

Friend or Foe?: A Plant's Induced Response to an Omnivore

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ABSTRACT Omnivorous natural enemies of herbivores consume plant-based resources and may elicit induced resistance in their host plant. A greater induction threshold for damage produced by omnivorous predators than for strict herbivores might be expected if omnivore performance is enhanced on noninduced plants, allowing them to reduce future levels of herbivory. Currently, it is not known if a plant responds to feeding by omnivorous predators and by herbivores similarly. To examine this question, we chose herbivore and omnivore species that produce the same kind of quantifiable damage to cotton leaves, enabling us to control statistically for the intensity of plant damage, and ask whether plant responses differed depending on the identity of the damaging species. We first compared changes in plant peroxidase activity, gossypol gland number and density, and leaf area in response to feeding by the spider mite *Tetranychus turkestanii* (Ugarov and Nikolski) (an herbivore) and by one of the mite's principal natural enemies, the western flower thrips *Frankliniella occidentalis* (Pergande) (an omnivore). Both species increased the activity of peroxidase, but when we controlled for the amount of damage, the peroxidase activity of mite-damaged plants was higher than that of thrips-damaged plants. We also found that thrips, but not spider mites, increased the density of gossypol glands in the second true leaf. In a second experiment we included an additional herbivore, the bean thrips *Caliothrips fasciatus* (Pergande), to see if the different responses of cotton to thrips and mite herbivory we first observed were attributable to differences in trophic function (herbivore versus omnivore) or to other differences in feeding generated by thrips versus mites. Cotton plants exhibited the same pattern of induced responses (elevated peroxidase, increased number of glands, reduced leaf area) to herbivory generated by the bean thrips (an herbivore) and western flower thrips (an omnivore), suggesting that trophic function was not a key determinant of plant response. Thrips-damaged plants again showed a significantly higher density of gossypol glands than did mite-damaged plants. Overall, our results suggest that (1) an omnivorous predator systemically induces resistance traits in cotton and (2) whereas there is evidence of taxonomic specificity (thrips versus mites), there is little support for trophic specificity (herbivorous thrips versus omnivorous thrips) in the elicitation of induced responses.

KEY WORDS *Frankliniella occidentalis*, *Tetranychus turkestanii*, *Gossypium*, biological control, pigment glands

In numerous natural and agricultural systems, attack by pathogens and herbivores has been shown to induce systemic plant responses. Reported responses include increases in direct defenses, such as increased trichome density (Dalin and Bjorkman 2003) and the production of feeding deterrents (McAuslane et al. 1997) and antinutritive compounds (Bi et al. 1997). The influence of these induced changes on the natural enemy community associated with the host plant has been investigated in many studies (Dicke and Sabelis 1988, De Moraes et al. 1998, Omer et al. 2001, Thaler 2002). Generally, these studies consider the effect of induced plant responses on predators and parasitoids as being mediated exclusively through effects on their prey (an indirect effect), or through attraction by damage induced volatiles. However, given the appar-

ent ubiquity of omnivory in arthropod communities (Coll and Guershon 2002), it is also important to recognize that natural enemies exist along a spectrum of omnivory, from primarily herbivorous to purely predaceous species. Indeed, it is thought that the ability of omnivores to feed on plant material during periods of prey scarcity may contribute to their success as biological control agents. Induced plant defenses may therefore act on natural enemies not only indirectly through effects on herbivores but also directly when natural enemies are omnivores that consume plant tissues.

Relative to constitutive defenses, whose levels may change over evolutionary time, induced responses can lead to increases in defense over an ecological time scale. This generates the possibility for important and relevant interactions between the induced plant, herbivores, and omnivorous natural enemies. The net

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effect of induced defenses on plant fitness, omnivore performance, and herbivore regulation may largely depend on the specificity of the response. Specificity of induced responses can be achieved through specificity of elicitation and/or of effect (Karban and Baldwin 1997, Agrawal 2000). Several studies have investigated specificity of plant responses to herbivores belonging to different species and/or feeding guilds (Turlings et al. 1998, Takabayashi et al. 2000, Traw and Dawson 2002, Van Zandt and Agrawal 2004). The degree of specificity observed varied among studies, yet qualitatively different responses have been documented even for closely related herbivores (De Moraes et al. 1998). If induced plant defenses specifically target herbivores, there may be no detrimental effect on omnivorous predators, and induced resistance should lead to a decrease in herbivore densities. If instead induced defenses are nonspecific or, worse, selectively reduce an omnivore's preference for or performance on an induced plant, induced resistance may erode biological control, leading to an increase in herbivore densities. We are unaware, however, of any study that has explicitly examined the specificity of plant responses to feeding by herbivores and their omnivorous natural enemies.

