Specificity of constitutive and induced resistance: pigment glands influence mites and caterpillars on cotton plants

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Abstract

Cotton plants contain suites of phytochemicals thought to be important in defense against herbivores, some of which are localized in pigment glands which contain gossypol and other terpenoid aldehydes. The simple genetic basis for the expression of these glands has led to the development of near-isogenic glanded and glandless genotypes. Glands may also be phenotypically induced by herbivory. We determined the consequences of constitutive and induced gland expression on two types of herbivores, spider mites (cell content feeders) and noctuid caterpillars (leaf chewers).

Induction of glands was strongly dependent on the density of attackers. Spider mite herbivory on cotyledons (1) increased the density (but not total number) of glands on cotyledons linearly, (2) increased the density and total number of glands on the first true leaf linearly, and (3) affected the density and total number of glands on the second true leaf non-linearly, compared to controls. Neither constitutive nor induced expression of glands affected mite population growth. An equal reduction of mite population size on induced glanded and glandless plants (50%) relative to uninduced controls indicated that factors other than glands were associated with induced resistance to mites. Constitutive gland expression had a strong negative impact on caterpillar performance, reducing growth by 45%. Induced resistance to caterpillars was three times stronger in glanded genotypes than in glandless genotypes, indicating that factors associated with induced resistance to caterpillars are strongly associated with glands. Three cotton varieties were highly variable in their constitutive and induced resistance to mites and caterpillars.

Thus, defense of cotton plants against herbivores can be roughly categorized as constitutive and inducible factors associated with terpenoid aldehyde containing pigment glands that are effective against caterpillars, and factors not associated with glands that are effective against mites.

Introduction

Plants are the basal resource for organisms in many food webs. As a consequence, most plants are consumed by numerous herbivores. Where herbivores reduce plant fitness, natural selection has presumably favored the evolution of defensive mechanisms in plants. Plant defenses can be expressed constitutively (all of the time) and facultatively (induced by some environmental cue) (Karban & Baldwin, 1997; Agrawal, 1998). Genotypically determined constitu-tive and induced expression of phytochemicals can mediate either specific or general resistance to attackers. Furthermore, different resistance mechanisms have the potential to interact with each other to increase (or decrease) defense (Berenbaum & Zangerl, 1996). Some plant defenses such as the tetracyclic triterpenoid cucurbitacins of the cucurbitaceae are effective against many attackers including spider mites, roaches, beetles, lepidopteran larvae, mice and vertebrate grazers (Da Costa & Jones, 1971; Metcalf & Lampman, 1989; Tallamy et al., 1997; Agrawal et al.,

1999b); other phytochemicals have more specific activity and are only effective against a particular class of herbivores. In most plants, with either generalized or specialized defenses, some herbivores inevitably circumvent or tolerate the defenses and consume the plants.

Most plants have multiple resistance mechanisms, some of which are general and some of which are more specific. For example, tomato plants produce many defensive compounds including alkaloids, proteinase inhibitors, pathogenesis-related proteins, and several oxidative enzymes (Duffey & Stout, 1996), only some of which may be effective in enhancing plant resistance to particular attackers (Stout et al., 1998, 1999; Thaler et al., 1999). Following attack by particular herbivores, expression of some or all of these phytochemicals may be enhanced (Stout et al., 1996, 1998, 1999). Thus, an important goal in understanding the evolutionary ecology of plant-herbivore interactions is determining the relative contribution of different constitutive and inducible defense systems to resistance against different attackers. In addition, we have little understanding of the interactions between different resistance mechanisms on plant attackers (Stout et al., 1998, 1999; Thaler et al., 1999). Thus, we examined the roles of alternative constitutive and induced defenses of cotton plants in resistance to mites and caterpillars.

We asked the following specific questions: (1) Do pigment glands in cotton plants increase in density on leaves of mite damaged plants compared to controls? (2) Is the increase in density of pigment glands on induced plants dependent on the density of herbivores? (3) Is the increase in density of pigment glands on induced plants due to changes in leaf size or due to increases in the total number of glands? (4) What is the role of constitutive and induced pigment gland production in plant resistance to herbivorous spider mites and caterpillars?

