</b>						
<i>Crematogaster africana</i> (N)	<i>Catenococcus loranthi</i>	Managed: cocoa plantation	Mistletoe	Exp(1)	Leaf damage,	10
<i>Azteca</i> sp. (N)	<i>Stictococcus sjostedi</i> <i>Coccus viridis</i>	Managed: orchard	<i>Tapinanthus bangwensis</i> Orange <i>Citrus reticulata</i>	Exp(1)	plant growth Leaf damage	11
<i>Formica</i> sp. (N)	<i>Publilia concava</i>	Natural: old field	Goldenrod	Corr	Plant growth, # seeds	12
<i>Formica rufa</i> (N)	<i>Periphyllus testudinaceus</i>	Natural: woodland	<i>Solidago altissima</i> Sycamore <i>Acer pseudoplatanus</i>	Exp(1)	Leaf damage	13
<i>Formica rufa</i> (N)	<i>Periphyllus testudinaceus</i>	Natural: woodland	Sycamore <i>Acer pseudoplatanus</i>	Exp(1)	Plant growth, # seeds	14



<i>Formica yessensis</i> (N)	<i>Tuberculatus</i>	Natural: chaparral	Oak	Corr	# acorns,	15
	<i>quercicola</i>		<i>Quercus dentata</i>		acorn damage	
<i>Dolichoderus thoracicus</i> (N)	<i>Cataenococcus</i>	Managed: plantation	Cocoa	Exp(1)	Fruit damage	16
	<i>hispidus</i>		<i>Theobroma cacao</i>			
<i>Anoplolepis custodiens</i> (N)	<i>Cerataphis variabilis</i>	Managed: plantation	Coconut	Corr	Palm lesions, # nuts,	17
			<i>Cocos nucifera</i>		nut damage	
<i>Formica</i> sp. (N)	<i>Chaitophorus</i>	Natural: woodland	Cottonwood	Exp(2)	Leaf damage	18
	<i>populicola</i>		<i>Populus fremontii</i>			
<i>Camponotus brutus</i> (N)	<i>Hilda undata</i>	Natural: woodland	Fig	Corr	Fruit damage	19
			<i>Ficus vallisclaudae</i>			
<i>Formica aquilonia</i> (N)	<i>Symydobius oblongus</i>	Natural: woodland	Birch	Exp(1)	Leaf damage	20
			<i>Betula pubescens</i>			
<i>Dolichoderus thoracicus</i> (N)	<i>Planococcus lilacinus</i>	Managed: plantation	Sapodilla	Exp(1)	Fruit damage	21
			<i>Manilkara zapota</i>			
<i>Formica aquilonia</i> (N)	<i>Pterocomma salicis</i>	Natural: woodland	Willow	Exp(3)	Leaf damage,	5
<i>Myrmica rubra</i> (N)			<i>Salix phylicifolia</i>		plant growth	
<i>Lasius japonicus</i> (N)	Complex of three	Natural: grassland	Vetch	Corr	# seeds	22
<i>Tetramorium tsushimae</i> (N)	aphid species <sup>d</sup>		<i>Vicia angustifolia</i>			

Complex of five species <sup>c</sup> (N)	<i>Guayaquila xiphias</i>	Natural: cerrado	Liana	Exp(1)	Damage to leaves	23
<i>Formica obscuripes</i> (N)	Unidentified <i>Aphis</i>	Natural: coastal dune	Willow	Corr	Leaf damage	24
	sp.		<i>Salix hookeriana</i>			
<i>Solenopsis invicta</i> (I)	<i>Aphis gossypii</i>	Managed: field crop	Cotton	Exp(2)	# cotton squares, bolls,	25
			<i>Gossypium hirsutum</i>		seeds; seedcotton mass	

<sup>a</sup> Status of ant as a native (N) or invasive (I) species at study site.

<sup>b</sup> Studies categorized as either correlative, ‘Corr’, or experimental, ‘Exp’. Experimental studies are further classified by the manipulation employed: (1) ant exclusion alone, (2) Hemipteran exclusion alone, (3) both ant and Hemipteran exclusion in a crossed design. The significance of this difference in methodology is discussed in the text.

<sup>c</sup> *Camponotus crassus*, *C. renggeri*, *C. rufipes*, *Cephalotes pusillus*, and *Ectatomma edentatum*

<sup>d</sup> *Aphis craccivora*, *Acyrtosiphon pisum*, and *Megoura crassicauda*.

Citations: 1-Banks & Macaulay 1976; 2-Buckley 1983; 3-Rico-Gray & Thien 1989; 4-Rico-Gray & Castro 1996; 5-Sipura 2002; 6-Renault et al. 2005; 7-Cooper 2005; 8-Fritz 1983; 9-Moya-Raygoza & Nault 2000; 10-Room 1972; 11-Jutsum et al. 1981; 12-Messina 1981; 13-Skinner & Whittaker 1981; 14-Whittaker & Warrington 1985; 15-Ito & Higashi 1991; 16-Khoo &

Ho 1992; 17-Löhr 1992; 18-Floate & Whitham 1994; 19-Dejean et al. 1997; 20-Karhu 1998; 21-Van Mele & Cuc 2001; 22-Suzuki et al. 2004; 23-Oliveira & Del-Claro 2005; 24-Crutsinger & Sanders 2005; 25-Chapter 3.

Figure 1. Potential interactions involving a mutualism between ants and honeydew-producing Hemipterans. Arrows indicate the direction of effects, positive (+) and negative (-). Note that the net effect of the mutualism on the host plant may be positive or negative depending on the ratio between the direct cost to the host plant of Hemipteran herbivory and the indirect benefit to the host plant of ant suppression of herbivory by other herbivores.

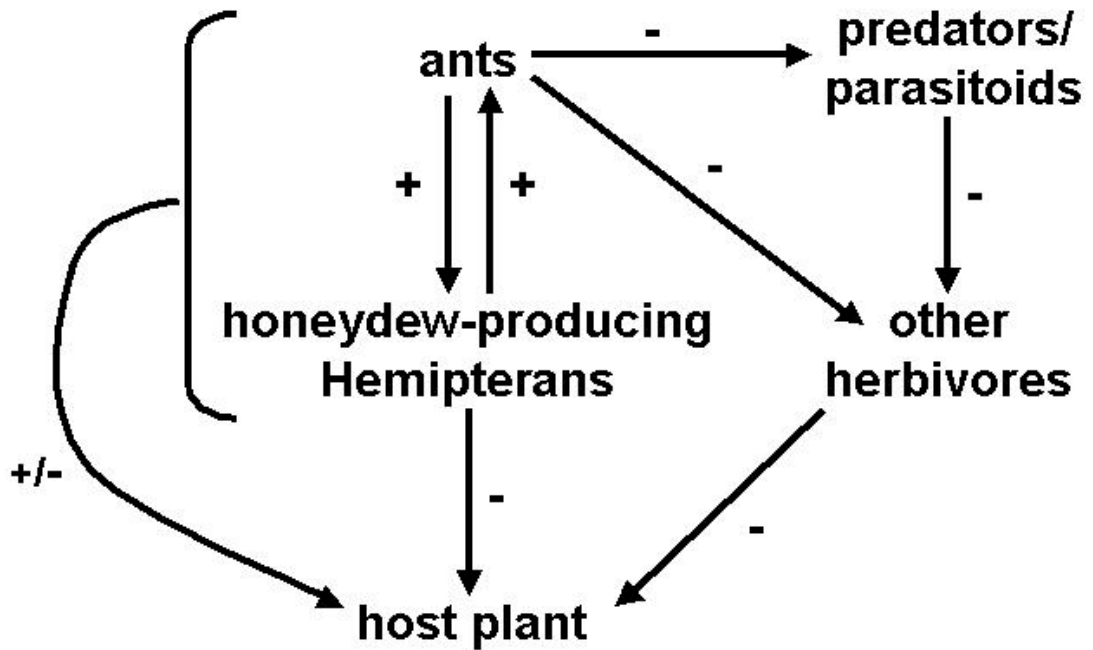
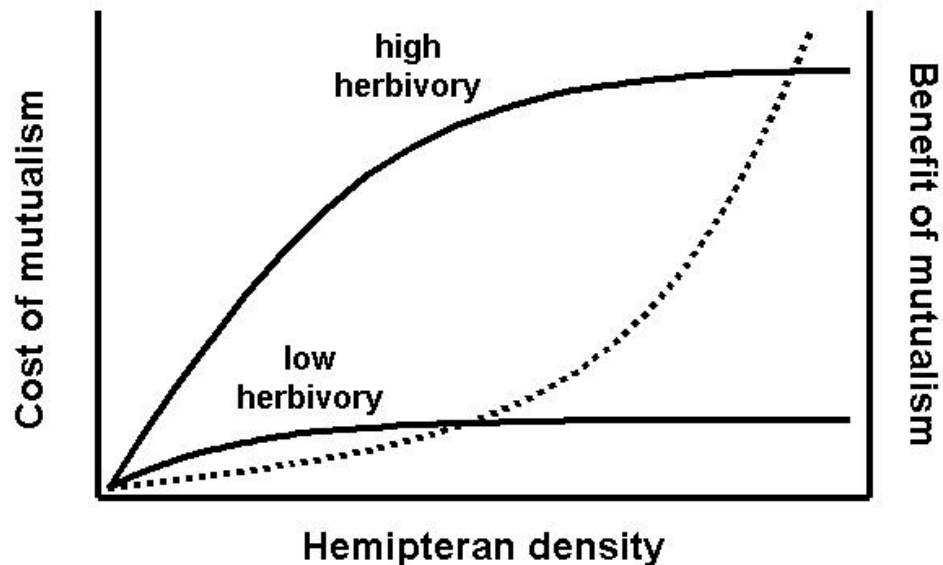


Figure 2. Conditionality in the consequences to a host plant of an ant-Hemipteran mutualism based on the intensity of non-Hemipteran herbivory. The cost to the plant of the mutualism (in terms of loss of fitness caused by plant damage by Hemipterans) is shown as the dotted curve and increases with increasing Hemipteran density. The benefit to the plant of the mutualism (in terms of increased plant fitness caused by ant suppression of plant damage by other herbivores) is shown as the two solid curves. At a high level of non-Hemipteran herbivory, the benefit of the mutualism to plant fitness is predicted to outweigh the cost at all but extremely high levels of Hemipteran density. At a low level of non-Hemipteran herbivory, however, the mutualism either provides only a slight fitness benefit (at lower Hemipteran densities) or results in a net loss of fitness (at higher Hemipteran densities).



**CHAPTER THREE:**  
**A FACULTATIVE MUTUALISM BETWEEN COTTON APHIDS**  
**AND AN INVASIVE ANT INDIRECTLY BENEFITS**  
**PLANT REPRODUCTION**

*Abstract.* Ants and aphids routinely engage in mutualistic interactions in which aphids barter a food resource (honeydew) for protection from predators by honeydew-collecting ants. The consequences of ant-aphid mutualisms to plants, particularly those involving invasive ant species, are poorly studied but could be negative or positive depending on the cost of herbivory by ant-tended aphids relative to the benefit of increased ant suppression of other herbivores. I conducted field and greenhouse experiments over two years in which I 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 beet armyworm caterpillars (*Spodoptera exigua*). I also manipulated caterpillar density to test whether the benefit of the mutualism varied with the level of caterpillar damage to plants. Greater numbers of fire ants foraged on plants with cotton aphids than on plants without cotton aphids, resulting in a significant reduction in caterpillar survival and caterpillar herbivory of leaves, squares, and bolls on plants with aphids. Consequently, cotton aphids indirectly

increased cotton reproduction. In one field experiment, plants with cotton aphids produced on average 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 on plants with aphids at low caterpillar density. My results highlight the potential benefit to an economically important plant of a mutualism between an aphid pest and an invasive ant species. Further, my results provide the first experimental evidence that the consequences to plants of ant-aphid mutualisms are context-dependent and can vary at different densities of non-aphid herbivores.

## INTRODUCTION

Ants commonly engage in mutualistic interactions with honeydew-producing Hemipteran insects, including aphids, scales, mealybugs, and Membracid treehoppers (Way 1963, Hölldobler and Wilson 1990). 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 and Wilson 1990). From the perspective of the plants hosting these interactions, ant-Hemipteran mutualisms can be either harmful or beneficial. Increased Hemipteran abundance in the presence of tending ants can result in decreased plant growth and reproduction because Hemipterans feed from plant phloem and often vector bacterial and viral pathogens (Buckley 1987, Delabie 2001). Hemipteran-tending ants, however, exhibit increased aggressiveness towards other arthropods on the plant (Way

1963), and thereby potentially protect plants from other, more damaging herbivores (e.g., Nickerson 1977, Cushman et al. 1998). The net consequence of an ant-Hemipteran mutualism to its host plant, therefore, could be negative or positive depending on the ratio of the direct cost of feeding by Hemipterans to the indirect benefit of increased ant suppression of other herbivores (Carroll and Janzen 1973, Fritz 1983, Buckley 1987, Lach 2003).

Surprisingly few studies have investigated the consequences of ant-Hemipteran mutualisms to plants considering the ubiquity of these interactions in terrestrial communities. Ant-Hemipteran mutualisms occur on most types of plants (including grasses, forbs, vines, shrubs, and trees) in most plant families from temperate to tropical latitudes (Buckley 1987, Blüthgen et al. 2000, Delabie 2001). Ant-Hemipteran mutualisms also commonly occur in managed (e.g., agricultural) as well as in natural systems (Buckley 1987, Way and Khoo 1992). In a review of the relatively few studies that explicitly investigated the effects on plants of mutualistic interactions between ants and Hemipterans, Styrsky and Eubanks (in review) found that the majority of studies documented positive effects in terms of decreased plant damage by non-Hemipteran herbivores and/or increased plant growth and reproduction. Messina (1981), for instance, reported that Membracid-tending *Formica* ants prevented defoliation of goldenrod plants by two Chrysomelid beetles, resulting in greater plant growth and seed production relative to neighboring plants that did not host Membracid aggregations. Similarly, mealybug-tending *Dolichoderus* ants protected cocoa pods and sapodilla fruits from damage by Mirids (Heteroptera) (Khoo and Ho 1992) and Pyralid larvae (Lepidoptera) (Van Mele and Cuc 2001), respectively, without any direct yield loss caused by the



mealybugs. Other studies, however, show that increased Hemipteran abundance in the presence of tending ants decreases plant fitness. Renault et al. (2005), for example, reported that the aster *Bidens pilosa* produced significantly fewer viable seeds on plants with ant-tended aphids compared to plants from which ants had been excluded.

All previous studies that specifically investigated the effects of ant-Hemipteran mutualisms on plant fitness have focused on mutualisms involving only native species of ants (Styrsky and Eubanks, in review). Invasive ants, that subset of unintentionally introduced ant species that establish populations in natural areas undisturbed by human activity, also tend honeydew-producing Hemipterans and may disrupt mutualisms involving native ants (Holway et al. 2002, Lach 2003, Ness and Bronstein 2004). Because of their comparatively greater abundance and aggression 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, Ness and Bronstein 2004), but they may also more effectively suppress other herbivores (Way et al. 1999, Lach 2003). Studies investigating 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.