It may be advantageous for the plant to be more tolerant of omnivore feeding. Such tolerance might be evidenced as a higher damage threshold for induction to damage generated by omnivores versus herbivores. Earlier studies have shown that the western flower thrips, *Frankliniella occidentalis* (Pergande) (an omnivore that feeds on plants and yet is known to be important in suppressing herbivorous mite populations), generates less damage on plants previously induced by jasmonic acid (cotton: Omer et al. 2001, tomato: Thaler et al. 2002) and the spider mite *Tetranychus turkestanii* (Ugarov and Nikolski) (cotton: Agrawal et al. 1999). Furthermore, thrips feeding on mite-induced cotton suffered greater mortality than did thrips feeding on uninduced controls (Agrawal et al. 1999). These results reveal the potential for negative effects of induction on an omnivore. However it is not known whether feeding by the omnivorous western flower thrips elicits induced responses in cotton plants or whether the cotton plant responds differently to foliar damage generated by omnivorous thrips versus strictly herbivorous arthropods.

We seek to address these questions using the mite-thrips-cotton system. Both arthropod species can occur singly or together at high densities on cotton seedlings at the cotyledon stage. In most systems, a direct, quantitative comparison of induction by herbivores and omnivores is not possible, because herbivores and omnivores feed on different parts of the plant, generate qualitatively different types of feeding damage, or produce damage that is not readily quantified. The thrips-mites-cotton system allows us to circumvent these problems. Spider mites and immature thrips are both commonly found on the same part of the cotton plant, the underside of leaves (Leigh et al. 1996). Thrips and mites both have short, stylet-like mouthparts, which they use to consume the contents

of epidermal and mesophyll cells (Chisholm and Lewis 1984, Tomczyk and Kropczynska 1985). Feeding by each generates small patches of dead leaf tissue, which are readily quantifiable. Thrips are larger than mites and produce more damage per capita. Thus, it is critical to compare plants sustaining equal total amounts of injury rather than plants harboring equal numbers of herbivores/omnivores.

Our initial experiment showed some differences between plant responses to feeding damage from mites and western flower thrips. This prompted the question of whether these differences were caused by trophic role (herbivore versus omnivore) or by other differences between mites and thrips. We therefore conducted a second experiment and included the bean thrips *Caliothrips fasciatus* (Pergande) (an herbivore) to provide an explicit comparison of herbivore versus omnivore while controlling for the effect of arthropod taxonomic order (Thysanoptera versus Acari). The bean thrips is an occasional economic pest of cotton (Rude 1996), and generates damage similar to that of mites and western flower thrips. It can co-occur with the western flower thrips and spider mites, but unlike the western flower thrips, it does not feed on mite eggs (unpublished data). We chose to use the Acala SJ-2 cotton cultivar because previous studies (Karban and Carey 1984, Brody and Karban 1992, Agrawal and Klein 2000) and our own preliminary data have shown that it is strongly inducible, particularly at the cotyledon stage.

To assess specificity of elicitation of induction by spider mites versus thrips, we sought a reliable means of quantifying induced responses in the host plant. We selected two complementary approaches: (1) the quantification of peroxidase activity and (2) the density of gossypol glands. Oxidative enzymes, such as polyphenol oxidase, lipoxygenase, and peroxidase, are known to be correlated with induced defenses to herbivory and pathogen attack (Bi et al. 1997, Thaler et al. 2001). Peroxidase has several important roles in cotton defensive chemistry. First, it catalyzes the conversion of the terpenoid hemigossypol to gossypol (Veech et al. 1976). Gossypol binds proteins and amino acids, reducing the nutritive value of ingested plant tissues (Felton et al. 1989, Felton 1996). Although levels of gossypol in foliage are relatively low, previous studies have shown that foliar concentrations increase in response to larval lepidopteran feeding damage (McAuslane et al. 1997). In addition, the flavanol catechin is condensed in the presence of peroxidase to produce the condensed tannin proanthocyanidin, which has antinutritive properties (Bell and Stipanovic 1978). Resistance of cotton cultivars to spider mites has been associated with high levels of condensed tannins (Bell 1986). Finally, peroxidase reacts with several aromatic compounds to generate reactive oxidative species (ROS) that are known to play a role in herbivore resistance (Bi et al. 1997, Kawano 2003).