Materials and methods

Study system. Resistance of cotton plants to herbivores has been well studied (Bell, 1984; Karban, 1991; Sadras & Felton, 2000). Exposure of the cotyledons to spider mite herbivory caused the newly formed leaves to be more resistant to subsequent attack by mites (Karban & Carey, 1984; Karban, 1987), thrips (Agrawal et al., 1999a), whiteflies (Agrawal et al., 2000), heteropteran bugs, (Agrawal et al., 2000),

caterpillars (Karban, 1988), and a vascular fungus (Karban et al., 1987), but not to bacterial blight (R. Karban & W. C. Schnathorst, unpubl.). All of these organisms regularly attack cotton plants under field conditions. Similarly, induced resistance has been reported following caterpillar herbivory (Alborn et al., 1996; Bi et al., 1997) and appears to be mediated by a reduction in nutritional quality of the foliage and increased activities of putatively defensive oxidative enzymes (Bi et al., 1997). More recently, it has been discovered that toxic and deterrent phytochemicals found in the pigment glands of cotton plants (terpenoid aldehydes, including gossypol) also increase in concentration in damaged plants compared to controls (McAuslane et al., 1997; McAuslane & Alborn, 1998). These glands can be seen as black dots on the leaves of many plants within the cotton tribe of the Malvaceae (Gershenzon & Croteau, 1992).

Pigment glands are an important component of resistance to many insect herbivores (Gershenzon & Croteau, 1992; Sadras & Felton, 2000). There is often a negative relationship between the number of glands on leaves and the preference or performance of herbivores feeding on those leaves (Meisner et al., 1978; Parrott, 1990; McAuslane & Alborn, 1998). Although the glands can provide resistance against herbivory, breeders have been interested in developing gland-free varieties of cotton to produce gossypol-free cottonseed for use as food for humans and livestock. Early on, McMichael (1960) determined that the presence of pigment glands was mediated by two loci, gl_2 and gl_3. Many near-isogenic glanded, $Gl_2Gl_2Gl_3Gl_3$, and glandless, $gl_2gl_2gl_3gl_3$, varieties of cotton have been bred; these pairs of lines are genetically identical except for those genes tightly linked with the genes responsible for the expression of pigment glands. Plants lacking glands have greatly reduced levels of gossypol and other terpenoid aldehydes (Hedin et al., 1991; McAuslane & Alborn, 1998) and are more susceptible to many herbivores (Parrott, 1990).

General procedures. Cotton plants were grown in 800 ml pots in U.C. soil mix (Redi Gro, Inc., Sacramento, CA) in a growth chamber maintained at 29 °C (day) and 25 °C (night) with a L16:D8 cycle. All experiments were conducted in a single growth chamber and plant position was completely randomized. Cotton plants were grown until the cotyledons had expanded and then randomly divided into two groups: (1) plants inoculated with 20–30 *Tetranychus turkestani* spider mites, and (2) undamaged control plants. After three

days of feeding, all mites were killed using a non-systemic miticide (dicofol, Kelthane 100 ppm, Rohm & Haas, Inc., Philadelphia, PA). Plants treated with mites as well as controls were dipped in the miticide. At this stage, the first true leaf was only a bud. After 7–10 days of growth, the plants were assayed for their pigment glands or challenged with mites or caterpillars (*Spodoptera exigua*). All mites were obtained from laboratory colonies maintained on cotton plants, and all caterpillars were obtained from a laboratory colony maintained on artificial diet. To count glands, a disk was removed from the middle of each leaf approximately 0.5 cm away from the midrib using a cork borer (0.125 cm^2). Prior to removal, we gently rubbed with our fingers the area from which we took the disk to remove a hazy film and make counting the glands easier. Pigment glands were then counted with the help of a dissection microscope.

Experiment 1 – Induction of increased pigment gland density in glanded plants. This experiment was designed to determine if gland density would increase on newly formed leaves of damaged plants compared to undamaged controls. Control and damaged plants of a glanded variety (*Gossypium hirsutum* var. Acala SJ-2) were grown until the first two leaves were fully expanded, after which the density of pigment glands on the first and second true leaf was measured. This experiment was conducted three times with 15 replicates of each treatment in each trial. The density of glands on the first and second true leaves was compared between control and induced plants using a two-factor multivariate analysis of variance (MANOVA) with induced resistance and trial as the main effects. We employed the MANOVA approach because our two response variables, the density of glands on the first and second true leaves, were not independent. Where MANOVAs were statistically significant we proceeded to conduct univariate analyses protected from type I errors.