Predictability of the consequences to plants of ant-Hemipteran mutualisms involving either both native and invasive ants may also be improved by increased understanding of how the consequences vary in different ecological contexts (Lach 2003). Only one study has specifically investigated conditionality in the effects of ant-Hemipteran mutualisms on plants. Sipura (2002) showed that the net consequence to

plants of a mutualism between the ant *Formica aquilonia* and the aphid *Pterocomma salicis* differed between two species of willow based on differences in foliar concentrations of phenolic glycosides, a trait that confers resistance against leaf-chewing herbivores. As a consequence of increased ant suppression of other herbivores, the ant-aphid mutualism resulted in decreased leaf damage and increased growth in the willow species with low resistance. In contrast, the ant-aphid mutualism negatively affected growth in the willow species with high resistance, presumably because the cost of aphid herbivory outweighed the very limited benefit of ant suppression of leaf-chewing insects.

Our understanding of the consequences of ant-Hemipteran mutualisms to plants is further limited by the fact that almost all previous studies have focused on mutualisms hosted by woody species of plants, primarily trees and shrubs in natural woodlands and, secondarily, trees of agricultural importance in orchards and plantations (Eubanks and Styrsky, in press). Ant-Hemipteran mutualisms may be more beneficial to host plants in woodlands and orchards because the relative stability of these habitats may promote long-term establishment of both ant and Hemipteran colonies (Way and Khoo 1992). Further, annual herbaceous plants may be particularly vulnerable to fitness costs associated with Hemipteran herbivory because of the allocation of resources to a single reproductive bout. Whether ant-Hemipteran mutualisms indirectly harm or benefit more ephemeral, herbaceous plants or plants in more ephemeral agricultural systems such as grain, fiber, or vegetable crops is practically unknown.

I present here the results of field experiments designed to investigate the consequences of a facultative mutualism between an invasive ant species, the red imported fire ant (*Solenopsis invicta*), and cotton aphids (*Aphis gossypii*) to an annual

host plant of agricultural importance, cotton (*Gossypium hirsutum*). I asked two questions: (1) Can the fire ant-cotton aphid mutualism indirectly benefit cotton reproduction as a consequence of increased fire ant predation of a leaf-chewing herbivore, the beet armyworm caterpillar (*Spodoptera exigua*)?, and (2) Does the outcome of the fire ant - cotton aphid mutualism to cotton reproduction vary with caterpillar density? I 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. Because the indirect benefit of increased 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, I further predicted that boll production would be greater on plants with aphids than on plants without aphids at high caterpillar density but not at low caterpillar density.

## 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 (Vinson 1997). They have since spread throughout the Gulf Coast states, east to the coastal areas of South Carolina, North Carolina, and Virginia, and were secondarily introduced to California where they continue to expand their range northward (Vinson 1997, Korzukhin et al. 2001). 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 and Bronstein 2004), including cotton aphids on cotton plants (Kaplan and Eubanks 2002, Diaz et al. 2004). Cotton aphids injure cotton plants by continuously feeding on plant phloem, which can result in stunted growth, loss of leaf surface area, premature leaf abscission, lower boll (fruit) retention, and reduced cotton lint mass (Fuchs and Minzenmayer 1995, Rosenheim et al. 1997). Cotton aphids are effectively protected from other predators by tending fire ants (Kaplan and 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 fairly tolerant to cotton aphid herbivory (Rosenheim et al. 1997), however, and could indirectly benefit from aphid-tending fire ants. Fire ants aggressively patrol cotton plants in the presence of cotton aphids and significantly reduce the abundance of more damaging, non-aphid herbivores including caterpillars and *Lygus* plant bugs (Diaz et al. 2004, Kaplan and Eubanks 2005). The model non-aphid herbivore used in this study, the beet armyworm caterpillar, is considered a secondary pest of cotton, but in sufficient numbers it can threaten 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 can be found together on cotton plants throughout the growing season.

### *Field experiments*

I conducted the field experiments in 2003 and 2004 at the E. V. Smith Research Center of the Auburn University Agricultural Experiment Station in Macon and Elmore Counties, Alabama, USA. The field experiment in each year consisted of a 2 x 2 factorial, randomized block design in which I manipulated cotton aphid density (aphids present or excluded) and beet armyworm caterpillar density (low or high) on cotton plants in 1.8 x 1.8 x 1.8-m field cages. The field cages were constructed of 32 x 32 mesh Lumite screening (opening size 530  $\mu\text{m}$ ; Bioquip, Gardena, California, USA) that prevented aphid movement in or out of the cages. I randomly assigned each treatment combination to one of four cages in each of seven blocks ( $n = 28$  cages total) in an 8-ha field of conventional cotton in Macon County in 2003. I followed the same procedure in 2004 but with eight additional cages in two additional blocks ( $n = 36$  cages total) in a 4-ha field of conventional cotton 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 seasons. The blocks were arranged linearly in both years so that each cage was approximately 35 m from a field edge.

I erected each field cage over two rows of cotton seedlings before they were colonized by any insects. In 2003, I thinned the seedlings in each cage to ten evenly spaced plants per row. By the middle of the season, however, branches on neighboring plants overlapped so extensively that sampling individual plants became very difficult, as

did moving around inside the cages without disturbing the plants (and fire ants). In 2004, therefore, I removed all the seedlings in one of the two caged rows to provide space in which to work and thinned the seedlings in the remaining row to five evenly spaced plants. The bottom edges of the cages were buried to block movement of all arthropods except fire ants. Fire ants were able to access the caged plants via foraging tunnels they constructed under the cage walls.

I established the experimental treatments in late June 2003 and 2004, when cotton aphids appeared naturally in the fields. I manipulated cotton aphid density by transferring to each plant in half of the cages approximately 300 aphids collected from the surrounding field (aphid-inclusion treatment). 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). Unfortunately, in 2003 cotton aphids colonized several of the ‘aphid-exclusion’ cages, and predatory larvae of ladybird beetles and lacewings infiltrated several of the ‘aphid-inclusion’ cages, causing aphid population crashes. This resulted in a range of cotton aphid densities in cages rather than the strict presence or absence of aphids (see below). Cotton aphids and aphid predators occasionally colonized cages in 2004, but by more frequently inspecting plants and removing unwanted individuals, I was able to maintain the two aphid density treatments.

I manipulated caterpillar density by transferring to plants caterpillars reared in the lab from commercially purchased eggs (Benzon Research, Carlisle, Pennsylvania, USA). Cages in the low-density and high-density treatments received 5 and 15 caterpillars per plant per week, respectively, in 2003. These densities are within the range of what is normally encountered in the field. Beet armyworm caterpillars were reared on artificial

diet (Product #F9219B, Bio-Serv, Frenchtown, NJ) until they reached their third instar before transferring them to several haphazardly chosen leaves on the caged plants. Incidental caterpillar mortality was very high, however, because caterpillars apparently had difficulty acclimating to walking on leaves (they repeatedly fell from the plants) and feeding on cotton foliage. Consequently, I could not effectively maintain two levels of caterpillar density in 2003. In 2004, I transferred beet armyworm caterpillars to plants immediately after the caterpillars hatched at rates of 10 and 30 caterpillars per plant per week in the low-density and high-density treatments, respectively. I used higher densities of caterpillars in 2004 to ensure that at least some caterpillars survived to begin feeding on the cotton foliage. Again, these caterpillar densities are representative of what occur naturally; beet armyworm caterpillar eggs are typically oviposited in clusters of approximately 50 to 200 eggs (Ruberson et al. 1994). The 10 or 30 caterpillars were transferred en masse to the underside of a single, haphazardly chosen, mainstem leaf in the middle of the plant canopy to mimic the oviposition behavior of the female beet armyworm moth (Ruberson et al. 1994). This procedure protected the caterpillars from heavy dew accumulations on the upper surfaces of leaves and allowed them to acclimate to their food source and to the surface of the plant. Consequently, incidental caterpillar mortality was much lower in 2004 than in 2003 and I was able to maintain the two caterpillar density treatments.

The cotton aphid and caterpillar density treatments were applied for seven weeks (early July to late August) in 2003 and for nine weeks (late June to late August) in 2004. These periods of time roughly coincided with the reproductive period of the plants (square development through early boll maturation), when cotton yield is most threatened

by cotton aphid and caterpillar herbivory (Rosenheim et al. 1997, Mascarenhas et al. 1999). I sampled individual plants approximately weekly for five weeks (mid-July to late August) in 2003 and for seven weeks (early July to late August) in 2004. I sampled ten randomly chosen plants (five from each row) in each cage in 2003 and all five plants in each cage in 2004. Each week, I estimated cotton aphid density (# aphids per leaf) and recorded the number of fire ants and caterpillars on each plant. I estimated cotton aphid density by averaging the number of aphids counted on six leaves, including the terminal leaf on branches four, eight, and twelve (from the top of the plant), and the mainstem leaf growing from the node giving rise to each of these branches. I also quantified caterpillar damage to plant foliage by summing estimates of the percent leaf area consumed for each mainstem leaf and dividing by the total number of leaves sampled. Finally, I quantified plant reproduction by recording the number of squares, flowers, and bolls on each plant, noting in 2004 whether the bolls were damaged or undamaged by caterpillars. I also quantified plant growth in 2004 by counting the number of mainstem nodes.

On 30 August 2003, one week after the final weekly sampling date, I collected ten randomly chosen plants from each cage and transported them to the lab. Stems, leaves, and bolls were dried separately at 60 C for three days and then weighed to the nearest 0.01 g to determine stem, leaf, and mean boll mass for each plant. To better estimate plant reproduction in 2004, developing bolls were allowed to mature and open before collecting them on 20 September, four weeks after the final weekly sampling date. I dried the bolls at 60 C for three 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, I counted the total number of seeds produced by each plant.



### *Greenhouse experiment*

Because I did not manipulate the presence and absence of fire ants independently of cotton aphid density in the field experiments (due to a limited number of field cages), I implicitly assume that any reduction in caterpillar abundance and caterpillar damage to plants with cotton aphids is the consequence of increased fire ant predation of caterpillars on those plants. This assumption is not unreasonable because fire ant abundance and predation of herbivores on cotton plants is strongly determined by the presence and abundance of cotton aphids (Kaplan and 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.

I tested this hypothesis in a greenhouse experiment in which I monitored beet armyworm 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 2004 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 I transferred approximately 100 cotton aphids from a lab colony to each of 16 plants. Two weeks later, I transferred five neonate beet armyworm caterpillars to each of the aphid-infested plants (mean  $\pm$  1 SD aphid density =  $249 \pm 163$  aphids per leaf) and to 15 plants without aphids. I counted the number of caterpillars on

each plant every 24 hours for 10 days. After 10 days, I collected the remaining caterpillars and weighed them individually to the nearest 0.01 g. I quantified caterpillar damage to plants by summing estimates of the percent leaf area consumed on each leaf and dividing by the total number of leaves on the plant.

### *Statistical analyses*

Because of the difficulties I had establishing the experimental treatments in 2003, mean cotton aphid densities varied substantially among the cages (0.1 to 24.2 aphids per leaf, averaged over all five sampling periods) and caterpillars densities in all cages were low. Because at least some caterpillars survived the transition to the field, however, I could still address the first of my two questions, whether cotton aphids indirectly benefit cotton reproduction by increasing fire ant suppression of caterpillar herbivory. Given the gradient of cotton aphid densities in the field cages, I used a series of regression analyses (SAS proc glm; SAS Institute, Inc. 2001) to test for: 1) a positive effect of cotton aphid density on fire ant abundance on cotton plants, 2) negative effects of fire ant abundance on the abundance of beet armyworm caterpillars and on percent caterpillar damage to leaves, 3) positive effects of cotton aphid density on the number of squares, flowers, and bolls on plants, and 4) a positive effect of cotton aphid density on mean boll mass. I also regressed stem mass and leaf mass on cotton aphid density to test for effects of cotton aphids on vegetative growth. Cage means averaged over all five sample dates (excepting mean boll mass, and stem and leaf mass, which were recorded only at harvest) were used in the analyses. Values for aphid density, fire ant abundance, and caterpillar abundance were  $\log(n + 1)$ -transformed to meet statistical assumptions.

The cotton aphid and caterpillar density treatments were successfully maintained for the duration of the experiment in 2004. I tested for treatment effects on cotton aphid density, fire ant abundance on plants, caterpillar abundance, percent caterpillar damage to leaves and bolls, and the number of squares, flowers, undamaged bolls, and nodes per plant using repeated-measures ANOVA (SAS proc mixed; SAS Institute, Inc. 2001). I analyzed the number of undamaged bolls because plants quickly aborted damaged bolls. I 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, percent caterpillar damage to leaves and bolls, and number of squares, flowers, and bolls, were  $\log(n+1)$ -transformed to meet statistical assumptions. Values for number of nodes, number of bolls, and number of seeds were log-transformed. Experiment-wise error rate was controlled in post-hoc pairwise comparisons of treatment means using sequential Bonferroni corrections.

In the greenhouse experiment, I tested for a treatment effect on mean caterpillar survival ( $n$  surviving / 5) using repeated-measures ANOVA. Survival data were arcsine square root-transformed prior to analysis. I used two-sample  $t$ -tests (SAS proc ttest; SAS Institute, Inc. 2001) to compare mean caterpillar mass and mean percent caterpillar damage to plants between treatments.

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

## RESULTS

### *2003 field experiment*

Mean fire ant abundance on plants increased significantly with increasing mean cotton aphid density ( $F_{1,26} = 3.03$ , one-tailed  $P = 0.045$ ,  $R^2 = 0.10$ ,  $b \pm 1SE = 0.16 \pm 0.09$ ) (Fig. 1A). Increasing fire ant abundance on plants did not result in a significant decrease in the mean number of beet armyworm caterpillars sampled ( $F_{1,26} = 0.69$ , one-tailed  $P = 0.21$ ,  $R^2 = 0.03$ ); however, mean percent caterpillar damage to leaves decreased significantly with increasing fire ant abundance on plants ( $F_{1,26} = 2.74$ , one-tailed  $P = 0.05$ ,  $R^2 = 0.10$ ,  $b \pm 1SE = -5.69 \pm 3.43$ ) (Fig. 1B).

Cotton aphid density had no effect on the mean number of squares produced by plants ( $F_{1,26} = 0.92$ , one-tailed  $P = 0.17$ ,  $R^2 = 0.03$ ). In contrast, both the mean number of flowers ( $F_{1,26} = 2.85$ , one-tailed  $P = 0.05$ ,  $R^2 = 0.06$ ,  $b \pm 1SE = 0.53 \pm 0.31$ ) and the mean number of bolls ( $F_{1,26} = 3.21$ , one-tailed  $P = 0.04$ ,  $R^2 = 0.08$ ,  $b \pm 1SE = 0.89 \pm 0.50$ ) increased significantly with increasing cotton aphid density (Figs. 1C,D). Similarly, mean boll mass increased significantly with increasing cotton aphid density ( $F_{1,26} = 5.50$ , one-tailed  $P = 0.01$ ,  $R^2 = 0.17$ ,  $b \pm 1SE = 0.47 \pm 0.20$ ) (Fig. 1E).

Although three of the four reproductive variables measured were associated positively with cotton aphid density, cotton aphids negatively affected vegetative growth. Both mean leaf mass ( $F_{1,26} = 6.32$ , two-tailed  $P = 0.02$ ,  $R^2 = 0.20$ ,  $b \pm 1SE = -5.16 \pm 2.05$ ) and mean stem mass ( $F_{1,26} = 6.44$ , two-tailed  $P = 0.02$ ,  $R^2 = 0.20$ ,  $b \pm 1SE = -11.87 \pm 4.68$ ) decreased significantly with increasing cotton aphid density (Fig. 1F).