In "glanded" cotton varieties, such as Acala SJ-2, the principal terpenoids (gossypol, hemigossypolone, and heliocides₁₋₄) are stored in lysigenous pigment glands. Leaves with a higher density of glands are thought to

be better defended against chewing herbivores (Agrawal and Karban 2000). An increase in the total number of glands per leaf or a decrease in leaf size after herbivory can lead to an increased density of glands.

Materials and Methods

Comparison of Induced Responses to Mites and Western Flower Thrips

Damage Treatments. Cotton plants, Acala-SJ2, were grown from untreated seeds (California Planting Cotton Seed Distributors, Shafter, CA) in plastic pots (9.84 by 9.84 by 8.89 cm; two seeds per pot) using Bandini planting mix (United Industries, St. Louis, MO). Pots were placed in plastic watering trays and watered by adding 2 liters of water with 10 drops of Schultz Plant Food 10–15–10 to each tray as needed. Plants were reared in laboratory growth chambers under a 16 L:8 D photoperiod and at a constant temperature of 28°C. Light was provided using GE Sunshine (General Electric, Fairfield, CT) fluorescent bulbs (24", full spectrum, 845 lumens, 5000°K; six to eight bulbs per growth chamber shelf). Ten days after planting, when the cotyledons were fully expanded, extra seedlings were removed so that only one seedling remained per pot. Each pot was randomly assigned to one of the following treatments, eight replicates per treatment: 15 western flower thrips (15 wft), 5 western flower thrips (5 wft), 15 mites (15 m), 5 mites (5 m), or a no arthropod control. Adult female thrips (*Frankliniella occidentalis*) and mites (*Tetranychus turkestani*) were obtained from laboratory colonies initiated ≈20 yr ago and maintained on bean pods and cotton seedlings, respectively. The arthropod densities selected fall within the natural range of densities observed on cotton seedlings in the field (unpublished data), and previous studies have documented induced responses using a similar number of mites per plant (Karbon 1985). Mites and thrips were confined with the seedling plants inside acrylic tube cages (7 cm diameter, 14 cm tall) that were covered on the top with mesh and sealed at the base with a foam core that surrounded the seedling stem. Mites and thrips were allowed to feed on the seedlings in growth chambers for 8 d, during which they had access to the fully expanded cotyledons and, in some cases, to the very small, unexpanded first true leaf. After this feeding period, all visible thrips (adults and immatures) were removed with an aspirator, after which all plants, including controls, were dipped in the miticide dicofol (Kelthane, 300 ppm). Plants were recaged and returned to the growth chambers. One thrips-induced plant (5 wft) was injured during recaging and discarded. Plants were rechecked 4 d later to remove any recently hatched thrips larvae. All cages were removed, and plants were again returned to the growth chamber to grow arthropod free for an additional 8 d. Inspection of the plants revealed no arthropods or arthropod damage to the second and third true leaves

at this time. The second and third true leaves of all plants were assayed for induced responses.

Quantification of Induced Responses. The assay of leaf peroxidase activity used in this study was developed from the protocol outlined by Bi et al. (1997). Briefly, one half of a leaf (cut along the mid-vein) was removed and ground in liquid nitrogen (the other half was used to quantify leaf area and gossypol gland density). Powdered leaf material was put into previously weighed 1.5-ml tubes containing 1 ml of 0.1 M ice-cold potassium phosphate buffer, pH 7.0, with 0.5 mM EDTA and 1% polyvinylpyrrolidone. All tubes were reweighed to determine the exact amount of powdered leaf tissue added and centrifuged for 20 min at 10,000g at 5°C. The resulting supernatant was decanted into 1.5-ml eppendorf tubes and kept refrigerated at 6°C until enzyme activity was assayed (<2 d). For the assay, 1 ml of substrate solution (1 mM H₂O₂, 2 mM guaiacol in 0.1 M potassium phosphate buffer, pH 7.0) was combined with 50 μl of enzyme solution, and the conversion of guaiacol to tetraguaiacol was monitored at 470 nm using a Spectronic 20 Genesys spectrophotometer connected to a laptop PC with software we developed to record the rate of conversion. Peroxidase activity units (AUs) are expressed as moles of tetraguaiacol produced per gram of leaf tissue per minute. Using the remaining half of each leaf, the number of gossypol glands in an area the size of a hole punch (0.25 cm²) in the middle of the leaf, adjacent to the mid-vein, was quantified. To generate an estimate of total glands per leaf, the number counted was multiplied by the area of the leaf and divided by 0.25 cm². To estimate leaf area, we measured the area of the half-leaf with a Li-Cor (Lincoln, NE) LI-3000 portable area meter and multiplied the area measured by two.