Experiment 2 – Density dependent induction of pigment gland density and total number. This experiment was designed to determine if (1) gland induction was dependent on the density of herbivores feeding on the plant and (2) if the increased density of glands seen on induced plants compared to controls was simply due to differences in the sizes of leaves. The size of newly formed leaves may be affected by herbivory on the cotyledons. We used *G. hirsutum* var. Acala SJ-2 for this experiment and inoculated the cotyledons

of each plant with 0–42 mites; the treatments were divided into 15 levels, each increasing by three mites (0, 3, 6, ... 42). This experiment was conducted twice with 2–3 replicates of each density in each trial. After the plants had two fully expanded true leaves, we measured the density of glands on the cotyledons and both true leaves as described above. In addition we measured the area of each of the leaves from which we took a gland density measurement by using a digital leaf area meter (Li-Cor 3000, Lambda Instruments, Corp., Lincoln, NE). Measurements of gland density (glands per cm^2) and leaf area (cm^2) allowed us to calculate an estimate of the total glands per leaf. Here we used multivariate analysis of covariance (MANCOVA) with mite induction density (level) as a continuous main effect and trial as a discrete main effect; the six response variables included the gland density and leaf size for each of the cotyledons, the first, and the second true leaves. The statistically significant MANCOVA was followed by univariate analyses. When examination of the residuals indicated a poor fit to a linear model, we fit a non-linear model to our data and tested the significance of the non-linear and linear parameters. Type III sums-of-squares were used in all analyses to calculate mean-squares.

Experiment 3 – Induction of resistance to spider mites in glanded and glandless plants. This experiment was designed to determine if (1) traits associated with glands mediate resistance to spider mites and (2) induced resistance to mites is mediated by gland induction and/or by induction of other compounds. The simple Mendelian basis of pigment gland expression in cotton (discussed above) has led breeders to develop near-isogenic varieties with and without glands. We obtained three independently developed near-isogenic pairs (designated Acala, Lambright, and 1209-619-7) of glanded and glandless cotton from Dr. A. E. Percival's lab at the USDA-ARS, Crop Germplasm Research, College Station, Texas. By replicating glandedness across near-isogenic lines we reduced the risk of confounding effects of glands with effects of other differences between glanded and glandless plants. When induced and control plants had grown two true leaves they were challenged by inoculating each plant with three adult female *T. urticae* spider mites. The rapid generation time of the mites (7–10 days) allowed us to measure their fitness and short-term population growth. Because the mites are relatively immobile, no cages were used around the plants, although leaves of different plants were not al-
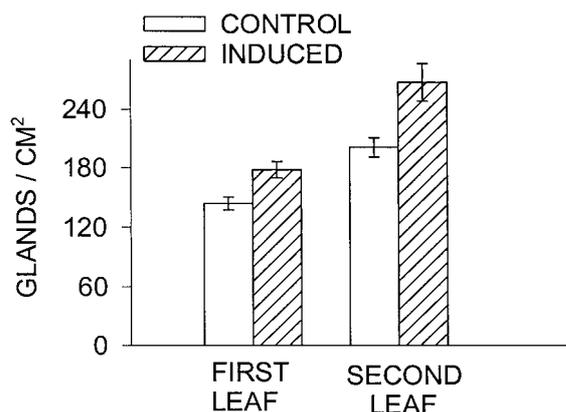
Figure 1. Effects of spider mite herbivory on the cotyledons of plants on the density of pigment glands on the first and second true leaves. Bars are mean ± 1 S.E.

lowed to touch one another. The mites were allowed to reproduce for 7 days, after which the plants were destructively sampled for all mites.

The design of the experiment was a 4 × 4 factorial with induced resistance and presence or absence of glands as main effects. Half of the plants were induced with mites as described in the general procedures. Plant variety and trial were also included in the four-factor analysis of variance; the population size of the spider mites was the single response variable. This experiment was conducted twice with 3–4 replicates of each treatment combination for each trial.

