

Plant trichomes indirectly enhance tritrophic interactions involving a generalist predator, the red imported fire ant

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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 we 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 more far more common than is currently appreciated.

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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 (Bottrell et al., 1998; Cortesero et al., 2000; Kennedy, 2003; Price et al., 1980). 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

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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; Krips et al., 1999; Lovinger et al., 2000; Sutterlin and van Lenteren, 1997; Treacy et al., 1986, 1987). As a result, trichomes decrease herbivore suppression by both parasitoids (Kaufman and Kennedy, 1989; McAuslane et al., 1995; Treacy et al., 1986) and predators (Barbour et al., 1993; Krips et al., 1999; Treacy et al., 1985, 1987). 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 12-spotted lady beetle, *Coleomegilla maculata* Timberlake (Coleoptera: Coccinellidae), predation of whitefly, *Trialetrodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), eggs by physically obstructing 4th-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, we 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 (Holway et al., 2002; Vinson, 1997; Wojcik et al., 2001). Although fire ants are reportedly significant predators of insect pests in several crops (reviewed in Holway et al., 2002; Taber, 2000), the utility of fire ants as biological control agents has not been well established because they may attack or disrupt other predators and parasitoids (Eubanks et al., 2002; Kaplan and Eubanks, 2002; Tedders et al., 1990; Vinson and Scarborough, 1989, 1991). 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, we present the results of field and greenhouse experiments in which we 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. We 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, we 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

We conducted the field experiment at the E.V. Smith Research Center of the Auburn University Agricultural Experiment Station in Macon County, Alabama, USA 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 × 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, USA) 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 isolate were planted in four 6.1 × 9.1 m plots arranged as quarters of a larger, 15.2 × 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 isolate within each plot was determined at random. Three meter 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, we suppressed fire ant densities in three of the six study fields by broadcasting approximately 40 g of Amdro 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 hydromethylnon, 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.

We 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-m² 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. We 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 isolate 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 (g/m²) for each isolate 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, we 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. We 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 ± 1 SE are presented in the text and P values ≤ 0.05 are considered significant in all analyses. Based on our predictions, we were 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, we collected at random six individual plants of each isolate 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-cm² section of leaf using a dissecting microscope with an ocular micrometer. Trichome density (number/cm²) 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 6th and 7th node, and trichomes were counted on the petiole and underside of one fully expanded leaf haphazardly chosen from the upper half of the plant. We tested for differences in trichome densities among the three isolines using a MANOVA (proc glm; SAS Institute, Inc., 2001). Means ± 1 SE are presented in the text.

2.2. Greenhouse experiment

We conducted the greenhouse experiment on the campus of Auburn University, Auburn, Alabama, USA 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 $2 \times 2 \times 3$ 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 gemmatilis* (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. We 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 isolate) 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 \times 33 \times 75 cm) that sat within a 37-L plastic tub 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 \times 43 \times 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 2nd-instar velvetbean caterpillars were placed on each plant in each treatment. At the same time, three 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).

We 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, we 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). We 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). We 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 ± 128 trichomes/cm²; petiole = 663 ± 70 ; leaves = 823 ± 128) than in the moderately pubescent isoline (stem = 477 ± 55 ; petiole = 335 ± 40 ; leaves = 350 ± 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).

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

	Wilks' λ	F	df	P	Standardized canonical coefficient ^a		
					Stem	Petiole	Leaf
Main effect							
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

^a 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.

3.1.2. Fire ants

The effects of the Amdro treatment and trichome density interacted to influence the abundance of fire ants on plants ($F_{2,457} = 3.74$, $P = 0.025$). Averaged across all sampling dates, fire ant abundance was statistically indistinguishable from zero in each soybean isoline 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