### 2004 field experiment

A few cotton aphids colonized plants in some of the ‘aphid exclusion’ cages, but mean aphid density was statistically indistinguishable from zero on every sampling date (Fig. 2A). Mean aphid density in the ‘aphids present’ cages fluctuated over the seven sampling dates but was significantly greater than aphid density in the ‘aphids excluded’ cages on all dates (aphid density x date interaction:  $F_{6,192} = 3.68$ ,  $P = 0.002$ ; Fig. 2A). Averaged over all sampling dates, mean aphid density in the ‘aphids present’ cages was  $37.1 \pm 2.2$  aphids per leaf, and in the ‘aphids excluded’ cages was  $5.1 \pm 2.2$  aphids per leaf ( $F_{1,24} = 133.47$ ,  $P < 0.0001$ ). Mean aphid density in the ‘aphids present’ cages did not differ between the low ( $37.5 \pm 3.1$  aphids per leaf) and high ( $36.8 \pm 3.1$  aphids per leaf) caterpillar density treatments ( $P = 0.83$ ).

Fire ants tended cotton aphids in the ‘aphids present’ cages within a week after establishing the experimental treatments. Fire ant abundance on plants with cotton aphids increased over time and was significantly greater on plants with aphids than on plants without aphids (i.e., plants in ‘aphid exclusion’ cages) on all sampling dates (aphid density x date interaction, Table 1) (Fig. 2B). 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 percent leaf damage by caterpillars increased over the seven sampling dates (date effects, Table 1). Caterpillars were significantly less abundant 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 x date

interaction, Table 1) (Fig. 2C). 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 to leaves did not vary with caterpillar density (Table 1). In contrast, an effect of cotton aphid density on caterpillar damage to bolls did vary with caterpillar density (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 density x caterpillar density interaction, Table 2), however, such that mean number of squares was significantly greater on plants with aphids only at high caterpillar density (Fig. 3A). 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 on plants with aphids at low caterpillar density ( $P = 0.35$ ) (Fig 3A). Similarly, the effect of cotton aphid density on number of undamaged bolls varied with caterpillar density (aphid density x caterpillar density x date interaction, Table 2) such that the increase in mean number of undamaged bolls over the season on plants with aphids was significantly greater only at

high caterpillar density (Fig. 3B). On the final weekly sampling date, 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 on plants with aphids at low caterpillar density ( $P = 0.41$ ) (Fig. 3B). The effect of cotton aphid density on mean number of flowers per plant did not vary with caterpillar density (Table 2).

In contrast to the three reproductive variables measured, cotton aphids negatively affected vegetative growth. Averaged over all sampling dates, cotton plants with aphids had significantly fewer nodes ( $16.7 \pm 0.1$ ) than did plants without aphids ( $17.4 \pm 0.1$ ; aphid density effect, Table 2).

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 mean number of bolls was significantly greater on plants with aphids only at high caterpillar density (Fig. 4A). 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 on plants with aphids at low caterpillar density ( $P = 0.80$ ). Plants with aphids also had significantly more seeds (Fig. 4B) and significantly greater seedcotton mass (Fig. 4C) than did plants without aphids (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 (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 ( $F_{9,261} = 56.20$ ,  $P < 0.0001$ ), but the average rate of decrease in survival was slower on plants with aphids (treatment x day interaction:  $F_{9,261} = 6.80$ ,  $P < 0.0001$ ). Consequently, a greater proportion of caterpillars survived on plants with aphids by the final three days of sampling (Fig. 5). As a result, percent 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 ( $45.5 \pm 3.3$  g) than on plants without aphids ( $54.1 \pm 4.7$  g), it did not differ statistically between the two treatments (equal variance  $t$ -test:  $t_{26} = 1.52$ ,  $P = 0.14$ ).

### DISCUSSION

Results of the field cage experiments show that a facultative mutualism between cotton aphids and an invasive ant species, the red imported fire ant, can increase the fitness of its host plant, cotton. In both 2003 and 2004, 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 to developing bolls on plants hosting the fire ant - cotton aphid mutualism resulted in increased cotton yield in terms of the mean number of bolls and seeds produced per plant, and mean seedcotton mass. This increase in cotton reproduction was realized in spite of direct negative effects of cotton aphids on plant growth (reduced stem and leaf biomass in 2003 and fewer mainstem nodes in 2004). 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. Caterpillar survival over time and caterpillar damage to leaves was actually greater in the presence of cotton aphids than in their absence in the greenhouse. 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.

These results are consistent with the few previous studies that have documented net positive effects on plants of ant-Hemipteran mutualisms involving native ants in terms of both reduced plant damage by non-Hemipteran herbivores (Jutsum et al. 1981, Khoo and Ho 1992, Löhrl 1992, Floate and Whitham 1994, Dejean et al. 1997, Karhu 1998, Van Mele and Cuc 2001, Sipura 2002, Oliveira and Del-Claro 2005) and increased plant growth and reproduction (Messina 1981, Whittaker and Warrington 1985, Ito and Higashi 1991, Löhrl 1992, Sipura 2002, Suzuki et al. 2004). One notable exception is a study by Buckley (1983) of the consequences of an ant-Membracid mutualism to the growth and reproduction of an extrafloral nectary-bearing host plant, *Acacia decurrens*. In the absence of Membracids, *Iridomyrmex* ants attracted onto *Acacia* saplings by extrafloral nectaries protected the plants from other herbivores, resulting in increased plant growth and seed set. In the presence of Membracids, however, the ants were apparently attracted away from the extrafloral nectaries and spent less time patrolling the plant, resulting in decreased ant protection of plants and, consequently, decreased plant growth and seed set. Cotton plants also bear extrafloral nectaries that attract ants, including fire ants. Fire ants exhibit a much stronger numerical response to cotton aphids

than to extrafloral nectaries, though, resulting in comparatively greater protection to plants from other herbivores (Kaplan and Eubanks 2002, 2005, this study).

This study represents the first experimental investigation of the consequences to plant fitness of an ant-Hemipteran mutualism involving an invasive ant species. Numerical dominance, extreme aggression, and broad omnivory characterize invasive ants, and are apparently traits that allow invasive ants to significantly alter the abundance and distribution of other organisms in invaded habitats (Holway et al. 2002). Several invasive ant species also possess a derived proventriculus that allows them to ingest large quantities of liquid food (Eisner 1957, Davidson et al. 2004), a trait that may explain the strong attraction of invasive ants to Hemipteran honeydew (Holway et al. 2002). A few studies have investigated the effects of invasive ants as biological control agents on plants that host honeydew-producing Hemipterans (reviewed in Lach 2003), but none have tested whether plant protection by ants from non-Hemipteran herbivores was specifically a consequence of mutualistic interactions between invasive ants and Hemipterans. Likewise, there have been no direct comparisons of the consequences to plants of mutualisms between Hemipterans and native versus invasive ants. Way et al. (1999) observed that defoliation of the pine *Pinus pinaster* by the larvae of the pine processionary moth (*Thaumetopoea pityocampa*) was less severe in trees patrolled by the invasive Argentine ant (*Linepithema humile*) relative to trees patrolled by native ants. The authors suggested that aggregations of honeydew-producing aphids facilitated protection of pines by *L. humile*, but they did not specifically test whether the ants had the same effect in the absence of aphids. Whether fire ants provide stronger protection to cotton plants relative to native aphid-tending ants is unknown but is very likely. Native

pyramid ants (*Dorymyrmex bureni*) are common along field margins on my study area and will tend cotton aphids, but they do not show the same numerical response to cotton aphids as do fire ants nor are they as abundant in the interior of cotton fields (personal observation). 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 and Bronstein 2004), studies comparing the consequences to plants of interactions between Hemipterans and native versus invasive ants are greatly needed.

Our study builds on previous work by providing experimental evidence of conditionality in the outcome of an ant-Hemipteran mutualism to its host plant. Messina (1981) and Fritz (1983) previously recognized this possibility, arguing specifically that the cost-to-benefit ratio of ant-Hemipteran mutualisms to plants may vary simply with the density of non-Hemipteran herbivores on the plant relative to Hemipteran density, as I show in this study. At high caterpillar density, caterpillar damage to developing bolls 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. 4A). This conditionality in the benefit of cotton aphids to boll production, however, 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 on plants without aphids than on plants with aphids at low caterpillar density. Statistically, however, the increase in mean boll mass on plants with aphids did not differ between the two levels of caterpillar density ( $F_{1,24} = 2.07, P = 0.16$ ).

Sipura (2002) is the only other study that has demonstrated that the consequences of ant-Hemipteran mutualisms to plants can vary in different ecological contexts (specifically, at different levels of host plant resistance to non-Hemipteran herbivores [see Introduction]). Indirect interactions between Hemipterans and other herbivores that are mediated by plants could also influence the effects of ant-Hemipteran mutualisms on plants. Consistent with the results reported here, Rodriguez-Saona et al. (2005) found that aphid damage to tomato plants increased consumption of leaves and survival in *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 aphid-free leaves. Because aphid herbivory may improve 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. 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, Wimp and Whitham 2001), 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), and the effects of plant genotype (Floate & Whitham 1994; see also Vrieling et al. 1991) and other biotic (e.g., pathogen infection) and abiotic factors (e.g., nitrogen availability, shade) on host plant quality (Cushman 1991). Because 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.

The net effects of ant-Hemipteran mutualisms to agriculturally important herbaceous plants have rarely been determined empirically but are thought to be strongly negative because of a high potential for Hemipteran outbreaks in plant monocultures and because Hemipteran-vectored plant viruses can be particularly devastating to yield (Buckley 1987). Cotton aphids do not transmit viruses to cotton in North America (Ebert and Cartwright 1997), but high densities of cotton aphids can reduce yield as a consequence of the loss of plant sap (Fuchs and Minzenmayer 1995, Rosenheim et al. 1997). I found no negative effect of aphids on cotton reproduction even at low caterpillar density. These results are surprising considering that cotton aphid density averaged over seven weeks of plant reproduction was moderately high (~40 aphids per leaf). There is no consensus on an action threshold for the treatment of cotton aphids in cotton but some states recommend the application of aphid pesticides when aphids reach densities of 50 aphids per leaf. From an applied perspective, therefore, the results of the field cage experiments are important because they suggest that: (1) though typically considered

herbivore pests themselves, cotton aphids (at least at moderate densities) indirectly benefit cotton reproduction by increasing fire ant suppression of other herbivores, (2) the efficacy of fire ant suppression of other herbivores increases with increasing cotton aphid density (see also Kaplan and Eubanks 2005), and (3) the indirect benefit of cotton aphids is greater at higher densities of other herbivores on cotton plants. I encourage additional studies of the consequences to plants of ant-Hemipteran mutualisms involving native and invasive ants in other annual agricultural systems to further evaluate the utility of ants as biological control agents of non-Hemipteran herbivores.

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**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 percent leaf damage and boll damage by caterpillars in the 2004 field cage experiment. *P*-values for significant treatment effects appear in bold.

Factor	d.f.	Fire ant abundance			Caterpillar abundance			Percent leaf damage			Percent boll damage <sup>a</sup>		
		<i>F</i>	<i>P</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>P</i>
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	< <b>0.0001</b>	11.87	<b>0.002</b>	14.21	<b>0.0009</b>	8.70	<b>0.007</b>				
Caterpillar density	1,24	0.43	0.52	20.09	<b>0.0002</b>	6.87	<b>0.01</b>	4.09	<b>0.05</b>				
Sampling date	6,192	3.61	<b>0.002</b>	21.84	< <b>0.0001</b>	10.15	< <b>0.0001</b>	1.96	0.15				
Aphid x Caterpillar	1,24	0.04	0.85	0.29	0.60	0.52	0.48	5.40	<b>0.03</b>				
Aphid x Date	6,192	2.62	<b>0.02</b>	2.66	<b>0.02</b>	1.36	0.23	1.13	0.33				
Caterpillar x Date	6,192	1.61	0.15	2.30	<b>0.04</b>	0.13	0.99	0.44	0.65				
Aphid x Caterpillar x Date	6,192	0.71	0.64	0.52	0.80	0.31	0.93	0.67	0.52				

<sup>a</sup> 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.

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

Factor	d.f.	# of squares		# of flowers		# of undamaged bolls		# of nodes	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
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	<b>0.0011</b>	11.50	<b>0.002</b>	7.73	<b>0.01</b>	21.71	<b>&lt;0.0001</b>
Caterpillar density	1,24	1.22	0.28	3.40	0.08	5.02	<b>0.03</b>	0.08	0.77
Sampling date	6,192	18.49	<b>&lt;0.0001</b>	152.23	<b>&lt;0.0001</b>	762.94	<b>&lt;0.0001</b>	184.38	<b>&lt;0.0001</b>
Aphid x Caterpillar	1,24	5.69	<b>0.03</b>	0.10	0.76	7.71	<b>0.01</b>	0.02	0.89
Aphid x Date	6,192	0.41	0.87	3.80	<b>0.001</b>	2.80	<b>0.01</b>	0.73	0.63
Caterpillar x Date	6,192	0.04	0.99	0.56	0.76	0.82	0.56	0.53	0.79
Aphid x Caterpillar x Date	6,192	0.15	0.99	1.35	0.24	3.11	<b>0.006</b>	0.56	0.76

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

Factor	d.f.	# of bolls			# of seeds			Seedcotton mass		
		<i>F</i>	<i>P</i>	<i>F</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>F</i>	<i>P</i>	
Block	8,24	5.96	0.0003	5.51	0.0005	7.25	<0.0001			
Aphid density	1,24	6.72	<b>0.02</b>	12.21	<b>0.002</b>	3.55	<b>0.04</b>			
Caterpillar density	1,24	4.66	<b>0.04</b>	4.58	<b>0.04</b>	2.02	0.17			
Aphid x caterpillar	1,24	4.93	<b>0.04</b>	0.88	0.36	0.14	0.71			

FIG. 1. Effect of cotton aphid density (mean # per leaf) on fire ant abundance on cotton plants (A), effect of fire ant abundance on mean percent leaf damage by beet armyworm caterpillars (B), and effect of cotton aphid density on number of cotton flowers (C), number of cotton bolls (D), boll mass (E), and leaf and stem mass (F) in the 2003 field cage experiment. Points represent field cage means. Cotton aphid density, fire ant abundance, percent leaf damage, and number of cotton flowers and bolls were averaged over five sampling dates. Boll, leaf, and stem mass were determined at harvest.