Experiment 4 – Induction of resistance to caterpillars in glanded and glandless plants. The design of this experiment was identical to the previous experiment (Experiment 3) except that a noctuid caterpillar, *S. exigua*, was the challenge organism after mite induction. Half of the plants were induced with mites as described in the general procedures. Three newly hatched caterpillars were put on to each plant when the plants had two true leaves. Each plant was caged with a spun-polyester Fibe-Air bag (Kleen Test Products, Brown Deer, Wisconsin), which allowed light to penetrate but did not allow the caterpillars to escape. After four days each plant was examined and caterpillars were randomly thinned so that there was only one left on each plant. After an additional four days the caterpillars were weighed to the 0.1 mg as a measure of their growth rate. Caterpillars were assumed to be of equal mass at egg hatch. This experiment was conducted twice and analyzed as described for the previous experiment.
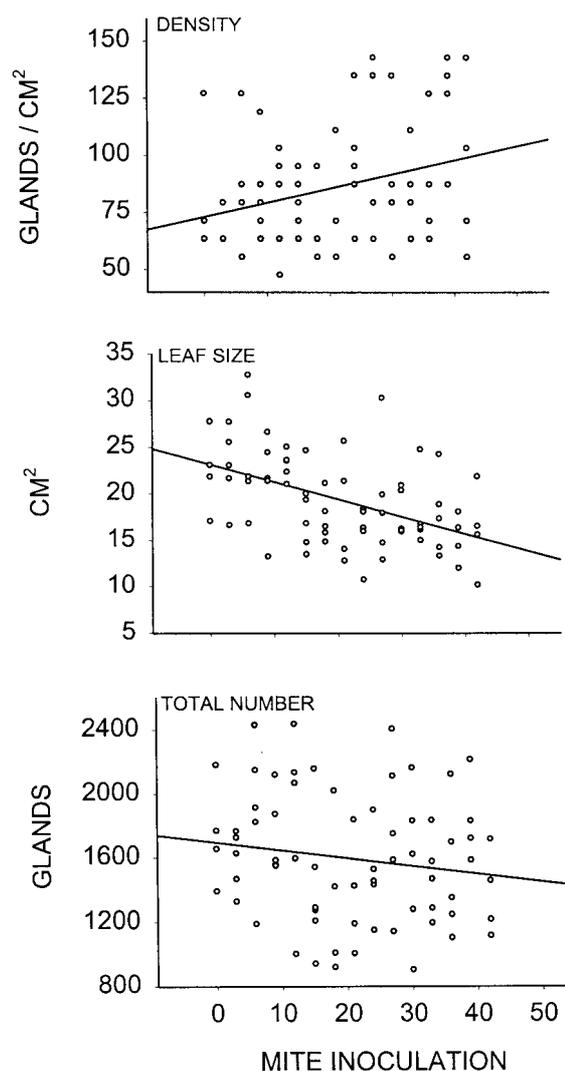

Figure 2. Effects of varying levels of spider mite herbivory on the cotyledons of plants on the density of pigment glands, leaf size, and total number of glands on the cotyledons of plants.

Results

A brief bout of mite herbivory to the cotyledons caused cotton plants to produce new leaves with higher densities of pigment glands compared with unattacked control plants (Table 1, Figure 1). The density of glands increased 24% and 34%, respectively, on the first and second true leaves of induced plants compared with controls (Figure 1). When we manipulated the density of mites on cotyledons we observed increasing gland density proportional to the level of attack (Figures 2–4). In the multivariate analysis of variance, initial density of mites (Wilks-$\lambda = 0.605$, d.f.

Table 1. Multivariate and univariate analysis of variance for the effects of spider mite herbivory (induced responses) on cotton cotyledons on pigment gland density on the first and second true leaves

Analysis	Source	d.f.	m.s. (*wilks-λ)	F	P
MANOVA	Induced responses (I)	2, 83	*0.790	11.028	<0.001
	Trial (T)	4, 166	*0.704	7.975	<0.001
	I*T	4, 166	*0.958	0.906	0.462
ANOVA (first leaf)	Induced responses (I)	1	26733.647	14.170	<0.001
	Trial (T)	2	26957.092	14.288	<0.001
	I*T	2	2495.122	1.322	0.272
	Error	84	1886.693		
ANOVA (second leaf)	Induced responses (I)	1	99924.269	10.246	0.002
	Trial (T)	2	44231.155	4.535	0.013
	I*T	2	4940.364	0.507	0.604
	Error	84	9752.358		

$= 6, 63$, $F = 6.857$, $P < 0.001$) and trial Wilks-λ $= 0.535$, d.f. $= 6, 63$, $F = 9.328$, $P < 0.001$) affected total glands per leaf and leaf size for the cotyledons and first and second true leaves. Univariate effects are shown in Table 2. For cotyledons (Figure 2), induction of gland density was coupled with reductions in leaf size, proportional to the level of attack; the net result was no effect of mite attack on the total number of glands. For the first true leaves (Figure 3), increasing mite density increased gland density and the total number of glands per leaf, as mite density had no effect on leaf size. For the second true leaves (Figure 4), there was no significant linear relationship between initial mite attack and gland density or total glands. However, when a squared term was included in the model, (Table 2) the coefficient of the squared term was significantly different from zero, indicating a non-linear relationship between mite attack and gland density. Induction of both gland density and total glands on the second true leaf peaked at an inoculation of 20 mites (Figure 4).

The presence of pigment glands had no detectable effect on mite populations, but induced resistance reduced population sizes by 50% in both glanded and glandless plants (Table 3, Figure 5a). The varieties differed in their inducibility to mites as indicated by a marginal effect of plant variety and a significant variety by induction interaction (Figure 6). The Lambright variety (glanded and glandless pair) failed to show any induced resistance to mites. A significant induction by trial interaction simply reflected variability in the strength (not direction) of induced resistance across trials.