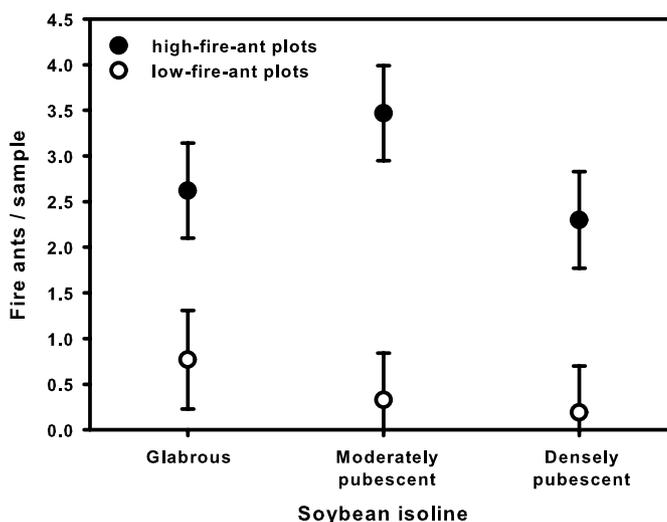


Fig. 1. Mean (\pm 1 SE) number of fire ants per beat-cloth sample of soybean foliage in high-fire-ant (untreated) and low-fire-ant (Amdro-treated) plots of three soybean isolines varying in trichome density in 2002. The application of Amdro effectively reduced the abundance of fire ants on plants and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

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, *Spis-sistilus 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 × 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 isolate as the season progressed (fire ant × isolate × 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 × isolate 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 × date interaction, Table 2). The beneficial effect of fire ants on *S. festinus* abundance varied with trichome density (fire ant × isolate 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 indepen-

Table 2

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

Factor	df	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 × date	6,457	3.69	0.001	3.20	0.004	6.79	0.0001
Isoline × date	12,457	5.30	<0.0001	4.13	<0.0001	1.50	0.12
Fire ants × isolate	2,457	0.18	0.83	4.96	0.007	3.32	0.04
Fire ants × isolate × date	12,457	3.51	<0.0001	0.64	0.81	0.58	0.86

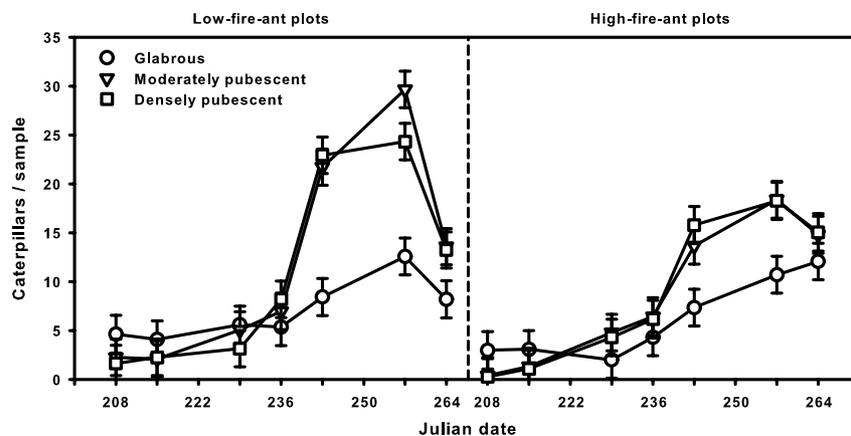


Fig. 2. Mean (± 1 SE) number of caterpillars per beat-cloth sample of soybean foliage over the 2002 growing season in high-fire-ant and low-fire-ant plots of three soybean isolines varying in trichome density. Caterpillars were least abundant on glabrous plants later in the season but fire ants most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