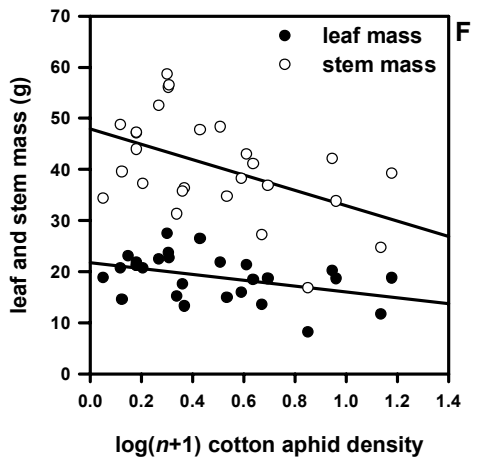
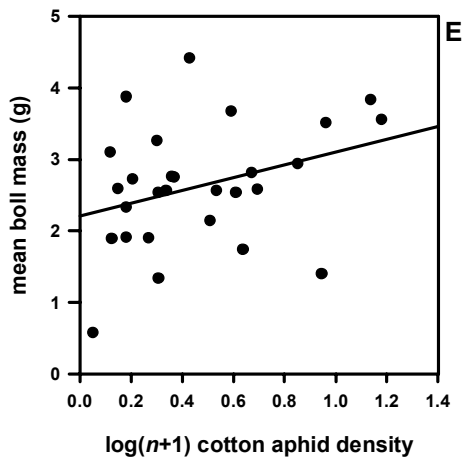
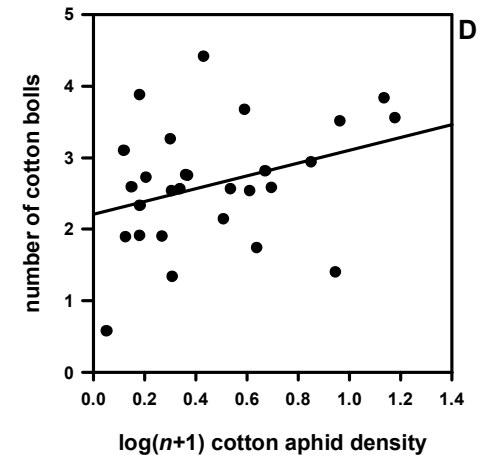
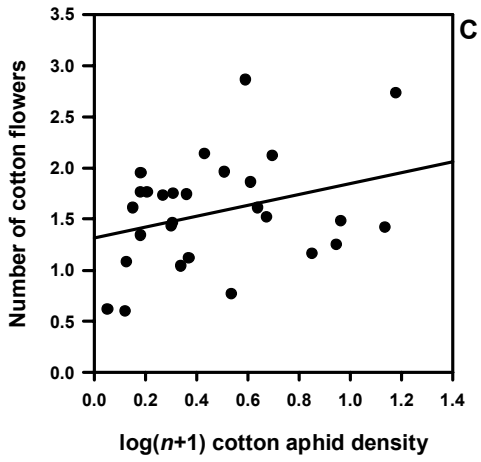
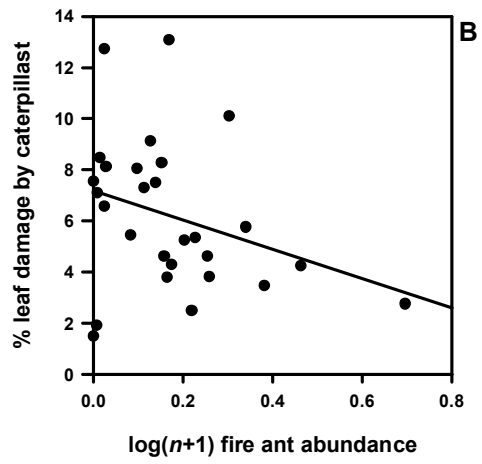
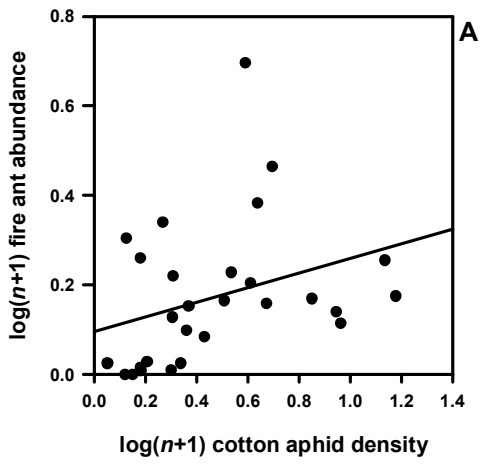


FIG. 2. Effect of the cotton aphid manipulation (aphids included or excluded from cotton plants in field cages) on mean cotton aphid density (mean number per leaf) (A), mean fire ant abundance per plant (B), and mean beet armyworm caterpillar abundance per plant (C) on seven sampling dates in the 2004 field cage experiment. Error bars represent 1 SE. Asterisks indicate significant differences between the two aphid treatments.

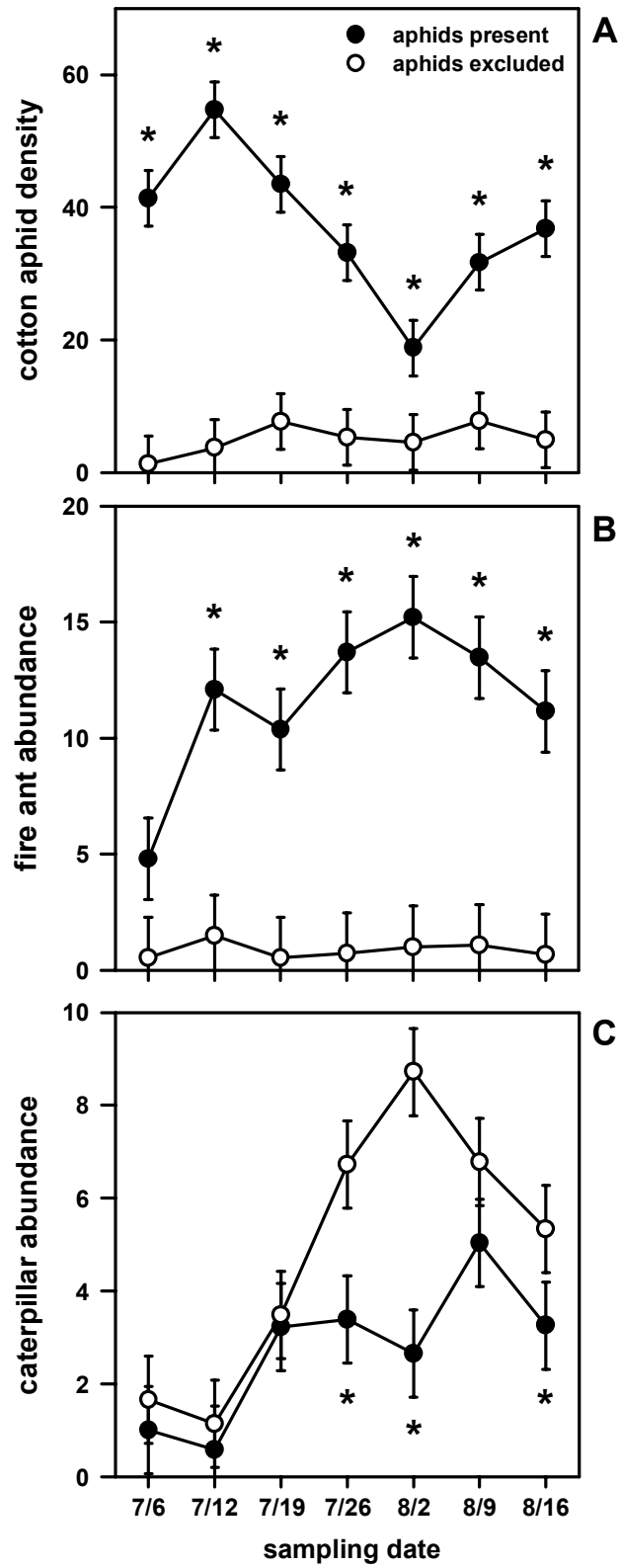


FIG. 3. Effect of cotton aphid density (aphids included or excluded from cotton plants in field cages) on mean number of cotton squares (flower buds) per plant at low and high beet armyworm caterpillar density averaged over seven sampling dates (A), and effect of cotton aphid density and caterpillar density on mean number of cotton bolls undamaged by caterpillars per plant on seven sampling dates (B) in the 2004 field cage experiment. Error bars represent 1 SE. Asterisk in (A) indicates significant difference between the two aphid treatments.

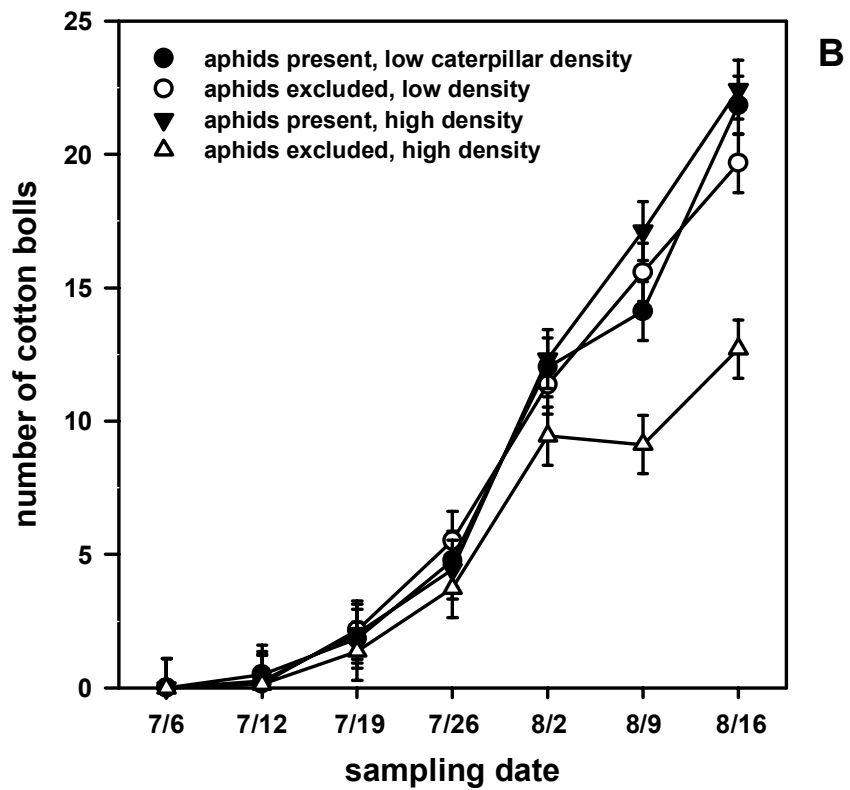
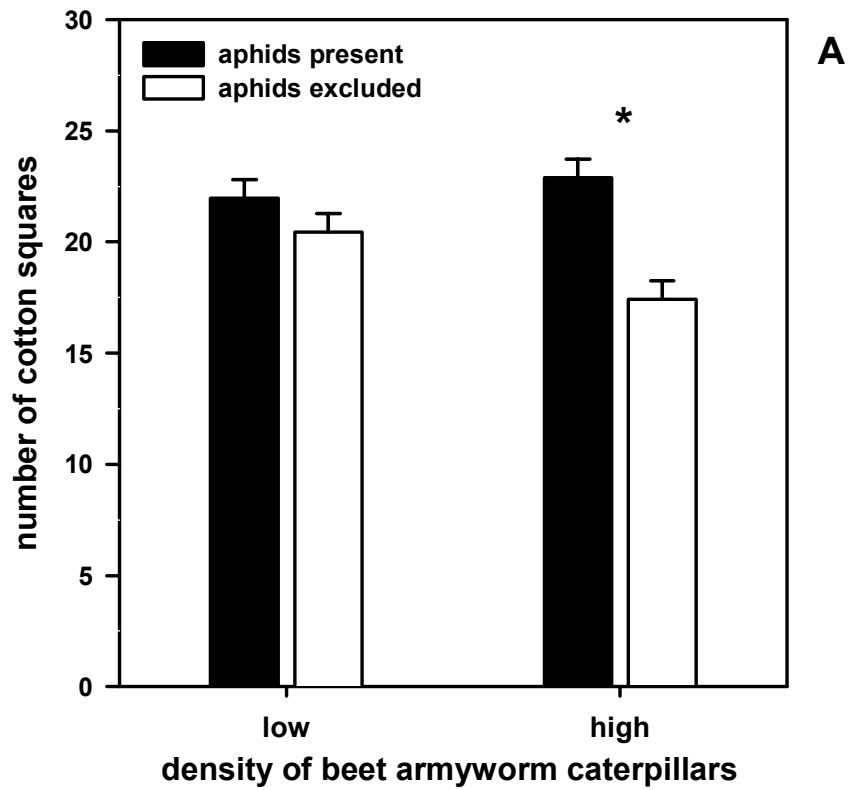


FIG. 4. Effect of cotton aphid density (aphids included or excluded from cotton plants in field cages) on mean number of cotton bolls per plant at harvest at low and high beet armyworm caterpillar density (A), and on mean number of cotton seeds per plant (B) and mean seedcotton mass per plant (C) at harvest averaged across the two caterpillar density treatments in the 2004 field cage experiment. Error bars represent 1 SE. Asterisks indicate significant differences between the two aphid treatments.

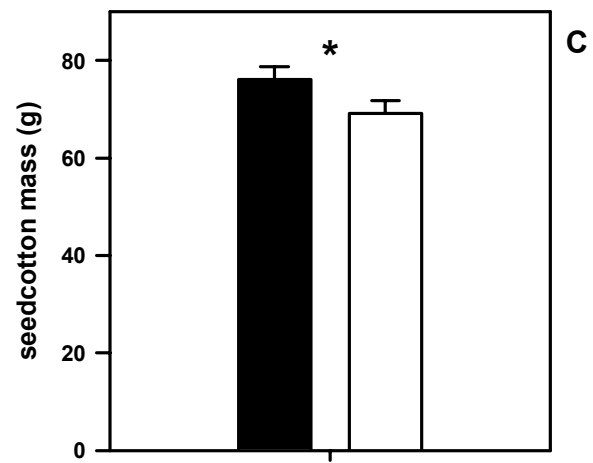
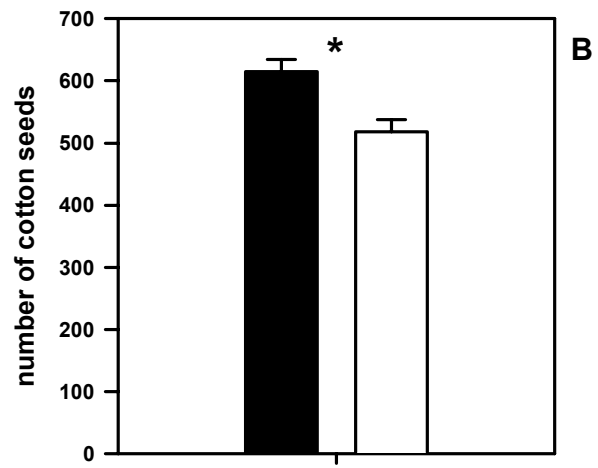
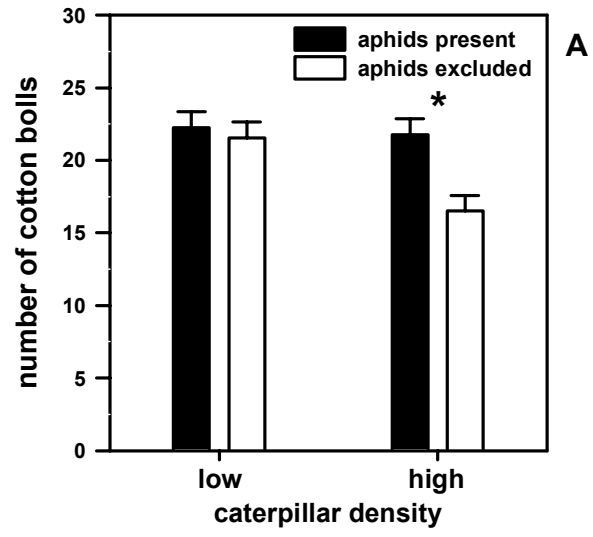
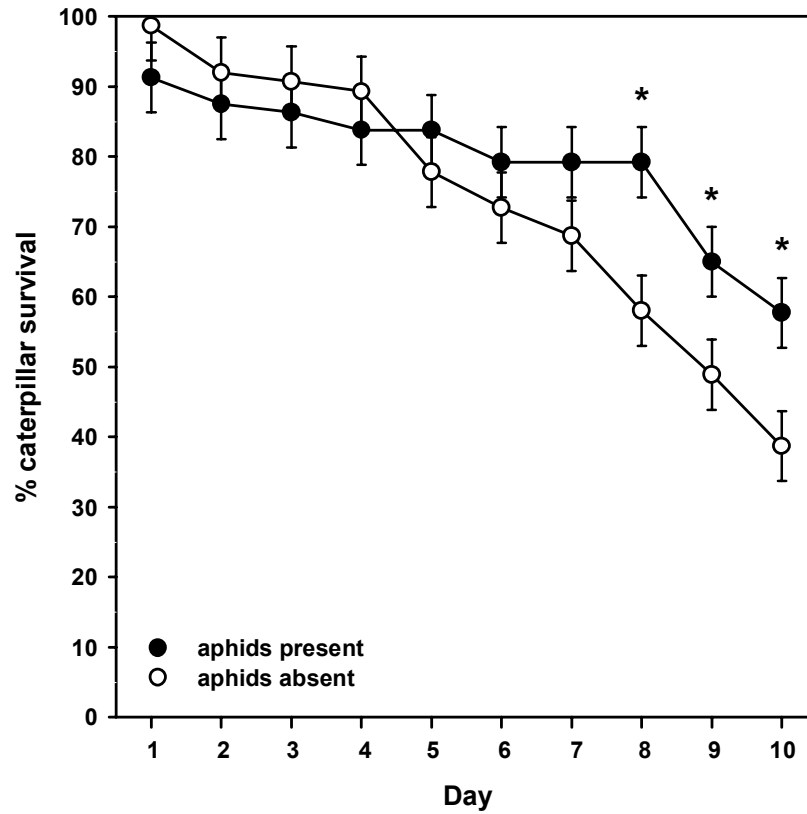