Plants with glands supported caterpillars that grew 45% less than those on glandless plants (Figure 5b, Table 4). Induction also had a significant negative effect on caterpillar performance, reducing growth by 15% on glandless plants, and by nearly 50% on glanded plants. However, we failed to detect a significant gland by induction interaction which would indicate that induction was stronger in glanded compared to glandless plants. As a post-hoc analysis, we looked for an effect of induction in the glanded and glandless varieties in separate analyses. As predicted, we did not detect a significant effect of induction on caterpillar growth in glandless plants (P = 0.339) although we did detect a significant effect in glanded plants (P = 0.002). We conclude from these analyses that induced resistance to caterpillars is, at minimum, more effective in glanded plants compared to glandless plants.

Varieties differed in induced resistance to caterpillars; although there was no significant main effect of variety, we did detect a variety by induction interaction for caterpillar weight (Figure 6, Table 4).

Discussion

Density dependent induced resistance. There is a simple genetic basis for the presence of glands in cotton plants (McMichael, 1960). In addition, these glands may be phenotypically induced by herbivory. We found that exposure of the cotyledons to spider mites increased the densities of glands on the cotyle-

Table 2. Univariate analysis of variance for effects of initial mite density and trial on density of pigment glands, leaf size, and total number of pigment glands per leaf

Leaf	Source	d.f.	Gland density				Leaf size				Total glands			
			m.s.	F	P	R^2	m.s.	F	P	R^2	m.s.	F	P	R^2
Cotyledons	Density	1	4503.691	7.620	0.007	0.131	410.988	26.378	<0.001	0.364	280195.053	1.840	0.094	0.063
(linear model)	Trial	1	1742.149	2.948	0.090		218.085	13.997	<0.001		439716.774	2.888	0.179	
	Error	69	591.026				15.581				152247.535			
First leaf	Density	1	6679.495	11.451	0.001	0.149	4.030	0.088	0.767	0.094	7697969.110	8.837	0.004	0.226
(linear model)	Trial	1	173.197	0.297	0.588		320.879	7.035	0.010		8947225.020	10.271	0.002	
	Error	68	583.331				45.612				871143.204			
Second leaf	Density	1	1155.179	0.172	0.679	0.133	20.540	0.421	0.519	0.006	62987.089	0.026	0.873	0.294
(linear model)	Trial	1	68255.707	10.182	0.002		0.455	0.009	0.923		69681200.00	28.317	<0.001	
	Error	68	6703.838				48.841				2460778.541			
Second leaf	Density	1	63974.595	10.94	0.002	0.255	158.327	3.33	0.072	0.048	19410059.962	8.92	0.004	0.385
	Density^2	1	63999.701	10.94	0.002		139.319	2.93	0.091		21516049.196	9.89	0.003	
(non-linear)	Trial	1	76344.389	13.05	<0.001		1.956	0.04	0.840		74247644.484	34.12	<0.001	
	Error	67	5848.680				47.491				2176371.491			

dons, and on the first and second true leaves. Similar patterns of induced increases in gland density were reported by McAuslane et al. (1997) on plants damaged by caterpillars (*S. exigua*). The density of glands is likely to be the appropriate measure of the plant resistance phenotype. Although small caterpillars are able to feed around the glands, as gland density increases (and caterpillars grow larger), caterpillars cannot avoid piercing the glands and suffering the toxic and deterrent effects of the contents (Parrott et al., 1983; Parrott 1990). The leaves differed in their responses. The total number of glands per leaf did not increase on damaged cotyledons. The total number of glands per leaf increased linearly with the extent of damage on the first true leaf. Induction of glands on the second true leaf was more complicated, with gland density and total number peaking at a 20 mite inoculation, and declining thereafter. This non-linear relationship between the extent of damage and glands on the second true leaf is suggestive of a cost of the heavy damage imposed at higher mite densities.

Induced resistance has been suggested as a possible mechanism for population regulation of herbivores (Underwood, 1999). One of several requirements for induction to work as a regulating mechanism is that the response of plants must be positively correlated with the extent of herbivory. Although density-dependent induced responses to herbivory have been reported in some systems (Karban, 1983; Bodnaryk, 1992;

Table 3. Effects of cotton variety, presence of pigment glands, induced responses to herbivory, and trial on spider mite populations. All three- and four-way interactions were included in the model, but all P ≫ 0.05

Source	d.f.	m.s.	F	P
Variety (V)	2	3489.566	3.016	0.057
Pigment glands (G)	1	961.563	0.831	0.366
Induced response (I)	1	12768.165	11.037	0.002
Trial (T)	1	2112.078	1.826	0.182
V*G	2	1273.384	1.101	0.340
V*I	2	5539.099	4.788	0.012
V*T	2	1906.278	1.648	0.202
G*I	1	355.567	0.307	0.582
G*T	1	83.855	0.072	0.789
I*T	1	5122.950	4.428	0.040
Error	53	1156.851		