The amount of damage sustained by the cotyledons of each plant during the induction treatment was estimated by placing a 5 by 3.5-cm transparent sheet marked with a 2 by 2-mm grid first over one and then the other half of the cotyledon and counting the number of damaged squares. The area of the cotyledons was measured using an area meter. With these data, we were able to generate an estimate of percent cotyledon surface area damaged. However, some cotyledons were so heavily damaged that they abscised. In cases where only one cotyledon abscised, we estimated percent damage using just the one remaining cotyledon. We were unable to estimate percent damage for two thrips-induced plants that abscised both of their cotyledons.

Statistical Analysis. Analysis of variance (ANOVA) was used to assess differences in leaf area, total number of gossypol glands, gland density, and peroxidase activity units among treatments. Significant ANOVAs were followed by Dunnett's test ($\alpha = 0.05$; JMP 5.2.1; SAS Institute 2004) for comparing multiple treatments to a control. Data for total number of glands, gland density, and peroxidase activity were log-transformed before analysis to improve their statistical properties.

To evaluate the response of plant peroxidase activity to mites and thrips given the same amount of

feeding damage, we analyzed a subset of the data restricted to a range of cotyledon damage values generated by both mite and thrips treatments (<40% leaf area damaged). This truncated data set was analyzed using an analysis of covariance (ANCOVA) model with peroxidase activity as the dependent variable, damage treatment (mite or thrips) as the independent factor, and percent leaf area damaged as the covariate (JMP 5.2.1; SAS Institute 2004).

Comparison of Cotton Responses to Mites and Two Species of Thrips

Cotton plants were grown from untreated seed in plastic posts (9.84 by 9.84 by 8.89 cm; four seeds per pot) using ACE soil mix (ACE Hardware). The photoperiod, lighting intensity, and watering regimen were the same as described above. Plants were rotated among growth chambers weekly to minimize effects of any possible differences among growth chamber environments. Seven days after planting, when the cotyledons were fully expanded, extra seedlings were removed so that only one remained per pot. Each pot was randomly assigned to one of the following treatments in a randomized complete block design with two replicates per tray: 10 female bean thrips, 7 female western flower thrips, 25 adult female spider mites, mechanical damage, or a no damage control. There were 20 replicates per treatment. Bean thrips were collected from alfalfa and maintained on cotton until the initiation of the experiment, completing at most one generation in the laboratory. Western flower thrips and mites were obtained from the laboratory colonies used in the first experiment. The mechanical damage treatment was established by firmly rubbing the underside of both cotyledons with a cotton swab covered in 800 grit carborundum powder (Karban 1985). (The mechanical abrasion treatment was intended as a positive damage control but failed to trigger any measurable induction as described in the Results. Therefore, it was combined with the negative control.) Plastic 2-liter soda bottles with two mesh-covered windows (7.62 by 10.16 cm) and the bottom removed were used to confine mites and thrips on the seedling plant. Mites and thrips fed on the caged seedlings for 7 d in the growth chambers. Thrips have a lower thermal optimum than mites (Lublinkhof and Foster 1977, Leigh et al. 1996). We exploited this difference to promote convergence of treatment damage levels. For the final 3 d of the 7-d feeding period, the temperature in all the growth chambers was decreased from 28 to 21°C to generate mean damage levels from mites and thrips that were more nearly equal. After the feeding period, all plants were dipped in a mixture of the miticide dicofol (Kelthane, 300 ppm) and the thripsicide spinosad (Entrust; 0.082 g/liter) to remove all arthropods, and the growth chamber temperature was returned to 28°C. To ensure that spinosad residues were sufficient to kill thrips larvae that were observed to hatch after the initial treatment, all plants were dipped again 3 d later. No growth, feeding, or damage by the hatching neonate thrips

larvae was observed. All cages were removed, and the plants were returned to the growth chamber. The plants grew less rapidly than in the previous experiment and were therefore allowed to grow insect free for an additional 23 d. At this time, the second and third leaves were assayed for induced responses.

