

POLYMORPHISM IN PLANT DEFENSE AGAINST HERBIVORY: CONSTITUTIVE AND INDUCED RESISTANCE IN *Cucumis sativus*

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Abstract—Theory predicts that plant resistance to herbivores is determined by both genetic and environmentally induced components. In this study, we demonstrate that the phenotypic expression of plant resistance to spider mite herbivory in *Cucumis sativus* is determined by genetic and environmental factors and that there is an interaction between these factors. Previous feeding by spider mites induced systemic resistance to subsequent attack over several spatial scales within plants, reducing the population growth of mites compared to that on control plants. Effects of induction were effective locally over the short term, but resulted in local increased susceptibility to spider mite attack after several days. However, this local induced susceptibility on the damaged leaf was associated with induced resistance on newer leaves. Induced resistance was correlated with increases in cucurbitacin content of leaves, but was not associated with changes in the density of leaf trichomes. Induced resistance to herbivory was not detected in plants of a genotype lacking constitutive expression of cucurbitacins, which were in general highly susceptible to mite attack. Allocation trade-offs between growth and defense are often invoked to explain the maintenance of variation in the levels of plant resistance. Contrary to current thinking, neither constitutive nor herbivore-induced plant resistance were associated with reductions in plant allocation to root and shoot growth. However, plants that had high levels of induced resistance to spider mites were the most susceptible to attack by a

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specialist beetle. Such ecological trade-offs between resistance to generalist herbivores and susceptibility to specialist herbivores may be important in the maintenance of variation of plant resistance traits. In summary, *C. sativus* exhibits strong genetic variation for constitutive and induced resistance to spider mites, and this variation in resistance is associated with ecological trade-offs.

Key Words: Cucumber, *Cucumis*, cucurbitacins, defense theory, *Diabrotica*, herbivory, induced responses, plant-insect interactions, plant resistance, spider mites, *Tetranychus*.

INTRODUCTION

Host-plant genotype and environment can play central roles in determining plant resistance to herbivory (Denno and McClure, 1983; Fritz and Simms, 1992). Intraspecific genetic variation can cause large differences in plant resistance to herbivores (Berenbaum et al., 1986; Simms and Rausher, 1989; Karban, 1992). Environmentally induced effects, such as plant responses to herbivory, have also been shown to negatively impact preference and/or performance of herbivores in over 100 systems (Karbon and Baldwin, 1997; Agrawal, 1998a; Agrawal et al., 1999a). In addition, plant genotype and environment frequently interact to influence plant resistance to herbivores (Agrawal, 1999a). Determining the factors that maintain variation in constitutive and induced levels of plant resistance has been a central issue in plant defense theory (Hermis and Mattson, 1992). Experimentally determining costs and benefits of expressing particular resistance phenotypes will help us to understand the selection pressures and constraints that have evolutionarily shaped plant defense.

Current thinking suggests that constitutive and environmentally induced expression of plant resistance traits diverts resources otherwise available for growth and reproduction (Hermis and Mattson, 1992). Such trade-offs have been commonly invoked and modeled as mechanisms behind the maintenance of genetic variation in plant resistance and inducible or facultative expression of plant resistance (environmentally induced variation). In a critique of this "allocation model," Mole (1994) pointed out that allocation to plant resistance may be trivial, especially in light of the myriad functions that plants perform. Plants do not simply grow or defend. In addition, there is increasing evidence that plants can increase nutrient uptake and photosynthetic rate to compensate for particular energetic burdens such as herbivory (van der Meijden et al., 1988; Trumble et al., 1993; Rosenthal and Kotanen, 1994; Sadras, 1995; Strauss and Agrawal, 1999).

Some authors have recently suggested non-mutually exclusive alternative hypotheses to the allocation model for explaining variation in plant resistance traits (Linhart, 1991; Parker, 1992; Simms, 1992; Karban, 1993; Rausher, 1996; van der Meijden, 1996; Agrawal and Karban, 1999). However, experimental tests