Agrawal & Dubin-Thaler, 1999), most studies have only investigated a few levels of herbivory; our study of gland induction adds a detailed demonstration of density dependent induced responses to herbivory. Higher levels of attack on cotton plants resulted in correlated levels of induced resistance to mites (Karban, 1987). Induced resistance to mites was also observed in the current experiment but was not correlated with gland number or density. However, factors associated with glands were important for induced resistance to
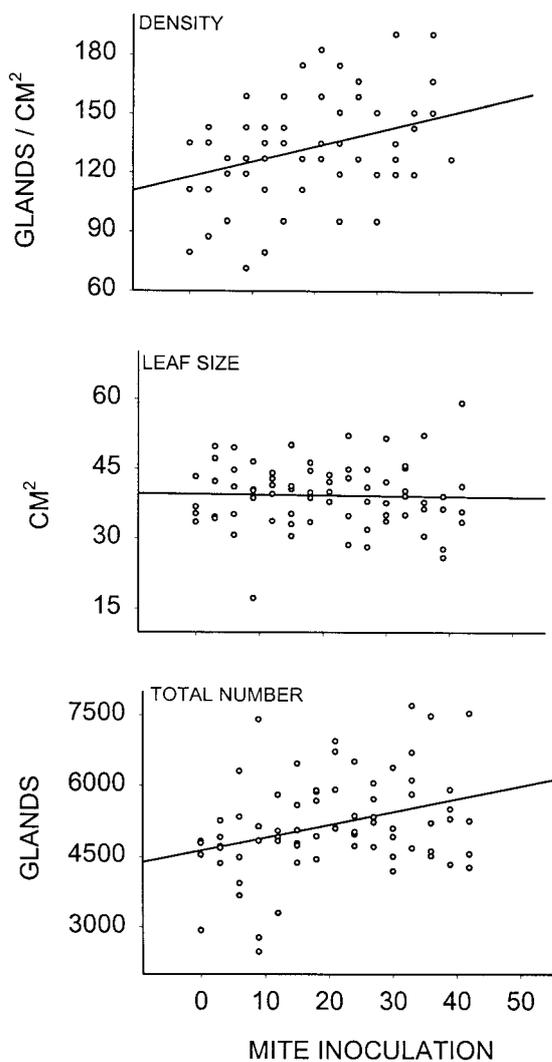
Figure 3. Effects of varying levels of spider mite herbivory on the cotyledons of plants on the density of pigment glands, leaf size, and total number of glands on the first true leaves of plants.

Figure 4. Effects of varying levels of spider mite herbivory on the cotyledons of plants on the density of pigment glands, leaf size, and total number of glands on the second true leaves of plants.

caterpillars. Thus, it appears that there are at least two different density dependent induced responses in cotton plants.

The role of glands in constitutive and induced resistance to mites and caterpillars. Spider mite population growth was not affected by constitutive or induced gland production. Mites feed by piercing cells and sucking up their contents. It is not known whether mites avoided the glands or were simply immune to the negative effects of the terpenoid aldehydes inside of the glands. Because our colony of mites has been reared on glanded cotton plants for more than 200 gen-
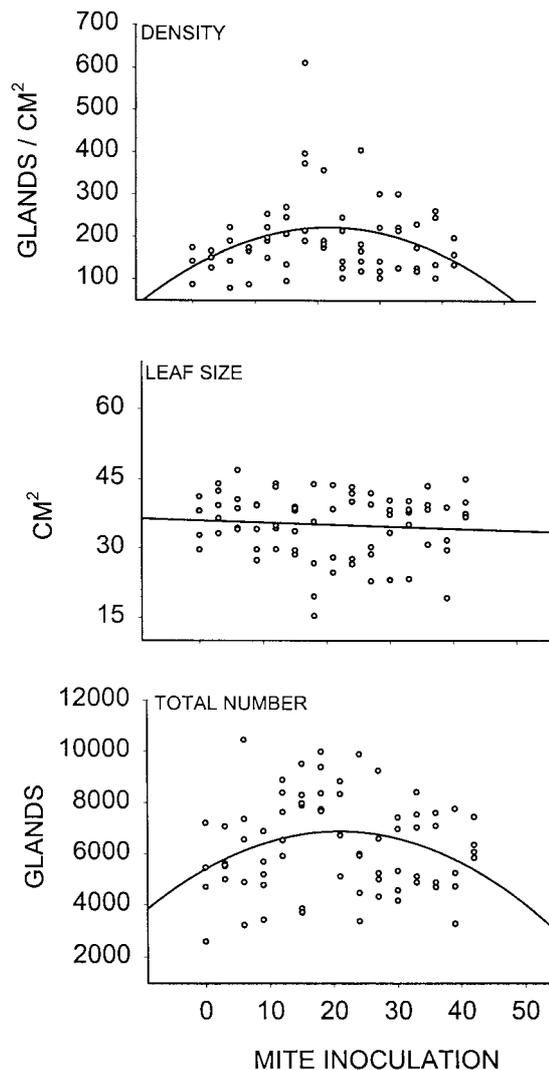
erations, it is possible that the mites have adapted to effects of the glands (Agrawal, 2000). It is, however, clear that the mites are still quite susceptible to other resistance mechanisms of the plant, as we found a 50% reduction in the population growth of mites on induced compared to control plants, regardless of whether the plants had glands or not.