FIG. 5. Mean ( $\pm 1$  SE) percent beet armyworm caterpillar survival over 10 days on cotton plants with and without cotton aphids in the 2004 greenhouse experiment. Asterisks indicate significant differences in caterpillar survival between the two aphid treatments.





**CHAPTER FOUR:**  
**WHEN INVASIVE SPECIES MEET: PREDICTING THE ECOLOGICAL**  
**CONSEQUENCES OF A MUTUALISM BETWEEN SOYBEAN APHIDS**  
**AND RED IMPORTED FIRE ANTS**

*Abstract.* Indirect interactions between honeydew-producing insects, other arthropods, and host plants mediated by direct interactions with ants are potentially important but often overlooked interactions in terrestrial arthropod communities. Previous work in cotton has shown that cotton aphids (*Aphis gossypii*) generate strong and pervasive indirect effects on other cotton arthropods and on cotton plants via their mutualistic interaction with an invasive predatory ant, the red imported fire ant (*Solenopsis invicta*). In comparison, fire ants are much weaker predators in soybean and other crops that do not host an abundant honeydew-producing insect. I conducted field and greenhouse experiments to test the hypothesis that the range expansion of an invasive aphid, the soybean aphid (*Aphis glycines*), into the range of fire ants in the southeastern United States will cause a dramatic increase in the community-level effects of fire ants in soybean. In field experiments in which I misted soybean plants with artificial honeydew as a surrogate for soybean aphid honeydew, fire ants were significantly more abundant on honeydew-misted plants versus water-misted control plants, resulting in decreased

caterpillar abundance and caterpillar damage to plants. Increased fire ant abundance on honeydew-misted plants had no appreciable effect, however, on other herbivore taxa or on non-ant predators. Results from greenhouse experiments with soybean aphids were mostly consistent with the field experiments. Significantly fewer caterpillars survived on soybean plants in the presence of fire ants that were tending soybean aphids than in the presence of fire ants alone. Increased fire ant abundance on plants with soybean aphids had no effect on the survival of predatory damsel bug nymphs, but did significantly reduce the survival of predatory big-eyed bug nymphs. Combined, my results suggest that the indirect negative effects of soybean aphids on the soybean arthropod community mediated by fire ants may be limited to one important group of herbivores, but that these effects may cascade down to benefit soybean plants.

## INTRODUCTION

Over the past two decades, ecologists have increasingly recognized the prevalence and importance of indirect interactions in ecological communities (Wootton 1994, Abrams et al. 1996, Schmitz et al. 2000, Halaj and Wise 2001, Werner and Peacor 2003, Ohgushi 2005, van Veen et al. 2006). Indirect interactions are interactions between two species mediated by a mutual interaction with a third species, as exemplified by apparent competition (interactions between two prey species mediated by a shared predator species; Holt 1977) and trophic cascades (interactions between species in two trophic levels [e.g., plants and predators] mediated by species in an intervening trophic level [e.g., herbivores]; Hairston et al. 1960, Paine 1980). Indirect interactions strongly influence community structure and function (e.g., Wootton 1993, Menge 1995, Martinsen

et al. 1998, Wimp and Whitham 2001, Schmitz 2003) but their significance to community dynamics has often been overlooked.

Many, if not most, terrestrial, plant-based arthropod communities include potentially important indirect interactions between honeydew-producing Hemipteran insects and other arthropods mediated by direct interactions with ants (Buckley 1987, Hölldobler & Wilson 1990, Oliveira and Del-Claro 2005, Styrsky and Eubanks, in review). Honeydew-producing Hemipterans (including aphids, scales, mealybugs, and treehoppers) excrete droplets of carbohydrate-rich honeydew derived from the plant sap on which they feed (Way 1963). Ants are highly attracted to honeydew as a food resource and aggressively recruit to aggregations of honeydew-producing insects, defending them from predators and parasitoids (Way 1963, Hölldobler and Wilson 1990). Hemipteran-tending ants assiduously patrol plants and attack or harass any arthropod they encounter, predator or herbivore (Way 1963, Buckley 1987); thus, by causing a dramatic increase in the abundance and aggression of ants on plants, honeydew-producing Hemipterans may generate potentially strong indirect effects on the abundance and distribution of other arthropod species utilizing the same host plant (Wimp and Whitham 2001, Kaplan and Eubanks 2005; reviewed in Styrsky and Eubanks, in review). These indirect effects may cascade down to plants if, for example, suppression of other herbivores by Hemipteran-tending ants causes a reduction in plant damage and a subsequent increase in plant fitness (Buckley 1987, Lach 2003; reviewed in Styrsky and Eubanks, in review). From an applied perspective, therefore, honeydew-producing herbivore pests of agriculturally important plants may be better considered beneficial

insects in cases in which they indirectly increase biological control of more damaging herbivore pests (reviewed in Way and Khoo 1992, Eubanks and Styrsky 2006).

Previous work has shown that cotton aphids (*Aphis gossypii*), a common pest of cotton (*Gossypium hirsutum*) in the southeastern United States, can have strong indirect effects on the abundance and distribution of several species of cotton herbivores and their predators via a mutualistic interaction with an invasive ant, the red imported fire ant (*Solenopsis invicta*; 'fire ants' from here forward). By manipulating densities of fire ants in Alabama cotton fields with naturally occurring populations of cotton aphids, Kaplan and Eubanks (2005) found that the mutualism between cotton aphids and fire ants negatively affected 27-33% of all herbivore taxa and 40-47% of all predator taxa. In addition to their indirect effects on cotton arthropod community structure, cotton aphids can also indirectly benefit cotton plants by precipitating a trophic cascade in which increased predation of caterpillars by tending fire ants results in decreased caterpillar damage to leaves and bolls (cotton fruits), and, consequently, increased yield (Chapter 3). In a field experiment in which I manipulated the presence and absence of cotton aphids on cotton plants, plants with cotton aphids produced on average 16% more bolls, 25% more seeds, and 10% greater seedcotton mass than plants without aphids as a result of the suppression of beet armyworm (*Spodoptera exigua*) caterpillars by aphid-tending fire ants (Chapter 3).

In comparison, the effects of fire ants as predators in other crops in Alabama (collards [*Brassica oleracea*], tomato [*Lycopersicon esculentum*], and soybean [*Glycine max*]) are not as strong or pervasive even though fire ant abundance on the ground in these three crops is roughly equivalent to that in cotton (Eubanks 2001, Eubanks et al.

2002, Harvey and Eubanks 2004, 2005, Cooper 2005, Styrsky and Eubanks 2006, J. D. Styrsky, unpublished data). Unlike cotton, however, collard, tomato, and soybean do not host an abundant honeydew-producing insect, perhaps precluding strong direct and indirect effects of ants on other foliage arthropods and their host plants, respectively. The importance of fire ants as predators in soybean may soon dramatically increase, however, as the range of an invasive honeydew-producing Hemipteran, the soybean aphid (*Aphis glycines*), expands south into the range of fire ants. Soybean aphids are native herbivores of soybean in eastern temperate and tropical Asia and were first detected in North America in Wisconsin in July 2000 (Ragsdale et al. 2004). By the end of the 2003 growing season, soybean aphids had spread to 21 states and three Canadian provinces, and recent models suggest that soybean aphids will become established in all soybean producing regions in the United States (Venette and Ragsdale 2004).

The spread of soybean aphids into the range of fire ants in the southeastern United States presents a unique opportunity to investigate the importance of indirect interactions between an invasive honeydew-producing insect and other arthropods mediated by an invasive ant. Similar to the effect of cotton aphids in cotton, I hypothesize that soybean aphids will cause a dramatic shift in the importance of fire ants as predators in soybean, thus generating strong indirect effects on the soybean arthropod community and on soybean plants themselves. I present here the results from field and greenhouse experiments designed to test this hypothesis. In anticipation of the spread of soybean aphids into Alabama, I conducted field experiments in which I treated plants with artificial honeydew as a surrogate for the honeydew excreted by soybean aphids. Artificial honeydew has been used in many previous studies to investigate indirect effects

of honeydew-producing insects on other arthropods mediated by predators that are attracted to honeydew (e.g., Evans and England 1996; see also Hagen 1986). I complemented these field experiments with greenhouse experiments in which I manipulated densities of soybean aphids and fire ants on plants to test whether the soybean aphid – fire ant mutualism increased fire ant predation of beet armyworm (*Spodoptera exigua*) caterpillars, an important soybean herbivore, and big-eyed bugs (Hemiptera: Geocoridae) and damsel bugs (Hemiptera: Nabidae), two important predator species in soybean.

## METHODS

### *Field experiments*

I conducted two field experiments at the E. V. Smith Research Center of the Auburn University Agricultural Experiment Station in Macon County, Alabama, USA from July through August 2004. I conducted both experiments in a 1.75-ha field of soybean plants (Pioneer 96B21; 76-cm row spacings, 30 seeds per m) in an area naturally infested with fire ants. In the first experiment, I established 20 plots in which I applied artificial honeydew (150 g sucrose / L water; Evans and England 1996) as a fine mist to 15-m sections of two adjacent rows of plants (experimental treatment) and water to 15-m sections of two additional rows of plants (control treatment). A single row of untreated plants separated the experimental and control rows in each plot. Plots were separated from each other by 10 m and were separated from the nearest field margin by at least 15 m. Artificial honeydew and water were applied twice weekly for 8 weeks (from

beginning bloom to full seed) at a rate of 0.5 L / 15 m of row using a hand-held, 6-L compression sprayer.

I sampled soybean plants for arthropods using a beat cloth in 10 plots and using a sweep net in the other 10 plots (Kogan and Pitrie 1980) approximately once weekly on a day immediately following one of the weekly applications of artificial honeydew and water. Plants on either side of a 1-m<sup>2</sup> cloth spread between two adjacent rows were beaten vigorously to dislodge all canopy arthropods. Arthropods that fell on the beat cloth were quickly identified to family or lower taxonomic status and counted. Flying insects cannot be sampled accurately using a beat cloth so I used sweep nets to sample long-legged flies (Diptera: Dolichopodidae) specifically, a very common predator in soybean. The sweep net samples consisted of 10 passes of a net through the upper 50% of the canopy of two rows of plants. After collecting beat cloth and sweep net samples, I measured the height of one haphazardly chosen plant and quantified caterpillar damage to leaves (beat cloth plots only) by summing estimates of the percentage leaf area consumed on three haphazardly chosen trifoliate leaves from the lower, middle, and upper canopy plus one additional leaflet and dividing by 10 (Kogan & Turnipseed 1980). I randomly sampled plants from each treatment in each plot twice on each sampling date ( $n = 6$  dates for beat cloth samples;  $n = 5$  dates for sweep net samples). Means of the two samples were  $\log(n + 1)$ -transformed for statistical analysis.

I tested for treatment effects on the abundance of fire ants and several herbivore and predator taxa on plants using repeated-measures ANCOVA (SAS proc mixed; SAS Institute, Inc, 2001). I used the same model to test for treatment effects on caterpillar damage to plants, herbivore diversity, predator diversity, and total arthropod diversity.

Plant height was included as a covariate to control for any associated variation in arthropod abundance.

In the second experiment, I established 10 plots in which I isolated three haphazardly chosen plants in a single row from the surrounding plant canopy. Neighboring plants were uprooted to provide approximately 15 cm of open space around each isolated plant, thereby preventing crawling insects from colonizing the isolated plants from neighboring plants. Five-m sections of undisturbed row separated each of the three isolated plants in each plot, and each plot was separated by 10 m. I applied artificial honeydew as a fine mist to two of the three plants and water to the third plant. Each plant was misted for three seconds. To distinguish the effects of fire ants alone from the effects of fire ants plus all other predators, I excluded fire ants from one of the two plants misted with artificial honeydew by applying a thin ring of Tanglefoot® to the base of the plant. Artificial honeydew and water were applied twice weekly for 8 weeks (from beginning bloom to full seed), and Tanglefoot was reapplied as necessary.

To each plant I also added five neonate beet armyworm caterpillars each week. Beet armyworm caterpillars were reared in the lab from commercially purchased eggs (Benzon Research, Carlisle, Pennsylvania, USA) and were transferred to one haphazardly chosen leaf on each plant a day prior to one of the weekly applications of artificial honeydew and water. Beet armyworm caterpillars are highly polyphagous and feed on a wide variety of crop plants, including soybean.

I visually sampled soybean plants for all arthropods approximately once weekly ( $n = 5$  sample dates) on a day immediately following one of the applications of artificial honeydew and water. I then measured the height of each plant and quantified caterpillar



damage to leaves using the same methodology as above. Any leaflets that had been damaged by caterpillars prior to the experiment were removed. Data were  $\log(n+1)$ -transformed for statistical analysis.