of these non-mutually exclusive hypotheses are lacking. One hypothesis suggests that variation in plant resistance traits should be maintained in both constitutive and induced resistance because some specialized herbivores may have counter-adapted to the defense (Linhart, 1991; Adler and Karban, 1994; van der Meijden, 1996; Agrawal and Karban, 1999). For example, if specialized herbivores make use of plant defense effective against generalist herbivores as host finding cues or as a source of chemicals that they can sequester for their own defense, then the "plant resistance traits" may only be beneficial to the plant in some environments (i.e., where generalist herbivores are more common than specialized herbivores). Anecdotal observations of such trade-offs between resistance and susceptibility to generalist and specialist herbivores have been reported from several plant-herbivore systems (Chambliss and Jones, 1966; Da Costa and Jones, 1971; Matsuda, 1988; Wink, 1988; Linhart, 1991; Pasteels and Rowell-Rahier, 1992; Vaughn and Hoy, 1993; Landau et al., 1994; Matsuki and MacLean, 1994; Giamoustaris and Mithen, 1995; Loughrin et al., 1995, 1996; Mithen et al., 1995; Alados et al., 1996; Jiang et al., 1997 van Dam and Hare, 1998).

In this study, we document differences in plant resistance to herbivory in two near-isogenic varieties of *Cucumis sativus* that differ qualitatively in production of cucurbitacins (hereafter, high cucurbitacins = bitter plants and no cucurbitacins = sweet plants). We first demonstrate differences in constitutive and inducible levels of plant resistance to generalist spider mites (*Tetranychus urticae*) in bitter and sweet plants and then investigate the phytochemical and physical basis of this resistance. We test two models, the allocation trade-off model and the specialist/generalist trade-off model, to explain the genotype and environmentally induced variation in resistance. Specifically, we asked: (1) What are the consequences for spider mite populations feeding on bitter or sweet, previously damaged or undamaged *C. sativus* plants? (2) Do cucurbitacins or trichomes increase locally and systemically in damaged plants? (3) What are the within plant spatial aspects of induced resistance in *C. sativus*? (4) Is constitutive or induced resistance to spider mites correlated with reduced allocation to root and/or shoot growth and biomass accumulation? (5) Is there a trade-off between resistance to generalist herbivores and susceptibility to specialist herbivores such that specialist herbivores (spotted cucumber beetles, *Diabrotica undecimpunctata*) prefer to feed on plants expressing high levels of induced resistance to spider mites?

Study System. *Cucumis sativus* (Cucurbitaceae) is commonly grown as cultivated cucumber. Many cucurbits vary qualitatively in the intraspecific presence or absence of cucurbitacin production (tetracyclic triterpenoids). Cucurbitacins are the most bitter tasting compounds known and can be detected by humans in dilutions of 1 ppb (Da Costa and Jones, 1971; Metcalf et al., 1980; Metcalf and Lampman, 1989). We used Marketmore varieties 76 (bitter) and 80 (sweet), that are genetically identical except for those genes tightly linked

with the gene responsible for the presence or absence of cucurbitacins (H. M. Munger, personal communication). Cucurbitacin production appears to be controlled by a two-allele single-locus gene, where cucurbitacin production is dominant. Cucurbitacins have been demonstrated to function defensively against several widespread generalist herbivores including spider mites, roaches, several beetles, lepidopteran larvae, mice, and vertebrate grazers (Da Costa and Jones, 1971; Gould, 1978; Metcalf and Lampman, 1989; Tallamy et al., 1997). Specialized feeders on *C. sativus*, such as diabroticite beetles, however, use cucurbitacins as kairomones, which cause "compulsive" feeding on plants with high levels of cucurbitacins (Chambliss and Jones, 1966; Metcalf et al., 1980, 1982; Metcalf and Lampman, 1989). In addition, diabroticite beetles sequester cucurbitacins, which may protect them against attack by natural enemies (Ferguson and Metcalf, 1985; Barbercheck et al., 1995). Growth rate, fecundity, and longevity of these beetles is unaffected by cucurbitacins (Hirsh and Barbercheck, 1996; Tallamy and Gorski, 1997).

METHODS AND MATERIALS

Cucumis sativus plants were grown in 125-cm³ plastic pots in greenhouse soil (U.C. mix, Redi-Gro, Inc., Sacramento, California) in laboratory growth boxes at ambient temperatures (25 ± 3°C) on a 14L:10D cycle. Two-spotted spider mites were collected from cotton, beans, roses, and morning glories in Davis, California, and were maintained in a laboratory colony on cotton plants.