Caterpillars were negatively affected by constitutive and induced expression of glands. Only the smallest caterpillars are able to avoid feeding on the glands. Induction of resistance by mite feeding strongly reduced caterpillar growth, but this effect was more than three times as strong in glanded plants compared to
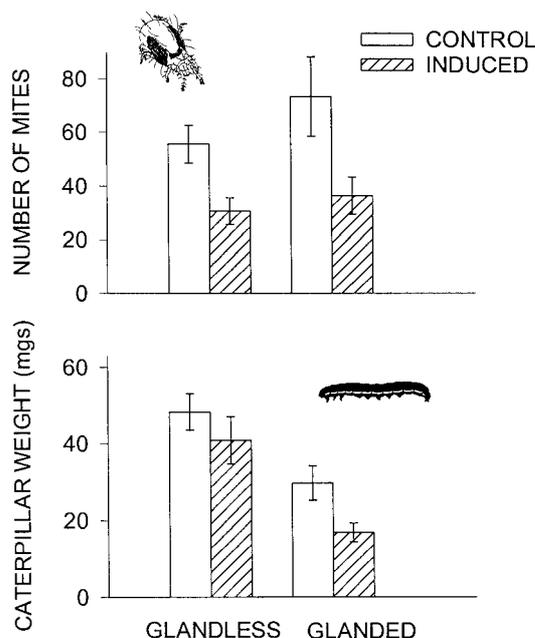
Figure 5. Effects of spider mite herbivory on the cotyledons of plants (induced resistance) on the (a) population growth of mites and (b) weight gain of army worm caterpillars on glanded and glandless varieties of cotton. Bars are mean ± 1 S.E.

Table 4. Effects of cotton variety, presence of pigment glands, induced responses to herbivory, and trial on the growth of *Spodoptera exigua* caterpillars. All three- and four-way interactions were included in the model, but all P ≫ 0.05

Source	d.f.	m.s.	F	P
Variety (V)	2	0.396	1.371	0.268
Pigment glands (G)	1	5.554	19.220	<0.001
Induced response (I)	1	3.210	11.109	0.002
Trial (T)	1	0.168	0.580	0.452
V*G	2	0.408	1.413	0.258
V*I	2	1.200	4.151	0.025
V*T	2	0.516	1.787	0.183
G*I	1	0.103	0.357	0.554
G*T	1	0.449	1.555	0.221
I*T	1	0.001	0.001	0.996
Error	33	0.289		

glandless plants. Previous experiments with glanded cotton plants have shown that damage by mites (Karban, 1988) and caterpillars (Alborn et al., 1996; Bi et al., 1997; McAuslane et al., 1997; McAuslane & Alborn, 1998) can induce resistance (reduced preference and performance) to caterpillars. Furthermore, damage to glanded plants resulted in *S. exigua* caterpillars
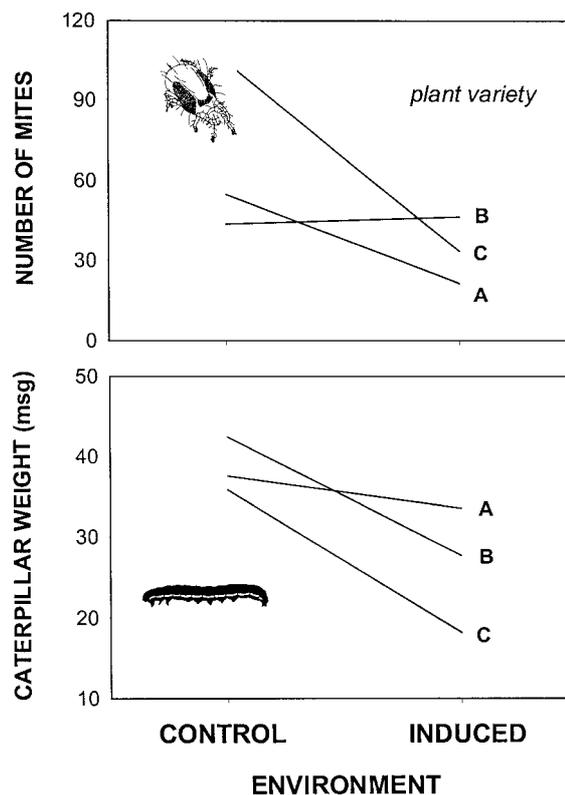