Quantification of Induced Responses. The amount of feeding damage sustained by the cotyledons during the induction treatment was estimated by placing a transparent sheet marked with a 1 by 1-mm grid first over the upper and then the lower surface of the cotyledon and counting the number of damaged squares. Because bean thrips damage was more dispersed relative to western flower thrips and mites, we used a finer scale to estimate damage in this experiment to avoid overestimating the amount of bean thrips damage. The area of the fresh, whole leaves was quantified using a Li-Cor LI-3000 area meter, and this measurement, together with the damage estimates, allowed us to calculate the percent damage to cotyledons.

The fresh leaf was cut in half, and peroxidase activity was determined using the method described above in the first experiment. The other leaf half was pressed and dried at room temperature. Gland density and total number of glands was quantified for the dried leaf using the methods described previously. Dry leaf area and fresh whole leaf area were strongly correlated, and residual analysis revealed that the proportional reduction in leaf area during drying did not differ among damage treatments.

Condensed tannins were analyzed using established techniques (Hedin et al. 1992, Bi et al. 1997, Hagerman 2002). Briefly, individual dried leaf samples were ground, and the tissue powder passed through a 200-mesh screen. Twenty-milligram samples of tissue powder from individual plant leaves were extracted in 4 ml of 70% MeOH by vortexing for 3 min and were centrifuged at 5000g for 20 min. To 5 ml of 1-butanol-HCl (95:5 vol:vol), we added 200 μ l of iron reagent (2% ferric ammonium sulfate in 2 N HCl) and a 1-ml aliquot of the supernatant. The reaction tube was placed in a boiling water bath for 60 min. After cooling, a 200- μ l aliquot was diluted with an equal volume of 1-butanol-HCl, and the absorbance was read at 550 nm using a Spectronic 20 Genesys (Thermo Scientific, Waltham, MA). Tannin concentrations in the samples were compared with cyanidin chloride and are expressed as cyanidin equivalents.

Statistical Analysis. For each leaf, multivariate ANOVA (MANOVA) using the full data set was used to identify treatment effects on plant response variables: leaf area, total number of gossypol glands, peroxidase activity, and condensed tannin concentration. We analyzed each leaf separately, because our previous work has shown that the magnitude and nature of induced responses may differ among leaf positions. Effects of damage treatments on individual response variables were analyzed using one-way ANOVA with tray number included as a blocking term. Data for total gossypol glands per leaf and gland density were log-transformed to improve their statistical properties.

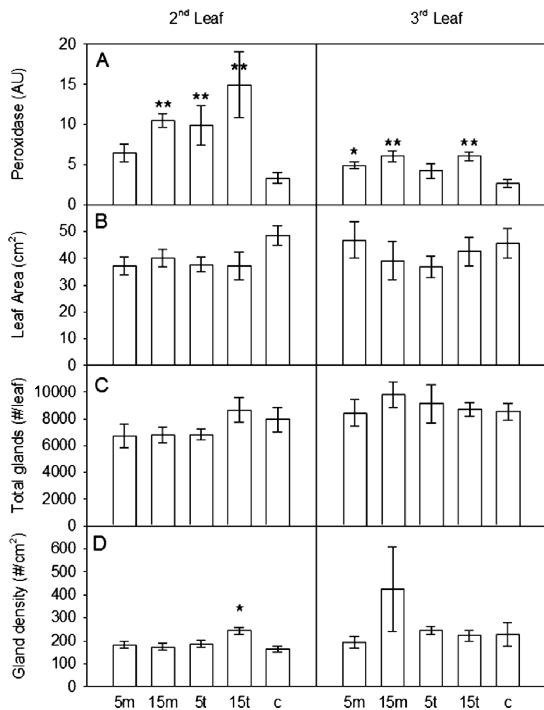


Fig. 1. Peroxidase activity (A), leaf area (B), total number of glands (C), and gland density (D) for second and third true leaves of cotton plants. Shown are means \pm SE. All treatments compared with control. Statistical significance determined by Dunnett's test ($\alpha = 0.05$): * $P < 0.05$; ** $P < 0.005$.

Planned univariate contrasts were conducted to compare responses of damaged plants to controls and to compare specific treatment groups. Post hoc contrasts between mites and thrips (bean thrips and western flower thrips combined) were also performed. The resulting table of *P* values was analyzed using the sequential-Bonferroni correction to establish table-wide significance (Rice 1989).