Genetic Variation in Constitutive and Induced Resistance to Spider Mites. This experiment was set up as a 2 × 2 factorial design to measure spider mite population growth on bitter and sweet plants and on plants with and without initial plant exposure to spider mites. The protocol for the initial spider mite exposure and the challenge with subsequent mites was as follows: When the cotyledons had fully expanded (after seven days), half the plants were inoculated with 15 adult female mites. They were allowed to feed on the cotyledons for three days, after which all of the plants (including undamaged controls) were dipped in dicofol (Kelthane, 100 ppm, Rohm and Haas, Philadelphia, Pennsylvania), a miticide that does not move systemically through the plant. At this time, the first true leaf was only a bud. Seven to 10 days later, when the first true leaf had fully expanded, this leaf was isolated by placing a thin ring of sticky Tanglefoot (Grand Rapids, Michigan) around the petiole near the base. Each first true leaf was inoculated with three adult female spider mites. Seven days later all stages of mites were counted on the leaves with the help of a dissecting microscope. This experiment was conducted three times with sample sizes of 10–20 plants for each treatment in each trial. Spider mite population growth on bitter and sweet, and induced and not induced plants was analyzed for the three trials by using a three-way ANOVA.

Cucurbitacin and Trichome Analysis. Sweet *C. sativus* plants did not contain cucurbitacins (H. M. Munger, personal communication; A. A. Agrawal, personal observation by taste). To evaluate local and systemic induction of cucurbitacins in bitter plants, experiments identical to those described above (without the challenge) were carried out with 12 plants in each of the two treatments. When the first true leaves had fully expanded, cotyledons and first true leaves were severed by using a razor blade, placed in coin envelopes, and put in a 0°C freezer. The following day, leaf tissue was freeze-dried and weighed. Because first true leaf samples were too small for chemical analysis, the 12 samples were randomly divided into four groups of three leaves each. Leaf samples were dried at 50°C over night and milled to a fine powder. We extracted 0.2 g of leaf powder in 0.5 ml of 95% methanol in 1.5 ml Eppendorf tubes. Homogenates were vortexed three times and allowed to sit overnight. The next day, homogenates were vortexed again, centrifuged, and the pellets were extracted two more times at 1-hr intervals with methanol. Combined methanol extracts were dried under N₂. Samples were redissolved in methanol and spotted on silica gel 254 thin-layer chromatography NP plates along with cucurbitacin C standards. Plates were developed in ethylacetate-toluene (75:25), dried, and cucurbitacin aglycones were quantified by using a Shimadzu SC9000U scanner (Shimadzu Scientific Instruments, Columbia, Maryland) (Halaweish and Tallamy, 1993). Cucurbitacin C was identified by comparing R_f values with those of the standard (visualization: UV). Cucurbitacin content was compared between induced and noninduced plant tissue by using one-tailed *t* tests to address the a priori hypothesis that cucurbitacin content would be higher in more resistant (induced) leaves.

An additional set of chemical analyses was conducted to test for phytochemical effects of localized damage to the first true leaf of bitter plants. We grew 40 plants until the first true leaf was fully expanded and then inoculated 20 of them with 15 spider mites each. The mites were allowed to feed for three days, after which all plants were treated with the miticide (dicofol). These plants were not challenged with mites, but were allowed to grow for three additional days, after which the damaged leaves were harvested, as above, for cucurbitacin analysis. Again, several leaves were pooled for each sample and the final sample sizes were: control, *N* = 6; induced *N* = 8.

In addition to the cucurbitacin analyses, we counted trichomes on the undersides of induced and control leaves to determine if part of the induced resistance to spider mites seen in the bitter plants were correlated with increased density of trichomes (*N* = 15–17 for each treatment). The total number of trichomes was counted in a 1-cm square at the base of each leaf, and the means were compared by *t* test.

Within-Plant Distribution of Induced Resistance. We conducted several experiments to determine spatial aspects of the induced resistance of *C. sativus* to spider mite herbivory. The protocol used in these experiments was identical to

that described above except as indicated. In the first experiment, we challenged plants by placing 30–50 mites on the first true leaf ($N = 11$ plants for each treatment). At this time, the second true leaf was beginning to expand, and neither leaf was isolated with Tanglefoot. The total number of mites was counted on the first and second true leaves one week later. The goal was to determine if spider mite herbivory induced systemic resistance throughout the entire growing plant and to test if induced resistance was robust to a challenge of mites mimicking outbreak conditions. In the second experiment, we induced the first true leaf (instead of the cotyledons) when it was fully expanded, inoculated the second true leaf with three adult female mites, and determined population growth after a week ($N = 10$ –12 for each treatment).