I tested for treatment effects on the abundance of fire ants, several other predator taxa, caterpillars, and on caterpillar damage to plants using repeated measures ANCOVA with plant height as a covariate. In both field experiments, experiment-wise error rate was controlled in post-hoc pairwise comparisons of treatment means using the Tukey-Kramer adjustment. Means  $\pm$  1 SE of the untransformed data are presented in the text and figures, and  $P$ -values  $\leq 0.05$  are considered significant in all analyses.

#### *Greenhouse experiments*

By chance, soybean aphids appeared in very low numbers (aggregations of 10-20 aphids on 1-3 leaves on approximately 20% of the plants visually searched in four hours) in several experimental soybean plots at the E. V. Smith Research Center by early September 2004. Because it was too late in the season to conduct another field experiment, I collected approximately 300 soybean aphids to establish a greenhouse colony at Auburn University, Auburn, AL. I used the progeny of these aphids in two greenhouse experiments in October 2004 and September 2005.

In the first greenhouse experiment, I manipulated the presence and absence of soybean aphids and fire ants on individual soybean plants using a 2 x 2 randomized complete block design in order to test the hypothesis that a mutualistic interaction between soybean aphids and fire ants increases fire ant predation of caterpillars, an abundant group of soybean herbivores. Seeds from the same soybean variety used in the

field experiments were sown in 20.3-cm pots and the seedlings ( $n = 28$ ) were allowed to grow until they developed their fourth set of true leaves. I transferred 50 soybean aphids to a single haphazardly chosen leaf on 14 individual plants and allowed them to settle and feed for 48 hours. No aphids were transferred to the other 14 plants. Half of the plants with soybean aphids ( $n = 7$ ) and half of the plants without soybean aphids ( $n = 7$ ) were then placed individually in plastic pans (53 x 43 x 13 cm) containing small colonies of approximately 2000 fire ants. The remaining plants ( $n = 7$  with soybean aphids and  $n = 7$  without soybean aphids) were placed individually in identical plastic pans without fire ant colonies. The plastic pans with fire ants were lined with Fluon® to prevent the ants from escaping, and small twigs were propped against the rims of the pots so that fire ants could access the plants. Fire ants were allowed 24 hours to discover and begin tending the soybean aphids.

At the initiation of the experiment, I transferred 10 neonate beet armyworm caterpillars to a single haphazardly chosen leaflet (without aphids) on each plant in each treatment. All 10 caterpillars were transferred to a single leaflet to mimic the natural tendency of neonate beet armyworms to aggregate after hatching. Beet armyworm caterpillars were lab-reared from commercially purchased eggs. I recorded the number of fire ants and caterpillars on each plant every 24 hours for the next four days. Missing caterpillars were considered depredated. I tested for treatment effects on fire ant abundance on plants ( $\log [n+1]$ -transformed) and on caterpillar survival ( $n$  surviving / 10) using repeated measures ANOVA. Raw data were used in the analysis of caterpillar survival because data transformation did not improve the fit of the model. At the end of the experiment, I recorded the number of leaflets on each plant that had been damaged by

caterpillar feeding. I tested for treatment effects on the percentage of caterpillar-damaged leaflets on each plant ( $n$  damaged leaflets /  $n$  total leaflets; log-transformed) using a two-factor ANOVA (SAS proc glm; SAS Institute, Inc, 2001).

The second greenhouse experiment was designed to test the hypothesis that a mutualistic interaction between soybean aphids and fire ants increases intraguild predation or harassment by fire ants of big-eyed bugs and damsel bugs, two of the most common predator taxa in soybean fields at our study area. Seeds from the same soybean variety used in the field experiments were sown in 20.3-cm pots and the seedlings ( $n = 48$ ) were allowed to grow until they developed their fourth set of true leaves. I transferred 50 soybean aphids to a single haphazardly chosen leaf on 16 individual plants and allowed them to settle and feed for 48 hours. I then applied artificial honeydew as a fine mist for 3 seconds to a second set of 16 plants. No treatment was applied to the remaining 16 control plants. Immediately thereafter, I haphazardly allocated one plant from each treatment to each of 16 plastic pans (53 x 43 x 13 cm) containing small colonies of approximately 2000 fire ants. Each pan was thus considered a single replicate. The plastic pans with fire ants were lined with Fluon® to prevent the ants from escaping, and small twigs were propped against the rims of the pots so that fire ants could access the plants. Fire ants were allowed 24 hours to discover and begin tending the soybean aphids.

At the initiation of the experiment, I divided the 16 pans into two equal groups. I transferred two big-eyed bug nymphs to each plant in one group ( $n = 8$  replicates) and two damsel bug nymphs to each plant in the other group ( $n = 8$  replicates). Third- and fourth-instar big-eyed bug and damsel bug nymphs were collected from agricultural

fields at the E. V. Smith Research Center, transferred to an environmental chamber, and provided only water for 24 hours prior to the experiment. Twenty-four hours after the initiation of the experiment, I recorded the number of fire ants and big-eyed bug nymphs or damsel bug nymphs on each plant. Missing bug nymphs were considered depredated. I tested for effects of the three treatments on fire ant abundance ( $\log [n+1]$ -transformed) on plants and on big-eyed bug and damsel bug survival ( $n$  surviving / 2; arcsine square root-transformed) using one-factor ANOVA (SAS proc glm). Experiment-wise error rate was controlled in post-hoc pairwise comparisons of treatment means using the Tukey-Kramer adjustment. Means  $\pm$  1 SE of the untransformed data are presented in the text and figures, and  $P$ -values  $\leq 0.05$  are considered significant in all analyses.

## RESULTS

### *Field experiment 1*

Averaged over all sampling dates, fire ants were significantly more abundant on plants misted with artificial honeydew ( $4.1 \pm 0.9$  fire ants per sample) than on control plants misted with water ( $1.1 \pm 0.9$ ) ( $F_{1,9} = 23.12$ ,  $P = 0.001$ ). Treatment effect did not vary with sampling date (treatment x time interaction:  $F_{5,45} = 2.0$ ,  $P = 0.1$ ). In addition to attracting fire ants onto plants, artificial honeydew also attracted other predators. The five most commonly sampled predator taxa (accounting for 97% of the total number of individuals sampled, excluding fire ants) were big-eyed bugs, damsel bugs, spiders, hooded beetles (Coleoptera: Anthicidae), and long-legged flies. Spiders were significantly more abundant on plants misted with artificial honeydew than on control plants on three of the six sampling dates (treatment x date interaction, Table 1A; Fig. 1).

Averaged over all sampling dates, big-eyed bugs were 25% more abundant on plants misted with artificial honeydew ( $6.4 \pm 0.3$  bugs per sample) than on control plants ( $5.1 \pm 0.3$ ) (Table 1A), and long-legged flies were 264% more abundant on plants misted with artificial honeydew ( $5.1 \pm 0.5$  flies per sample) than on control plants ( $1.4 \pm 0.5$ ) (Table 1A). Application of artificial honeydew had no effect, however, on the abundance of damsel bugs (honeydew-misted plants:  $4.7 \pm 0.3$  bugs per sample; control plants:  $3.9 \pm 0.3$ ) or hooded beetles (honeydew-misted plants:  $3.5 \pm 0.3$  beetles per sample; control plants:  $3.0 \pm 0.3$ ) on plants (Table 1A).

The four most commonly sampled herbivore taxa (accounting for 90% of the total number of individuals sampled) were Lepidopteran larvae (including velvetbean [*Anticarsia gemmatalis*], soybean looper [*Pseudoplusia includens*], green cloverworm [*Plathypena scabra*], and armyworm [*Spodoptera* spp.] caterpillars), three-cornered alfalfa hoppers, *Spissistilus festinus* [Hemiptera: Membracidae], stink bugs [Hemiptera: Pentatomidae], and *Lygus* plant bugs [Hemiptera: Miridae]). The application of artificial honeydew and subsequent increase in predator abundance on plants did not affect the abundance of stink bugs (honeydew-misted plants:  $0.8 \pm 0.2$  bugs per sample; control plants:  $0.9 \pm 0.2$ ) or plant bugs (honeydew-misted plants:  $0.8 \pm 0.1$  bugs per sample; control plants:  $0.9 \pm 0.1$ ) in the plant canopy (Table 1B). Averaged over all sampling dates, however, alfalfa hoppers were marginally significantly more abundant on plants misted with artificial honeydew ( $1.1 \pm 0.1$  hoppers per sample) than on control plants ( $0.8 \pm 0.1$ ) (Table 1B). In contrast, there were significantly fewer caterpillars on plants misted with artificial honeydew than on control plants as the season progressed (treatment x date interaction, Table 1B; Fig. 2A). Consequently, plants misted with artificial honeydew

were significantly less damaged by caterpillars over the season than control plants (treatment x date interaction, Table 1B; Fig. 2B).

The application of artificial honeydew and subsequent increase in fire ant abundance on plants did not measurably affect herbivore diversity (honeydew-misted plants:  $5.2 \pm 0.3$  taxa per sample; control plants:  $5.1 \pm 0.1$ ) or total arthropod diversity (honeydew-misted plants:  $9.7 \pm 0.3$  taxa per sample; control plants:  $9.9 \pm 0.3$ ) in the plant canopy (Table 2). Averaged over all sampling dates, however, predator diversity was slightly but significantly reduced on plants misted with artificial honeydew ( $4.4 \pm 0.1$  taxa per sample) relative to control plants ( $4.8 \pm 0.1$ ) (Table 2).

### *Field experiment 2*

Fire ant abundance on individual plants varied among the three treatments in the second field experiment (Table 3). Fire ants were completely excluded from honeydew-misted plants that had a ring of Tanglefoot applied around the base of the stem (Fig. 3A). Fire ants actively foraged on the honeydew-misted plants without the Tanglefoot ant barrier, however, and were significantly more abundant on those plants than on water-misted control plants (Fig. 3A). The abundance of all other predators combined (big-eyed bugs, damsel bugs, long-legged flies, and spiders) on the plants also varied among the three treatments, but differently from that of fire ants (Table 3). Total predator abundance did not differ between honeydew-misted plants with and without fire ants, and was significantly greater on each set of honeydew-misted plants than on control plants (Fig. 3B). The only predator taxon affected individually by the three treatments, however, was spiders (Table 3). Spiders were marginally significantly more abundant

( $P = 0.05$ ) on honeydew-misted plants without fire ants ( $1.0 \pm 0.1$  spider per plant) than on control plants ( $0.5 \pm 0.1$ ). Spider abundance did not statistically differ between honeydew-misted plants with fire ants ( $0.9 \pm 0.1$ ) and control plants ( $P = 0.19$ ), or between honeydew-misted plants with and without fire ants ( $P = 0.69$ ).

Both the abundance of beet armyworm caterpillars and caterpillar damage to leaves varied among the three treatments (Table 3). Caterpillars were significantly less abundant on honeydew-misted plants with fire ants than on water-sprayed control plants ( $P = 0.02$ ) or on honeydew-misted plants without fire ants ( $P = 0.01$ ) (Fig. 3C).

Similarly, leaf damage by caterpillars was significantly lower on honeydew-misted plants with fire ants than on control plants ( $P = 0.002$ ) or on honeydew-misted plants without fire ants ( $P = 0.04$ ) (Fig. 3D). Neither caterpillar abundance ( $P = 0.77$ ) nor caterpillar damage to leaves ( $P = 0.26$ ) measurably differed between control plants and honeydew-misted plants without fire ants (Fig. 3C, D).

### *Greenhouse experiment 1*

Fire ants began tending soybean aphids within 12 hours after individual plants had been placed in the pans with fire ant colonies. Averaged over the four day sampling period, fire ants were significantly more abundant on plants with soybean aphids ( $14.0 \pm 1.3$  ants per plant) than on plants without soybean aphids ( $0.2 \pm 1.4$ ) in the two sets of plants that were exposed to fire ants ( $F_{1,13} = 86.32$ ,  $P < 0.0001$ ). Treatment effect did not vary across the sampling period (no treatment x day interaction:  $F_{3,75} = 0.56$ ,  $P = 0.64$ ).

The effects of the fire ant treatment and soybean aphid treatment interacted over the four-day sampling period to influence beet armyworm caterpillar survival (fire ant x

soybean aphid x day interaction, Table 4). Caterpillar survival remained very high over time on plants that were not exposed to fire ants, whether soybean aphids were present or absent (Fig. 4). In contrast, caterpillar survival declined over time on plants that were exposed to fire ants. Fewer caterpillars, however, survived on plants with soybean aphids (and tending fire ants) than on plants without soybean aphids (and far fewer fire ants) (Fig. 4). By the final sampling day, caterpillar survival on plants with fire ant-tended soybean aphids ( $31 \pm 4\%$ ) was half that of caterpillar survival on plants exposed to fire ants but without soybean aphids ( $62 \pm 5\%$ ;  $P < 0.0001$ ) (Fig. 4).

The effects of the fire ant treatment and the soybean aphid treatment did not interact to influence caterpillar dispersal and feeding on plants ( $F_{1,52} = 0.21$ ,  $P = 0.65$ ). The percentage of leaflets damaged by caterpillar feeding was significantly lower on plants exposed to fire ants ( $40 \pm 3\%$ ) than on plants not exposed to fire ants ( $72 \pm 3\%$ ) whether soybean aphids were present or not ( $F_{1,52} = 64.46$ ,  $P < 0.0001$ ). Caterpillars damaged a slightly lower percentage of leaflets on plants with soybean aphids ( $52 \pm 3\%$ ) than on plants without soybean aphids ( $60 \pm 3\%$ ) whether fire ants were present or not, but the difference was not quite statistically significant ( $F_{1,52} = 3.30$ ,  $P = 0.07$ ).

### *Greenhouse experiment 2*

Fire ants were significantly more abundant on plants with soybean aphids and on plants misted with artificial honeydew than on control plants ( $F_{2,21} = 22.16$ ,  $P < 0.0001$ ) in the greenhouse experiment with big-eyed bugs (Fig. 5A). Consequently, big-eyed bug nymph survival was significantly greater on control plants than on plants with soybean aphids or on plants misted with artificial honeydew ( $F_{2,21} = 3.68$ ,  $P = 0.04$ ) (Fig. 5A).



Fire ants were also significantly more abundant on plants with soybean aphids and on plants misted with artificial honeydew than on control plants ( $F_{2,21} = 7.10$ ,  $P = 0.004$ ) in the greenhouse experiment with damsel bugs (Fig. 5B). Damsel bug nymph survival, however, did not differ statistically among the three treatments ( $F_{2,21} = 0.43$ ,  $P = 0.66$ ) (Fig. 5B).

## DISCUSSION

Previous studies of the effects of fire ants as predators in agricultural systems in Alabama have found that fire ants exert much stronger and more pervasive direct negative effects on the arthropod community in cotton than in collard, tomato, or soybean crops (Eubanks 2001, Eubanks et al. 2002, Harvey and Eubanks 2004, 2005, Cooper 2005, Kaplan and Eubanks 2002, 2005, Styrsky and Eubanks 2006). This pattern is attributed to the fact that cotton hosts an abundant honeydew-producing insect, the cotton aphid, which increases the abundance and aggressiveness of fire ants on plants, thereby generating strong indirect effects on other cotton arthropods and on cotton plants (Kaplan and Eubanks 2005, Chapter 3). Here, I present the results of field and greenhouse experiments designed to determine the potential for similar indirect effects in soybean following the range expansion of an invasive aphid, the soybean aphid, into the range of fire ants. My results suggest that soybean aphids may dramatically increase the direct effects of fire ants on some soybean arthropod taxa, thereby indirectly benefiting soybean plants.