Plants in the mite herbivory treatment sustained a somewhat higher mean amount of damage than did plants in the two thrips herbivory treatments. We therefore conducted two-way ANCOVA on a subset of the data for which the amount of cotyledon damage

generated by mites and thrips overlapped (<30% damage). This resulted in the exclusion of six mite and three bean thrips replicates. All analyses were also repeated with these replicates included to assess whether or not their exclusion altered the results. Planned univariate contrasts were used to compare means among treatment groups, and a post hoc comparison of mites and thrips (BT + WFT) was also performed. The resulting table of *P* values was again analyzed using the sequential-Bonferroni correction.

Results

Comparison of Induced Responses to Mites and Western Flower Thrips

Feeding by mites and thrips elevated peroxidase activity levels in both the second and third leaf relative to control plants (Fig. 1A). Peroxidase activity was significantly higher for the second true leaf (9.06 ± 1.16 AU) than the third true leaf (4.81 ± 0.34 AU; $F = 24.76$; $df = 1,37$; $P < 0.0001$; statistical model included plant number to control for between-plant differences in overall peroxidase activity). The effect of plant number ($F = 3.54$; $df = 38,37$; $P = 0.0001$) indicates that average peroxidase levels viewed across the second and third leaves varied between plants. The interaction between damage and leaf number was not significant, suggesting that increases in peroxidase activity levels in response to feeding were consistent for both leaves. Neither mites nor thrips produced a significant change in leaf area or the number of gossypol glands per leaf (Fig. 1B and C), although averaging across the four damage treatments revealed that the area of the second true leaf was significantly reduced for damaged plants compared with undamaged controls ($F = 6.45$, $df = 1,33$; $P = 0.016$). The density of gossypol glands was elevated in the second leaf of plants damaged by thrips (15 t) but not mites (Fig. 1D).

When the data set was reduced to consider only plants with similar total damage to the cotyledons (<40% damage), thrips and mites were found to generate very similar responses. We found no difference in leaf area, number of gossypol glands per leaf, or gland density for the second or third true leaf of plants damaged by mites or thrips (data not shown). How-

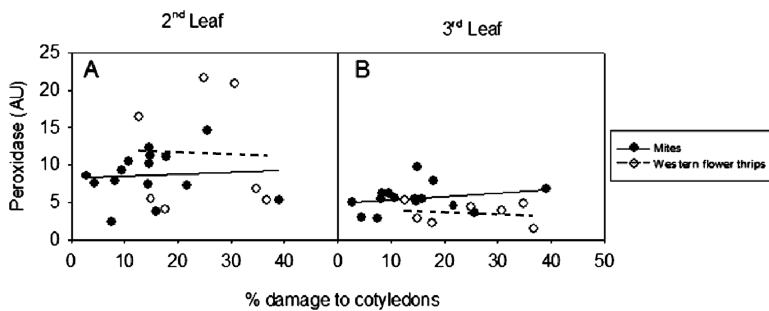


Fig. 2. The relationship between peroxidase activity in the second (A) and third (B) true leaves and percent damage to the cotyledons by mites (●) and thrips (○). ANCOVA regression lines are shown for each species.

Table 1. Multivariate analysis of variance for the effects of arthropod feeding on cotton cotyledons on peroxidase activity, leaf area, total number of gossypol glands, and condensed tannin content of the second and third true leaves

Manova	Source	df	F	P
Leaf #2	trt	12, 117	3.23	<0.001
	tray	36, 167	4.92	<0.001
	trt*tray	108, 177	1.14	0.210
Leaf #3	trt	12, 199	2.40	<0.001
	tray	36, 170	6.33	<0.001
	trt*tray	108, 181	1.02	0.450

ever, elevated peroxidase was expressed at the third leaf (mite mean: 5.49 ± 0.44 ; thrips mean: 3.56 ± 0.53 ; $F = 6.53$, $df = 1,20$; $P = 0.019$; Fig. 2B), but not at the second leaf (Fig. 2A). The covariate, percent damage, was not significant over this range of the data.