In the third set of experiments, we tested for localized effects of induction by creating two 3-cm² Tanglefoot enclosures on the tops of all first true leaves (see Gould, 1978). The two square enclosures were contiguous and shared one common border. On treatment plants, we inoculated one enclosure with 15 mites and then removed them by using the miticide (dicofol) after three days (one enclosure on the control leaves was also treated with the miticide). After three days, the second enclosure on all plants was inoculated with three female mites, and mites were counted after one week. In this experiment, the challenge mites were exposed to a section of the leaf unexposed to initial treatment with mites. This experiment was repeated two times with 11–13 samples for each treatment, each trial. Because the results were contrary to our predictions, we conducted an additional experiment on localized effects of herbivory on subsequent plant resistance. We inoculated the first true leaf with 15 spider mites, and after three days of feeding we removed all mites and eggs by hand by using a paint brush and a dissecting microscope (no Tanglefoot or miticide was used on the leaf). After three days, we inoculated the same leaf and controls with three mites and measured mite population growth as above ($N = 19$ –20 for each treatment). In this experiment, the challenge mites were exposed to the entire first true leaf (as were the initial treatment mites). The goal of this experiment was to test for localized effects of mite feeding on subsequent mite population growth without the use of Tanglefoot or a miticide.

A final experiment on localized effects of damage was conducted to test if induced responses conferred rapid resistance to herbivory. In the above experiments, challenge mites were placed on the plant six days after the original inducing mites were placed on the plant; in this experiment, challenge mites were placed on the plant after one day of mite feeding. The experiment was conducted by using the Tanglefoot enclosures and miticide procedures described above. Treatment plants ($N = 18$) were exposed to feeding by 15 mites for the first 24 hr, but control plants ($N = 16$) received no mites for the first 24 hr. After the mites were removed by hand, all plants were inoculated with three female mites, and mites were counted after one week. Mite population sizes in these experiments were compared by using t tests.

Growth Rate of Bitter and Sweet Plants. To test if plant resistance and associated cucurbitacin production were correlated with growth rate and/or allocation to root and shoot biomass, we set up a 2×2 factorial experiment that used bitter and sweet plants initially exposed or not exposed to spider mites ($N = 24$ –25 for each treatment). As before, plants in the induction treatment were exposed to mites for three days. All plants were treated with the miticide, but no plants were subsequently challenged with spider mites. Plants were grown for two weeks following initial spider mite exposure and then were harvested. Roots were washed with water to remove all soil and all plant parts were dried for three days at 60°C in a drying oven. Dried plant material was weighed to 0.1 mg, and differences in weight were analyzed by using two-way ANOVAs.

Feeding Preference of a Specialized Feeder. Spotted cucumber beetle (*Diabrotica undecimpunctata*) adults were collected from cotton flowers in Davis, California, and used immediately for experiments. Individual *C. sativus* plants that were induced or not induced were prepared as above by damaging cotyledons and then plants were paired in 20-liter ventilated boxes. Two days after damage, one beetle was introduced to each cage and allowed to feed for 24 hr; the percent leaf area consumed was estimated on each plant by using an acetate grid ($N = 10$ pairs). Measurements of percent leaf area consumed were arc-sine square root transformed and compared by paired t test.

RESULTS

Genetic Variation in Constitutive and Induced Resistance to Spider Mites.

Spider mites had approximately twice the population growth on sweet plants lacking cucurbitacins than on bitter plants with cucurbitacins (Figure 1, Table

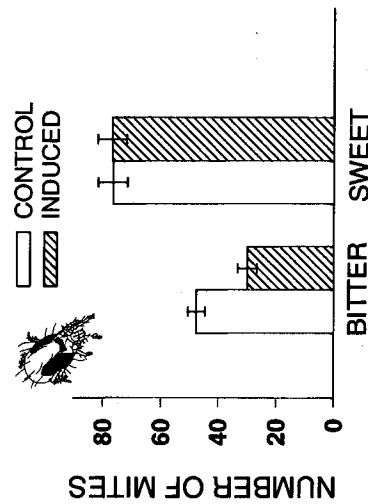


FIG. 1. The influence of plant genotype and induction treatment on population growth of herbivorous spider mites (mean ± SE).

TABLE 1. ANALYSIS OF VARIANCE TABLE FOR EFFECTS OF PLANT GENOTYPE AND INDUCTION TREATMENT ON SPIDER MITE POPULATION GROWTH

Source	df	ms	F	P
Genotype	1	37534.818	106.786	<0.001
Induction	1	2542.079	7.232	0.008
Genotype × induction	1	2361.010	6.717	0.011
Trial	2	4463.464	12.698	<0.001
Error	138	351.497		

1). Although previous mite damage (induction) had no effect on subsequent plant resistance in sweet plants, bitter plants showed a 40% increase in resistance following an initial mite attack (Figure 1, Table 1). Overall, there were highly significant effects of genotype (sweet vs. bitter), induction, and genotype × induction interaction on population size of the mites, indicating that although damage induced resistance in bitter plants, sweet plants were not inducible (Figure 1, Table 1).