Figure 6. Reaction norm plots of caterpillar and mite performance on control and induced plants. Each line in the figure represents a variety of cotton (A = Acala, B = Lambright, and C = 1209-619-7). Means for performance were averaged over all other factors, including the fact that each variety had both glanded and glandless plants.

having a 33-fold preference for control over induced plants; while in glandless plants, caterpillars only showed a 2.6-fold preference for control over induced plants (McAuslane & Alborn, 1998). Our performance assays support these findings. The larger effect of induced resistance in glanded varieties may be due to at least two non-mutually exclusive mechanisms: (1) induction of glands per se and of their contents may result in greater toxicity to the caterpillars, and (2) induction of factors other than terpenoids in glanded plants may enhance resistance due to synergistic effects involving glands and the other components of induced resistance.

Mechanisms of induced resistance in cotton. Several phytochemical mechanisms may result in constitutive and induced resistance (Bell, 1984; Duffey & Stout, 1996; Sadras & Felton, 2000). In our study, the mechanisms that negatively impacted mites and caterpillars

appeared to be distinct. The terpenoid aldehydes, including gossypol, in the pigment glands of cotton are effective against several lepidopteran herbivores (Parrott, 1990; Sadras & Felton, 2000). Gossypol and its phytochemical relatives are compounds that are thought to act by binding to proteins in the caterpillar gut, thereby reducing growth and performance of the caterpillar (Gershenzon & Croteau, 1992). Two chemicals in cotton, gossypol and caryophyllene oxide, had synergistic effects on the performance of *Heliothis virescens* caterpillars (Gunasena et al., 1988). Bi et al. (1997) studied cotton plants damaged by caterpillars and found significant induction of several oxidative enzymes, cell wall lignification, and reductions in the nutritive properties of the plant. In addition, foliar increases in gossypol, hemigossypolone, and heliocides (all terpenoid aldehydes) were found in damaged plants compared to controls (McAuslane & Alborn, 1998).

Spider mites were unaffected by glands and their terpenoid aldehyde constituents. At this point little is known about factors that do contribute to resistance against mites. Earlier demographic work suggested that reduced plant nutritional quality may be one of the primary mechanisms of induced resistance to mites in cotton (Brody & Karban, 1989); physiological studies have supported this suggestion (Bi et al., 1997). Further work on the induction of oxidative enzymes and other potential mechanisms in glanded and glandless plants, and their direct effects on mites would be useful.

Induced terpenoids and indirect defense of cotton. Plants may induce 'indirect defenses', by enhancing volatile production and attracting natural enemies of herbivores. Volatiles, including terpenes, emanating from damaged plants have long been recognized as potential cues for natural enemies of herbivores (Sabelis & van de Baan, 1983). Glandless cotton plants produced minimal, if any, volatile terpenoids (Elzen et al., 1985), while glanded plants produced them constitutively. Glanded cotton plants that are infested with herbivores release terpenoid volatiles that are attractive to the natural enemies of herbivores (De Moraes et al., 1998). Glandless plants are susceptible to caterpillars and do not emit volatiles which attract parasitoids. We suggest that the positive association between glands and volatile production may not be incidental, and that plants that are not subject to caterpillar herbivores should employ neither direct (foliar glands) nor indirect (volatile production) defenses. Alternatively, in the cotton system, direct and indirect defenses may be physiologically linked because of their common terpenoid origin (Gershenzon & Croteau, 1992).

Genetic variation in induced resistance to two herbivores. Genetic variation in induced plant resistance has been reported from nearly 20 plant-herbivore systems (Agrawal, 1999). As in previous experiments with cotton (Brody & Karban, 1992), we found strong varietal variation in constitutive and induced resistance to mites and caterpillars. The rank order of constitutive resistance to mites and caterpillars was exactly opposite among varieties, suggesting a genetic trade-off in resistance to these two attackers (left side of Figure 6a, b). Trade-offs in plant resistance to different attackers have been proposed as an ecological cost, and a potential mechanism maintaining genetic variation in resistance. However the pattern of a trade-off did not hold for induced resistance, where there was no clear relationship of resistance to the two attackers (right side of Figure 6a; b). For both caterpillars and mites, one variety appeared to lack induced resistance, although it was a different variety for each herbivore.

Acknowledgements

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