Comparison of Cotton Responses to Mites and Two Species of Thrips

Full Data Set. Relative to the undamaged control (c), the mechanical abrasion (ma) treatment did not induce a change in peroxidase activity (ma mean second/third leaf: $2.94 \pm 0.92/2.85 \pm 0.39$; c mean second/third leaf: $2.80 \pm 0.46/2.63 \pm 0.40$; $df = 1,35/1,34$; $F = 0.05/0.15$; $P = 0.82/0.70$), leaf area (ma mean second/third leaf: $43.39 \pm 2.13/62.04 \pm 2.31$; c mean second/third leaf: $44.33 \pm 1.75/59.32 \pm 2.74$; $df = 1,35/1,35$; $F = 0.11/0.58$; $P = 0.74/0.45$), total gland number (ma mean second/third leaf: $6132.72 \pm 360.83/10045.41 \pm 523.11$; $df = 1,33/1,34$; $F = 0.023/0.07$; $P = 0.88/0.79$), gland density (ma mean second/third leaf: $213.47 \pm 10.75/269.68 \pm 21$; c mean second/third leaf: $218.00 \pm 14.92/270.67 \pm 24.71$; $df = 1,35/1,35$; $F = 0.06/0.00$; $P = 0.81/0.98$), or condensed tannin levels (ma mean second/third leaf: $4.77 \pm 0.41/4.22 \pm 0.39$; c mean second/third leaf: $4.78 \pm 0.33/4.48 \pm 0.54$; $df = 1,35/1,33$; $F = 0.00/0.15$; $P = 0.98/0.70$). Therefore, the mechanical abrasion treatment will be included with the undamaged control hereafter. Multivariate ANOVA revealed that arthropod feeding elicited induced responses in the cotton plants (Table 1). However, for our most direct comparison of omnivore to herbivore (western flower thrips versus bean thrips), all plant responses were remarkably similar (Fig. 3). Univariate tests revealed no differences between thrips treatments for peroxidase activity, leaf area, total number of glands, gland density, or condensed tannin percentage. We therefore grouped the thrips species together (hereafter "thrips" refers to bean thrips + western flower thrips) and performed post hoc comparisons between mites and thrips for each induced response. Relative to thrips damaged plants, mite damaged plants had reduced leaf area (second leaf: $F = 6.75$; $df = 1,84$; $P = 0.011$; third leaf: $F = 6.60$; $df = 1,83$; $P = 0.012$), fewer gossypol glands per leaf (second leaf: $F = 14.12$; $df = 1,77$; $P = 0.0003$), and lower gossypol gland density (second leaf: $F = 7.65$; $df = 1,83$; $P = 0.007$; Fig. 3B–D). Peroxidase activity increased in response to feeding damage by mites (second leaf: $F = 10.72$; $df =$

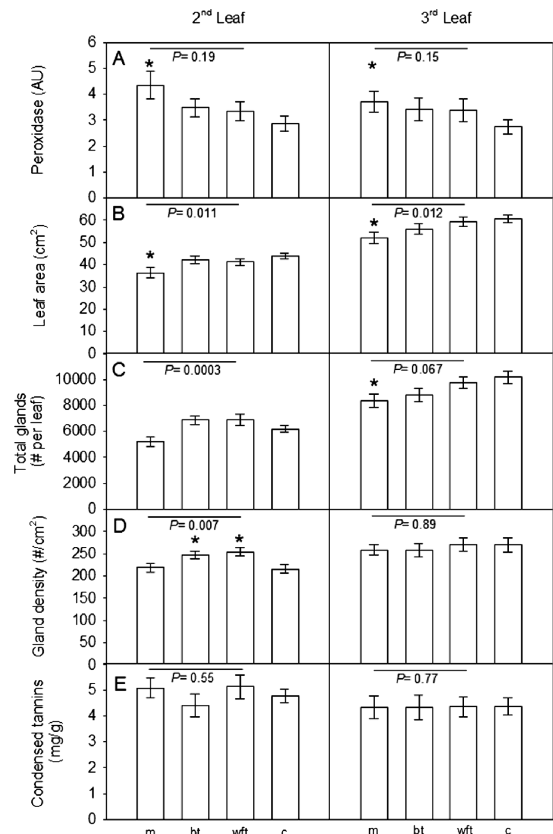


Fig. 3. Leaf area (A), peroxidase activity (B), total gossypol glands (C), gossypol gland density (D), and condensed tannin percentage (E) for the second and third true leaves of cotton plants damaged by spider mites (m), bean thrips (bt), western flower thrips (wft), and on an arthropod-free control (c). Shown are means \pm SE. Within each panel, the results of pairwise contrasts of individual treatments versus the arthropod-free control are shown with asterisks, after applying the sequential Bonferroni correction. Also within each panel, horizontal lines and associated P values show the results of pairwise contrasts of spider mites versus thrips (bean thrips and western flower thrips combined).