Cucurbitacin and Trichome Analysis. Induced resistance caused by initial mite attack was associated with a significant increase in the cucurbitacin content of leaves of bitter plants (Figure 2). The cucurbitacin content of induced bitter plants increased locally in the (damaged) cotyledons by 30% ($t = 2.328$, $df = 20$, $P = 0.015$) and systemically in the (undamaged) first true leaves by 50% ($t = 2.368$, $df = 6$, $P = 0.028$) over controls. The number of trichomes on induced and control plants did not differ (mean \pm SEM of control plants = 31.643 ± 1.856 ; induced plants = 30.353 ± 1.025 ; $t = 0.637$, $df = 29$, $P = 0.529$).

Within Plant Distribution of Induced Resistance. Induced resistance to spider mites was spatially dynamic within individual plants: when previously dam-

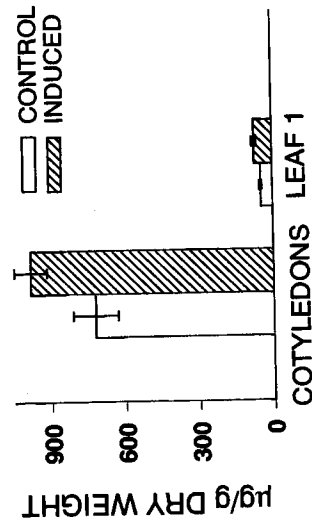


FIG. 2. Local and systemic effects of spider mite herbivory on cucurbitacin-c content of leaves (mean \pm SE).

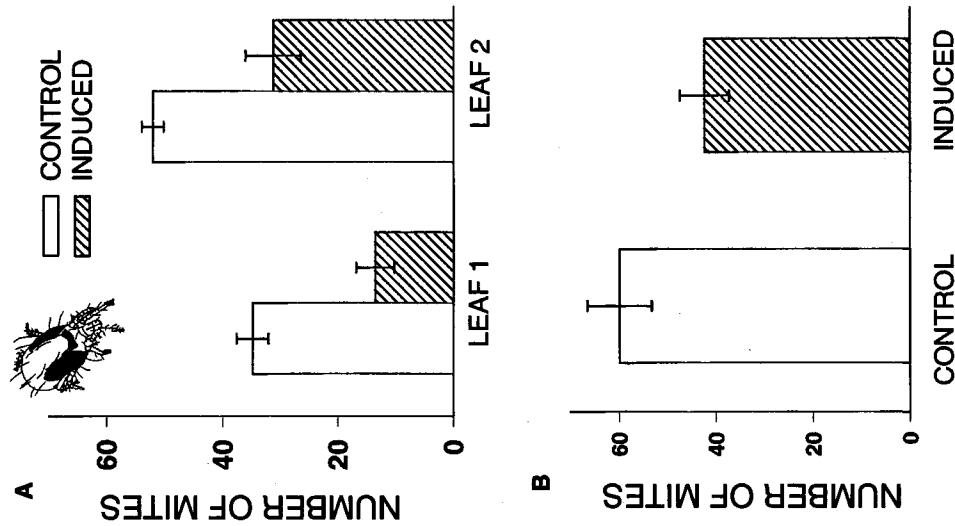


FIG. 3. Within-plant spatial aspects of induced resistance to spider mite herbivory (mean \pm SE): (A) Effects of initial spider mite damage to the cotyledons on systemic resistance on the first and second true leaves under outbreak conditions. At the time of initial spider mite attack, the first and second true leaves were not formed. (B) Effect of initial spider mite damage to the first true leaf on spider mite population growth on the second true leaf.

aged plants were faced with a challenge of 30–50 spider mites, induced resistance persisted and decreased mite population growth twofold compared to controls (Figure 3A) ($F_{1,40} = 39.358$, $P < 0.001$). The induced resistance was systemic throughout the entire plant; only the cotyledons were damaged, and mite

population growth was decreased on both the first and second true leaves (Figure 3A). The second true leaf consistently supported higher spider mite populations than did the first true leaf. When only the first true leaf was damaged, the quality of second declined and supported smaller mite populations compared to undamaged controls (Figure 3B, $t = 2.134$, $df = 19$, $P = 0.046$).