In the first field experiment, significantly fewer caterpillars (the most abundant herbivore taxon in my soybean plots) foraged on plants misted with artificial honeydew

than on water-misted control plants (Fig. 2A), resulting in significantly less caterpillar damage to leaves on honeydew-misted plants (Fig. 2B). Whether the reduction in caterpillar abundance and damage was caused specifically by the greater number of fire ants on honeydew-sprayed plants relative to control plants is uncertain because the application of artificial honeydew attracted other generalist predators, including big-eyed bugs, long-legged flies, and spiders. The purpose of the second field experiment, therefore, in which I excluded fire ants from some honeydew-misted plants using Tanglefoot, was to isolate the effects of fire ants on caterpillars and to determine whether fire ants negatively affected the non-ant predator guild. Significantly fewer beet armyworm caterpillars foraged on honeydew-misted plants with fire ants than on honeydew-misted plants from which fire ants were excluded (Fig. 3C). Similarly, honeydew-misted plants with fire ants were significantly less damaged by caterpillars than were honeydew-misted plants from which fire ants were excluded (Fig. 3D). These results indicate that fire ants attracted to artificial honeydew had much stronger effects than all non-ant predators combined, and suggest that the reduced caterpillar abundance on honeydew-misted plants in the first field experiment was caused by fire ant predation specifically. Further, combined non-ant predator abundance was no lower on honeydew-misted plants with fire ants than on honeydew-misted plants from which fire ants were excluded, indicating that fire ants had no measurable effect as intraguild predators (Fig. 3B).

Results of the greenhouse experiments are mostly consistent with results from the field experiments. Beet armyworm caterpillar survival on plants was most reduced when fire ants were attracted onto plants by soybean aphids (Fig. 4), thus providing direct

evidence that soybean aphids can generate strong indirect effects on another important group of herbivores as a consequence of engaging fire ants in a mutualistic interaction. The percentage of soybean leaflets damaged by caterpillars in the presence of fire ants did not differ, however, between plants with or without soybean aphids, suggesting that the soybean aphid – fire ant mutualism did not appreciably affect caterpillar dispersal on plants. Inconsistent with the field data, increased fire ant abundance on plants with soybean aphids and on plants misted with artificial honeydew reduced the survival of big-eyed bug nymphs compared to control plants with almost no fire ants (Fig. 5A). In contrast (but consistent with the field data), increased fire ant abundance on plants with soybean aphids and on honeydew-misted plants had no effect on the survival of damsel bugs nymphs (Fig. 5B).

Combined, the results of the field and greenhouse experiments suggest that a facultative mutualism between soybean aphids and fire ants will have limited effects on soybean arthropod community structure. The application of artificial honeydew to plants strongly affected the abundance of only one important group of herbivores (caterpillars) via increased fire ant predation, and neither herbivore diversity nor total arthropod diversity differed between honeydew-misted versus control plants. In contrast, increased fire ant abundance on plants misted with artificial honeydew did slightly reduce predator diversity, despite having no appreciable effect on the abundance of non-ant predators. Previous studies of the community-level consequences of mutualistic interactions between honeydew-producing Hemipterans and ants have reported much stronger effects. Fowler and MacGarvin (1985), for example, found that aphid-tending ants reduced total herbivore species richness in birch (*Betula pubescens*) by 28%. Similarly, in an

experiment in which aphids were removed from cottonwood (*Populus angustifolia*) saplings, aphid-tending ants abandoned the trees resulting in an 80% increase in total arthropod abundance and a 57% increase in total species richness (Wimp and Whitham 2001). Kaplan and Eubanks (2005) did not report whether a mutualism between cotton aphids and fire ants affected cotton arthropod diversity in large-scale field manipulations of fire ant densities, but aphid-tending fire ants significantly reduced the abundance of 27-33% of the herbivore taxa sampled and 40-47% of the predator taxa sampled.

Low taxonomic resolution may have compromised the strength of our analyses of arthropod diversity in the first field experiment. Sampling plants for arthropods using a beat cloth hinders identification of individuals to species because the dislodged arthropods must be identified and counted very quickly before they run off of the beat cloth. In addition, many early-instar insects (particularly caterpillars) are too small to identify to species in the field with the naked eye. Both Fowler and MacGarvin (1985) and Wimp and Whitham (2001) used a visual sampling technique that allowed finer taxonomic resolution, which may account for their ability to have documented strong indirect negative effects of ant-tended aphids on arthropod species richness. Further, fire ants may respond more aggressively to actual aggregations of honeydew-producing aphids than to a sheen of artificial honeydew on the plant surface because aphid aggregations may be perceived by fire ants as more defensible, and aphid honeydew may be more valuable to fire ants as a food resource. Artificial honeydew, therefore, may elicit a more anemic response in fire ants than would soybean aphid honeydew, weakening the overall effect of fire ants as predators of other soybean arthropods.

Fire ants attracted onto plants by artificial honeydew did not have strong and pervasive effects on the soybean arthropod community, but they indirectly protected soybean plants from feeding damage caused by caterpillars. These results are consistent with previous work in cotton which demonstrated that cotton aphids indirectly reduced caterpillar damage to plants by increasing fire ant predation of leaf- and boll-feeding caterpillars (Chapter 3). Several other studies also report strong indirect effects of honeydew-producing Hemipterans on plant fitness via increased predation of other herbivores by Hemipteran-tending ants (reviewed in Styrsky and Eubanks, in review). Messina (1981), for instance, reported that ants that were attracted onto goldenrod plants (*Solidago altissima*) by aggregations of a honeydew-producing treehopper prevented plant defoliation by two leaf beetles (Chrysomelidae), resulting in greater plant growth and seed production relative to neighboring plants that did not host treehoppers. Similarly, myrmecophilous aphids on oaks (*Quercus dentata*) indirectly protected the trees from damage by acorn-boring caterpillars by attracting predaceous wood ants (*Formica yessensis*) (Ito and Higashi 1991).

Although soybean aphids may enhance the beneficial effects of fire ants as predators of caterpillar pests in soybean, fire ants attracted to artificial honeydew in the field experiments had no measurable effect on other soybean pests, including stink bugs and plant bugs, and even caused a slight increase in the abundance of another Hemipteran pest, the three-cornered alfalfa hopper. Alfalfa hoppers also feed on phloem sap and produce honeydew, but they typically occur in low densities, eliciting a much weaker numerical response by fire ants than do cotton aphids in cotton, for example (J. D. Styrsky, unpublished data). The greater abundance of fire ants on honeydew-misted

plants in this study may have increased fire ant protection of alfalfa hoppers and, consequently, alfalfa hopper abundance. At high densities, alfalfa hoppers can cause significant yield losses by feeding on the petioles and racemes during the pod-setting and pod-filling stages (Kogan and Turnipseed 1987); thus, soybean aphids may indirectly harm soybean plants by increasing ant protection of alfalfa hoppers.

More importantly, the direct negative effects of soybean aphids themselves on soybean plants may outweigh any indirect benefit of increased fire ant predation of caterpillars. Soybean aphid feeding can cause reduced photosynthetic capacity (Macedo et al. 2003), stunted growth, and reduced pod set (Nielsen and Hajek 2005), resulting in seed yield losses as high as 40% (DiFonzo and Hines 2002). Soybean aphids also vector two important viruses of soybean plants, *Soybean mosaic virus* and *Alfalfa mosaic virus* (Clark and Perry 2002). By protecting soybean aphids from predators, fire ants may facilitate soybean aphid outbreaks, thereby exacerbating the negative effects of soybean aphids on plants. In an experimental study of the effect of mutualistic interactions between aphids and fire ants in tomato (*Lycopersicon esculentum*), for example, the presence of fire ants caused a 240% increase in aphid abundance on plants, resulting in a 65% increase in the level of infection of plants by the aphid-vectored *Cucumber mosaic virus* (Cooper 2005). The net consequence of the soybean aphid – fire ant mutualism to soybean yield in any particular season and location, therefore, will depend on the magnitude of the indirect benefit of increased fire ant suppression of non-aphid pests relative to the magnitude of the direct cost of soybean aphid feeding damage. Because soybean plants are actually very tolerant of damage by foliage-feeding herbivores (Kogan

and Turnipseed 1987), increased fire ant suppression of caterpillars in the presence of soybean aphids may not often outweigh damage caused by soybean aphids.

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Table 1. Results of repeated measures ANCOVA of the effects of artificial honeydew on A. predator abundance on soybean plants and B. herbivore abundance and caterpillar damage to leaves in field experiment 1. Significant treatment effects in bold.

<b>A. Predators</b>		Big-eyed bugs	Damsel bugs	Hooded beetles	Long-legged flies <sup>a</sup>	Spiders
Factor	d.f.	<i>F</i> ( <i>P</i> )	<i>F</i> ( <i>P</i> )	<i>F</i> ( <i>P</i> )	<i>F</i> ( <i>P</i> )	<i>F</i> ( <i>P</i> )
Treatment	1,9	5.35 ( <b>0.05</b> )	3.60 (0.09)	3.70 (0.09)	51.71 (< <b>0.0001</b> )	21.51 ( <b>0.001</b> )
Sampling date	5,45	1.69 (0.16)	2.23 (0.07)	12.78 (<0.0001)	2.22 (0.09)	6.87 (<0.0001)
Plant height	1,98	0.01 (0.94)	4.18 (0.04)	3.52 (0.06)	0.02 (0.90)	0.02 (0.88)
Treatment x Date	5,45	0.60 (0.70)	0.33 (0.89)	0.46 (0.80)	1.31 (0.28)	3.47 ( <b>0.01</b> )
<b>B. Herbivores</b>		Stink bugs	Plant bugs	Alfalfa hoppers	Caterpillars	Caterpillar damage
Factor	d.f.	<i>F</i> ( <i>P</i> )	<i>F</i> ( <i>P</i> )	<i>F</i> ( <i>P</i> )	<i>F</i> ( <i>P</i> )	<i>F</i> ( <i>P</i> )
Treatment	1,9	0.00 (0.97)	0.42 (0.53)	4.78 ( <b>0.05</b> )	21.90 ( <b>0.001</b> )	13.64 ( <b>0.005</b> )
Sampling date	5,45	1.62 (0.17)	3.72 (0.007)	2.33 (0.05)	33.86 (<0.0001)	10.41 (<0.0001)
Plant height	1,98	1.63 (0.20)	8.24 (0.005)	0.83 (0.37)	0.00 (0.96)	0.07 (0.80)
Treatment x Date	5,45	0.67 (0.65)	1.03 (0.41)	0.09 (0.99)	3.44 ( <b>0.01</b> )	2.53 ( <b>0.04</b> )

<sup>a</sup> Sampled on five dates rather than six; thus, d.f. date and treatment x date = 4,36, and d.f. plant height = 1,80.

Table 2. Results of repeated measures ANCOVA of the effects of artificial honeydew on taxa diversity of herbivores, predators, and all arthropods on soybean plants in field experiment 1. Significant treatment effects in bold.

Factor	d.f.	Herbivores	Predators	All arthropods
		<i>F (P)</i>	<i>F (P)</i>	<i>F (P)</i>
Treatment	1,9	1.37 (0.27)	6.11 ( <b>0.04</b> )	0.15 (0.71)
Sampling date	5,45	2.47 (0.05)	3.63 (0.008)	2.27 (0.06)
Plant height	1,98	0.27 (0.61)	9.05 (0.003)	0.59 (0.44)
Treatment x date	5,45	0.59 (0.71)	1.47 (0.22)	0.90 (0.49)

Table 3. Results of repeated measures ANCOVA of the effects of artificial honeydew on the abundance of fire ants, other predators, and caterpillars, and on caterpillar damage to soybean plants in field experiment 2.

Factor	Fire ants		All other predators		Caterpillars		Caterpillar damage	
	d.f.	$F(P)$	$F(P)$	$F(P)$	$F(P)$	$F(P)$	$F(P)$	$F(P)$
Treatment	2,27	5.29 ( <b>0.01</b> )	6.80 ( <b>0.004</b> )	4.05 ( <b>0.03</b> )	5.93 ( <b>0.007</b> )			
Sampling date	4,106	1.52 (0.10)	8.44 (<0.0002)	3.76 (0.007)	13.35 (<0.0001)			
Plant height	1,106	2.45 (0.33)	32.00 (<0.0001)	0.89 (0.35)	5.99 (0.02)			
Treatment x Date	8,106	0.88 (0.99)	0.49 (0.86)	0.36 (0.94)	0.90 (0.61)			
<hr/>								
Factor	Big-eyed bugs		Damsel bugs		Spiders		Long-legged flies	
	d.f.	$F(P)$	$F(P)$	$F(P)$	$F(P)$	$F(P)$	$F(P)$	$F(P)$
Treatment	2,27	2.99 (0.07)	1.46 (0.25)	3.37 ( <b>0.05</b> )	1.24 (0.30)			
Sampling date	4,106	5.76 (0.0003)	1.25 (0.30)	8.81 (<0.0001)	7.89 (<0.0001)			
Plant height	1,106	6.83 (0.01)	0.80 (0.37)	1.68 (0.20)	18.88 (<0.0001)			
Treatment x Date	8,106	1.14 (0.34)	0.92 (0.50)	0.33 (0.95)	0.97 (0.46)			

Table 4. Results of repeated measures ANOVA on the effects of fire ants and soybean aphids on the survival of beet armyworm caterpillars on soybean plants in greenhouse experiment 1. Significant treatment effects in bold.

Factor	<i>F</i>	df	<i>P</i>
Block	0.97	13,39	0.50
Fire ants	32.43	1,39	<b>&lt;0.0001</b>
Soybean aphids	3.84	1,39	0.06
Day	54.80	3,149	<0.0001
Fire ants x day	26.04	3,149	<b>&lt;0.0001</b>
Soybean aphids x day	3.43	3,149	<b>0.02</b>
Fire ants x soybean aphids	3.87	1,39	0.06
Fire ants x soybean aphids x day	3.02	3,149	<b>0.03</b>



FIG. 1. Mean ( $\pm 1$  SE) number of spiders per beat-cloth sample of soybean foliage misted with artificial honeydew or water (control treatment) in 2004. Spiders were significantly more abundant on plants misted with artificial honeydew on the dates indicated with asterisks.