1,83; $P = 0.0015$; third leaf: $F = 8.88$; $df = 1,82$; $P = 0.0038$) relative to controls. Thrips feeding also increased peroxidase activity (second leaf: $F = 5.56$; $df = 1,83$; $P = 0.021$; third leaf: $F = 3.69$; $df = 1,82$; $P = 0.058$), but this increase did not achieve table-wide significance after the sequential-Bonferroni correction (Fig. 3A). The peroxidase activity levels elicited by mites and thrips feeding were not different. Condensed tannin levels were unaffected by arthropod feeding (Fig. 3E).

Subset of Overlapping Damage. Two-way ANCOVA of the subset of data for which the treatment damage levels converged (<30%) provided further support for taxonomic specificity in the elicitation of induced responses. Mite feeding increased peroxidase activity in the second leaf marginally more than thrips feeding (mite mean: 4.47 ± 0.64 ; thrips mean: 3.40 ± 0.26 ; $F = 3.2$; $df = 1,48$; $P = 0.08$). The second leaf of mite-

induced plants had fewer gossypol glands per leaf than thrips induced plants (mite mean: 5120.29 ± 526.33 ; thrips mean: 6967.49 ± 272.03 ; $F = 6.56$; $df = 1,44$; $P = 0.014$). Mite damaged plants also had lower gland densities than thrips damaged plants (mite mean: 210.29 ± 10.89 ; thrips mean 250.59 ± 6.61 ; $F = 8.91$; $df = 1,48$; $P = 0.0045$).

Discussion

Our study showed that feeding by an omnivorous arthropod, the western flower thrips, does elicit induced resistance traits in cotton plants, namely elevated peroxidase activity and an increase in the number and density of gossypol glands. Although previous studies have reported induced defenses after damage by herbivorous thrips (Olson and Roseland 1991, Gopichandran et al. 1992, Ananthakrishnan 1993) and induced volatile emissions after feeding by the putative omnivore *Lygus hesperus* (Knight) (Rodriguez-Saona et al. 2002), this is the first study to report systemically induced increases in plant defensive chemicals by an omnivorous natural enemy (but see Belliure et al. 2005).

The induction threshold does not seem to be lower for our focal omnivore, the western flower thrips, than for two herbivores. For bean thrips, which is perhaps the best comparison, because it is taxonomically closest, we saw virtually identical responses for all variables. For mites, we saw mostly similar responses once the covariate of percent cotyledon damage was included in the statistical model. Thus, we found no evidence supporting the hypothesis that plants may be more tolerant of damage that is generated by omnivorous arthropods that may be plant mutualists by virtue of suppressing herbivores on their host plant.

Why might cotton plants be selected to express induced resistance traits in response to feeding by the western flower thrips? One possibility is suggested by the work conducted by Agrawal et al. (1999): on induced cotton plants, the western flower thrips reduced their plant feeding and shifted to functioning more as a carnivore, consuming more spider mite eggs. This possibility has drawn further support from our own ongoing work. In a greenhouse study, we examined the specificity of the effects of systemically induced defenses on the performance of mites and the western flower thrips. We found that thrips performance on cotton plants increased on plants previously damaged at the cotyledon stage but only in the presence of spider mite prey (K.O.S. and J.A.R., unpublished data). Thus, plants may respond to feeding damage generated by omnivores with resistance traits that diminish the negative effects of the omnivore on its host plant and simultaneously enhance the omnivore's role as a defensive mutualist.

Although the cotton plants' responses to omnivore and herbivore feeding damage were similar, cotton does seem to differ in its response to damage generated by mites versus thrips (bean thrips and western flower thrips combined), even when corrected for the total amount of cotyledon damage generated. Perox-

idase activity was greater, the total number of gossypol glands was lower, and gossypol gland density was reduced for the second leaves of mite-damaged plants compared with thrips-damaged plants. Thus, our study adds to a growing body of evidence showing that plants respond to different herbivores with the expression of different suites of resistance traits.

Our results reveal that cotton can be selective in its responses to arthropods generating qualitatively very similar forms of leaf herbivory. Plants, including cotton, have an array of inducible defenses, and it is possible that studies measuring other traits may find different patterns of specificity. For the resistance traits measured here, however, it seems that taxonomic order is more important than trophic role in determining the nature of the induced responses.

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