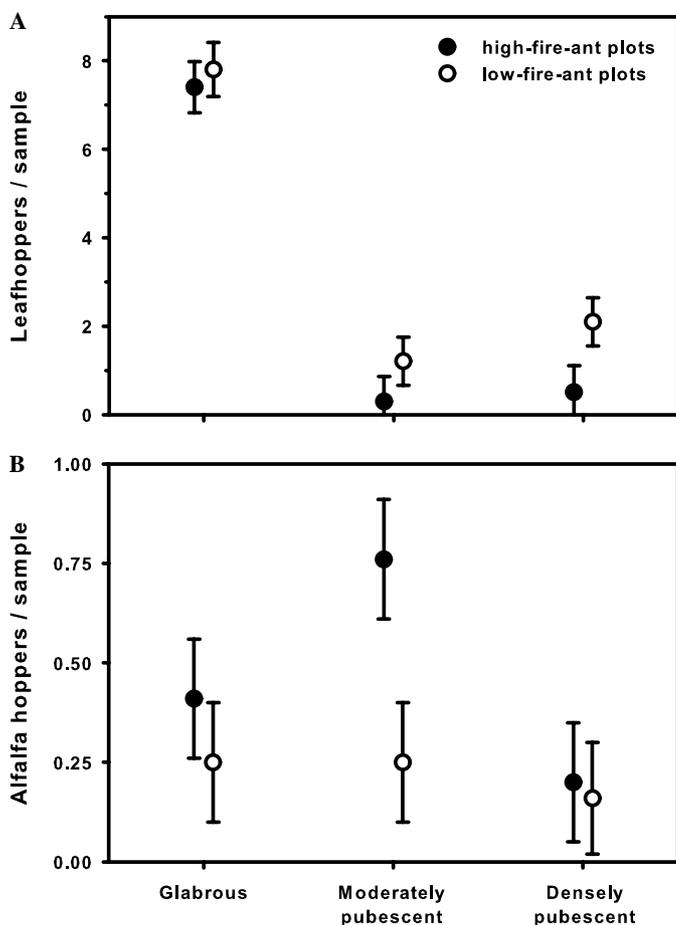


Fig. 3. Mean (± 1 SE) number of (A) leafhoppers and (B) three-cornered alfalfa hoppers per beat-cloth sample of soybean foliage in high-fire-ant and low-fire-ant plots of three soybean isolines varying in trichome density in 2002. Fire ants most reduced leafhopper abundance on pubescent plants. Fire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

dently 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 \times 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 ± 0.3 bugs/sample) and densely pubescent plants (3.0 ± 0.3) than on glabrous plants (1.9 ± 0.3 ; $P < 0.0001$ for both comparisons). In contrast, damsel bug abundance did not differ between pubescent plants and glabrous plants (1.5 ± 0.5 bugs/sample); however, damsel bugs were more abundant on moderately pubescent plants (1.7 ± 0.5) relative to densely pubescent plants (1.3 ± 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

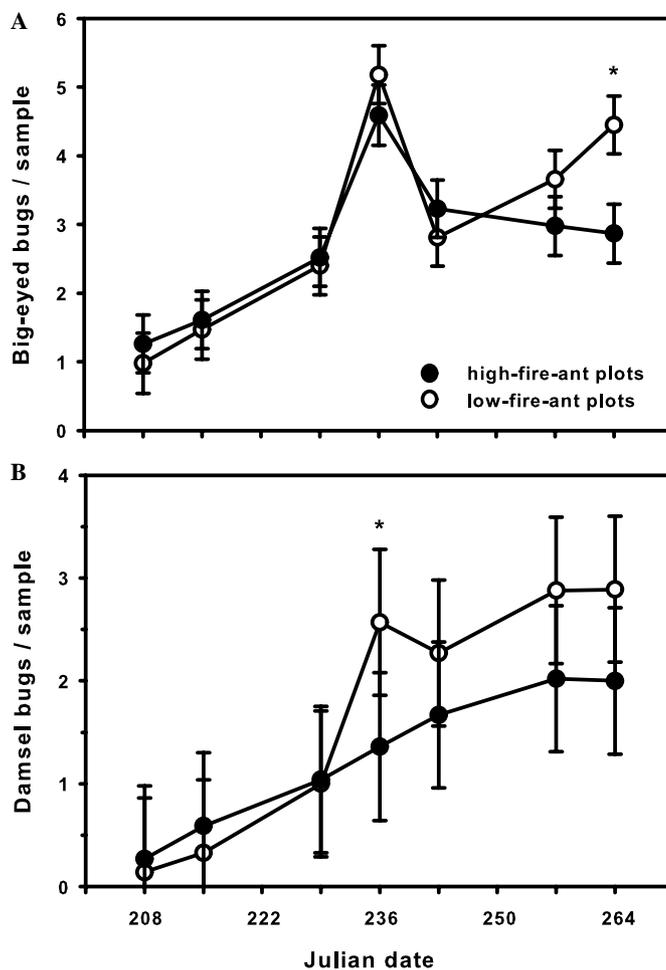


Fig. 4. Mean (± 1 SE) number of (A) big-eyed bugs and (B) damsel bugs per beat-cloth sample of soybean foliage over the 2002 growing season in high-fire-ant and low-fire-ant plots of soybean plants. Natural enemies were significantly more abundant in the low-fire-ant plots on the dates indicated with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

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 \times 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 \times isolate \times 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).