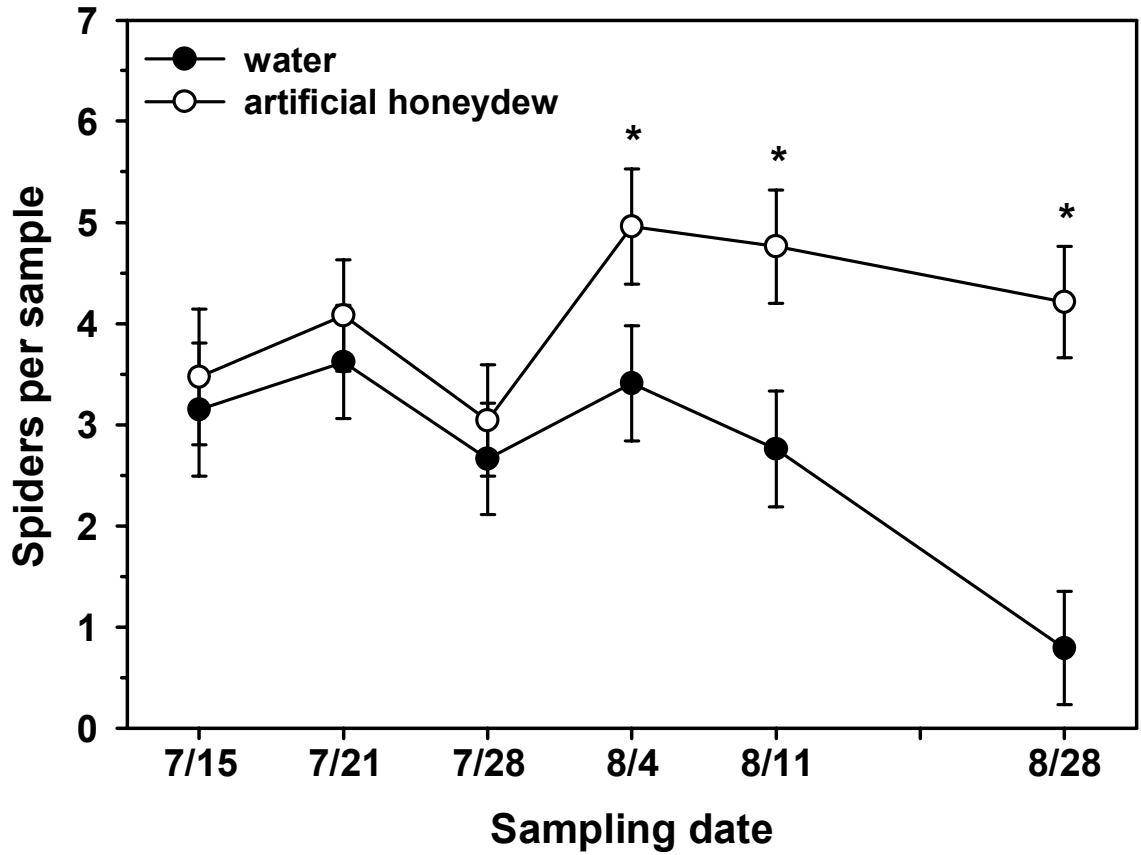


FIG. 2. (A) Mean ( $\pm 1$  SE) number of caterpillars per beat-cloth sample of soybean foliage, and (B) mean ( $\pm 1$  SE) percent leaf damage by caterpillars on soybean plants misted with artificial honeydew or water (control treatment) in 2004. Caterpillar abundance and caterpillar damage to leaves were significantly reduced on plants misted with artificial honeydew on the dates indicated with asterisks.

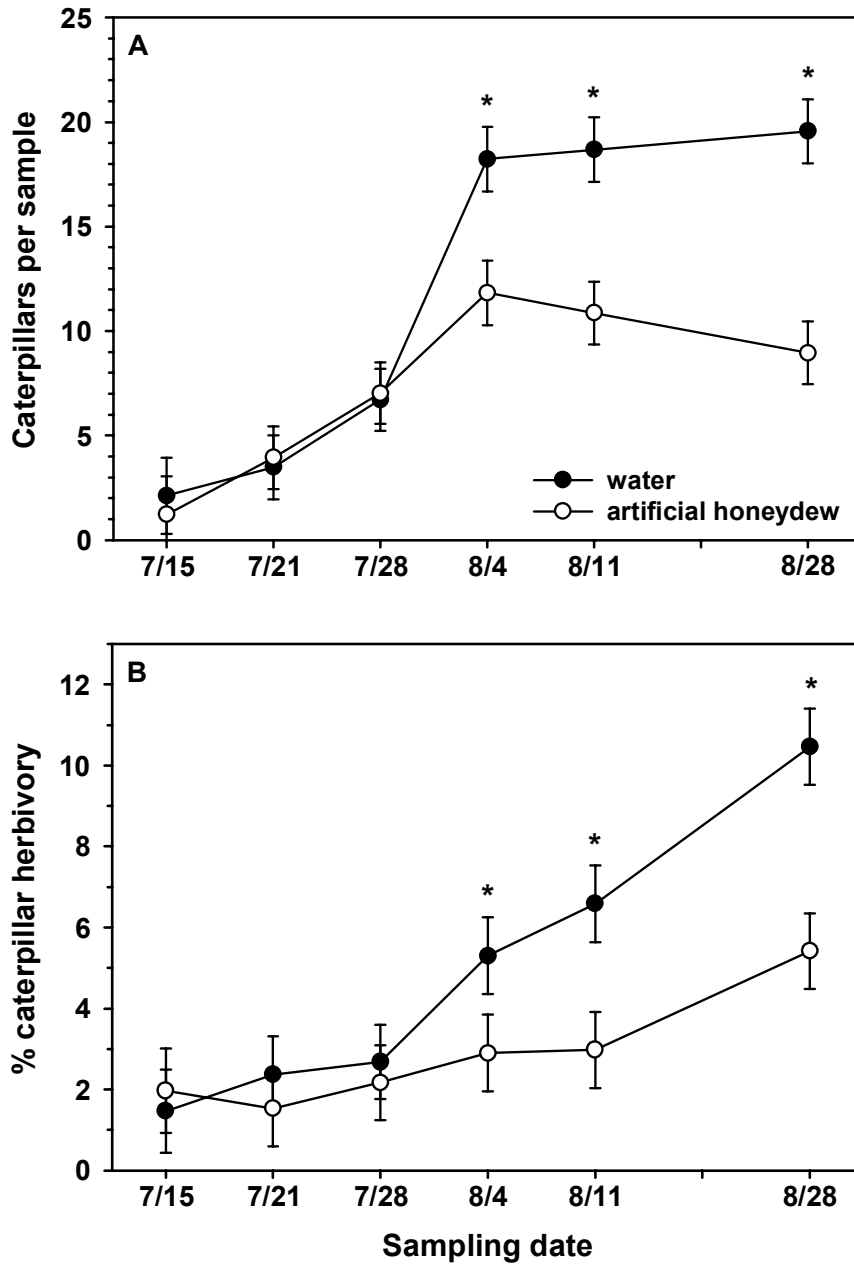


FIG. 3. Mean ( $\pm 1$  SE) number of (A) fire ants per plant, (B) non-ant predators per plant, and (C) caterpillars per plant, and (D) mean ( $\pm 1$  SE) percent leaf damage by caterpillars on individual soybean plants in three treatments in 2004: plants misted with water (control), plants misted with artificial honeydew, and plants misted with honeydew from which fire ants were excluded with Tanglefoot. Within each panel, different letters above bars indicate significant differences among treatment means.

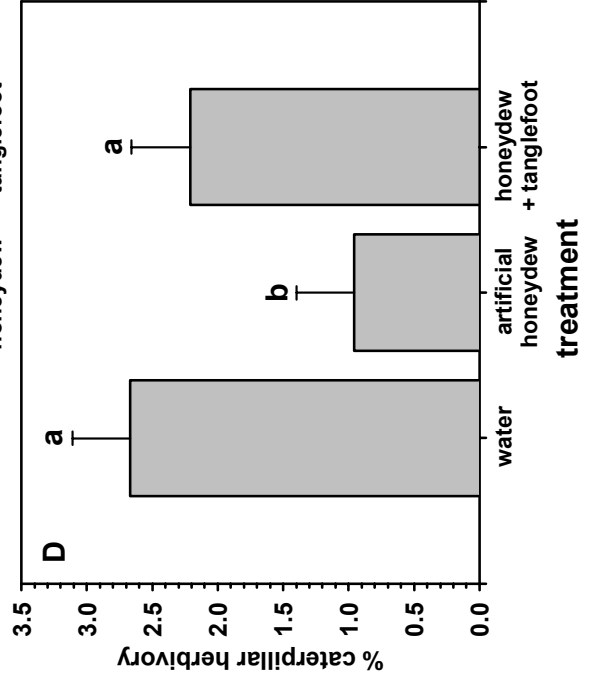
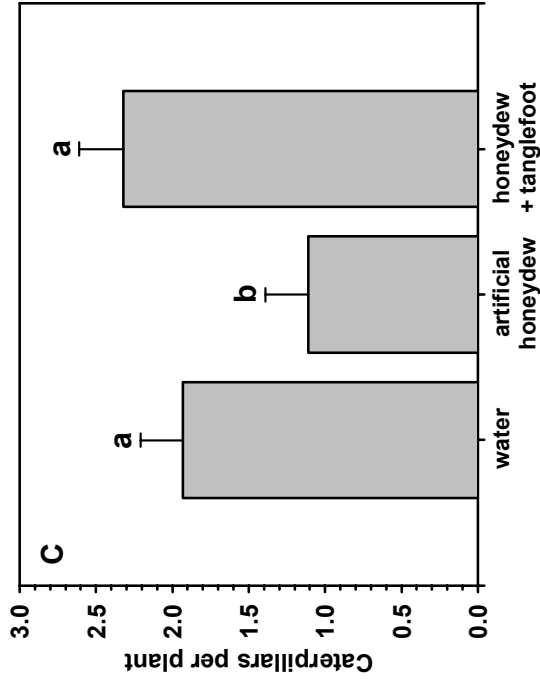
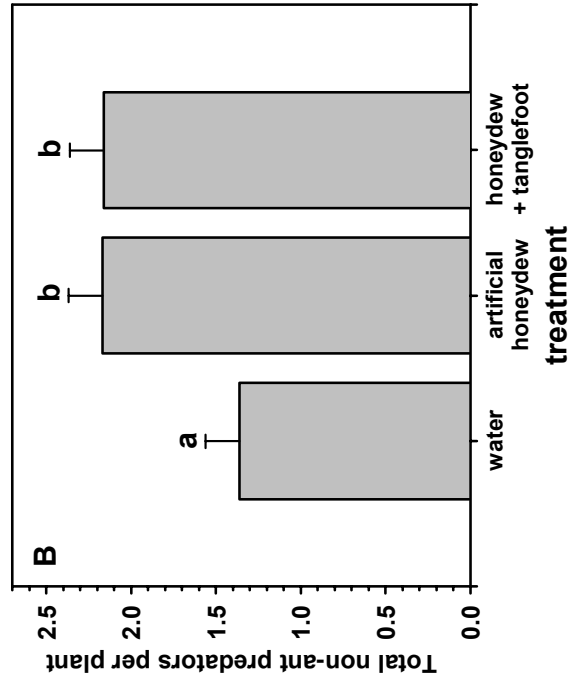
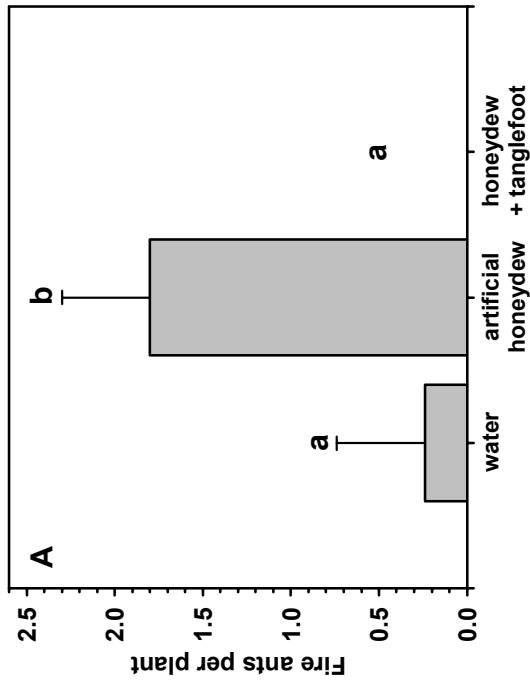


FIG. 4. Mean ( $\pm 1$  SE) percent survival of beet armyworm caterpillars over four days on soybean plants with and without soybean aphids either exposed or not exposed to fire ants in the 2004 greenhouse experiment. The presence of fire ants reduced caterpillar survival whether soybean aphids were present or absent, but caterpillar survival was most reduced on plants with fire ant-tended aphids.

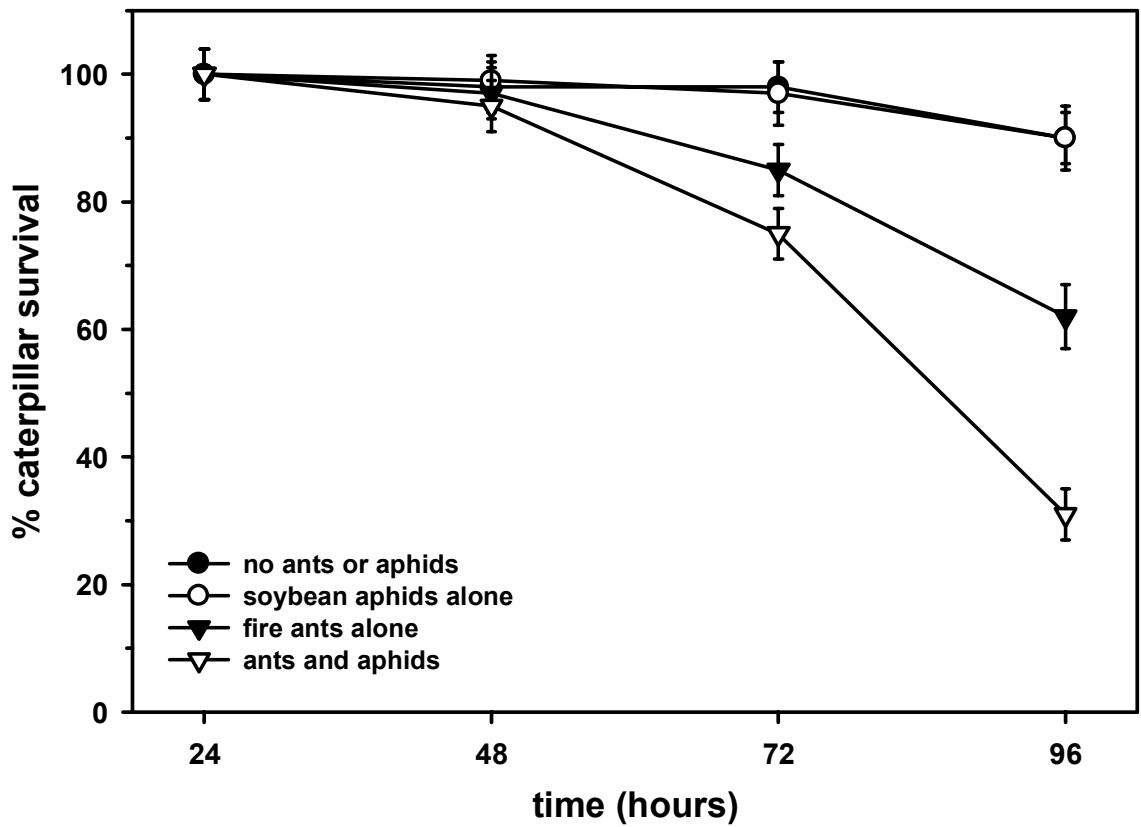


FIG. 5. Mean ( $\pm 1$  SE) number of fire ants per plant and mean ( $\pm 1$  SE) percent survival of (A) big-eyed bug nymphs and (B) damsel bug nymphs on individual soybean plants in three treatments in the 2005 greenhouse experiments: unmanipulated (control) plants, plants misted with artificial honeydew, and plants hosting soybean aphids. Within each panel, different letters above bars of the same color indicate significant differences among treatment means.