Table 3

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

Factor	df	Big-eyed bugs		Damsel bugs		Spiders	
		F	P	F	P	F	P
Fire ants	1,4	0.17	0.70	0.23	0.65	0.72	0.44
Isoline	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 × date	6,457	3.05	0.006	5.22	<0.0001	0.76	0.60
Isoline × date	12,457	2.46	0.004	1.32	0.20	0.79	0.66
Fire ants × isolate	2,457	0.73	0.48	0.88	0.41	0.18	0.84
Fire ants × isolate × date	12,457	0.71	0.75	0.92	0.53	1.23	0.26

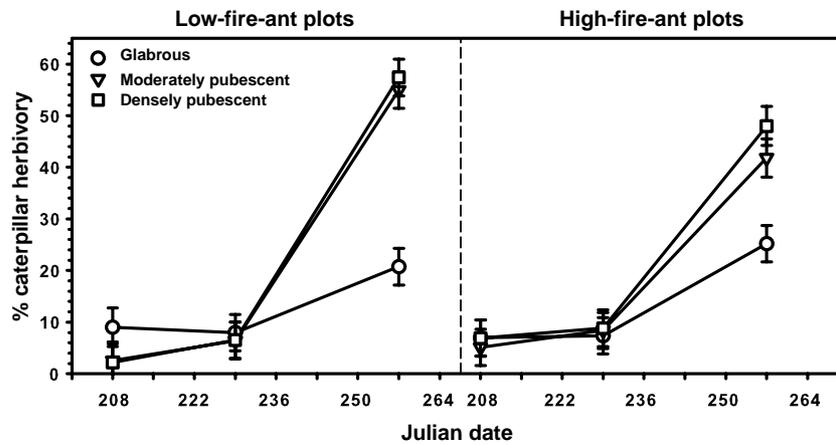


Fig. 5. Mean (± 1 SE) percent leaf damage by caterpillars over the 2002 growing season in high-fire-ant and low-fire-ant plots of three soybean isolines varying in trichome density. Fire ants suppressed caterpillar herbivory only on pubescent plants.

Although the effects of fire ant abundance and trichome density interacted to influence caterpillar herbivory, the effect of the fire ant \times isolate 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 ± 0.4 g/m²) was no greater than that of either pubescent isolate. Yield differed statistically only between moderately pubescent plants (5.7 ± 0.3 g/m²) and densely pubescent plants (4.8 ± 0.3 g/m²; $P = 0.03$). 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 ± 0.9 g/m²) relative to low-fire-ant plots (4.8 ± 0.9 g/m²).

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 ± 0.3 ants/plant; moderately pubescent: 0.9 ± 0.3 ; densely pubescent: 1.2 ± 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 \times isolate 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 (Cortesero et al., 2000; Bottrell et al., 1998). 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, we 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. Our 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. We are 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; Lucas et al., 2004; Obrycki and Tauber, 1984), lacewing larvae and adults (Fordyce and Agrawal, 2001; Treacy et al., 1985, 1987), an anthocorid bug (Coll et al., 1997), a predaceous stinkbug (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. We 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.

We 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 (Harvey and Eubanks, 2004; Risch and Carroll, 1982; Way and Khoo, 1992). 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

In previous work, we have 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 appe-

ciably increase intraguild predation in this study, our 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 stinkbug, *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae), an important pod-feeding pest (Krispyn and Todd, 1982). Our 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 *Spissitilus festinus* nymphs for their honeydew, thereby protecting them from other predators. *S. 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). We 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.

5. 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. We 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 (Hölldobler and Wilson, 1990; Holway et al., 2002; Way and Khoo, 1992). We 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, our 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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