Damage to the first true leaf resulted in increased susceptibility of the first true leaf to subsequent attack in the experiments that used Tanglefoot enclosures (Figure 4A; induction effect: $F_{1,44} = 6.234$, $P = 0.016$; trial effect: $F_{1,44} = 0.036$, $P = 0.851$). These effects were found in plants that were challenged locally with mites six days after the initial attack. Similar results were found when we locally challenged leaves with mites after removing the initial mites by hand (Figure 4B, $t = -7.422$, $df = 37$, $P < 0.001$), not using Tanglefoot or the miticide. Leaf cucurbitacin analysis on plants treated exactly as in the above two trials revealed that there was two times the level of cucurbitacins in locally damaged leaves compared to undamaged leaves (mean \pm SEM $\mu\text{g/g}$ dry weight cucurbitacin, controls = 5.5 ± 1.016 , induced = 10.175 ± 1.406 ; $t = -2.695$, $df = 12$, $P = 0.020$). This result was contrary to our predictions because increased susceptibility to mites was correlated with increases in cucurbitacin content of leaves. However, when leaves that were damaged by spider mites were challenged 24 hr after the initial inoculation, we found increased resistance on plants that were previously damaged (Figure 4C, $t = 2.506$, $df = 32$, $P = 0.018$). These results suggest that local induced responses can result in short-term increased resistance followed by a rapid decay and increased susceptibility.

Growth Rate of Bitter and Sweet Plants. Neither root nor shoot biomass accumulation was affected by constitutive or induced levels of plant resistance (Figure 5, all $F_{s1,40} < 1.13$, all $P_s > 0.291$).

Feeding Preference of a Specialized Feeder. *Diabrotica undecimpunctata* beetles ate significantly more leaf tissue of previously damaged plants compared to undamaged controls (Figure 6, $t = -3.695$, $df = 9$, $P = 0.005$).

DISCUSSION

Genetically determined expression of constitutive and induced plant resistance traits can have important fitness consequences for herbivores and the plants they feed on (Agrawal, 1999a). In the present experiment, spider mites produced nearly two times as many progeny on plants that did not produce cucurbitacins compared to near-isogenic plants that constitutively produced cucurbitacins. In addition, systemically induced production of cucurbitacins was correlated with reductions in mite population growth on previously damaged plants compared to controls. Sweet plants did not exhibit induced resistance to herbivory by spider mites. This finding has two important implications: First, the induced resistance

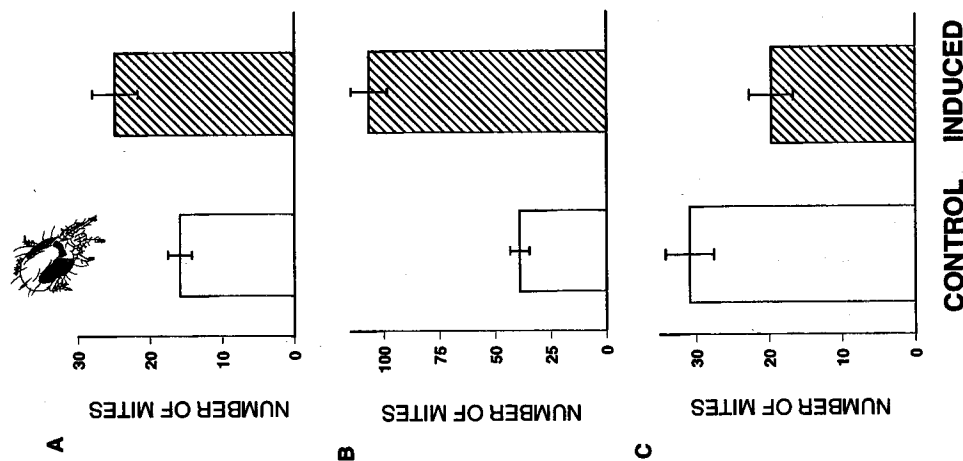


FIG. 4. Localized effects of induction (mean \pm SE): (A) Effect of initial spider mite damage to the first true leaf on subsequent mite population growth on the same leaf, six days after the original inoculation. Initial mites and challenge mites were confined by using Tanglefoot enclosures. Initial mites were removed with a miticide. (B) Effect of initial spider mite damage to the first true leaf on population growth of mites subsequently attacking the same leaf, six days after the original inoculation. Initial mites and challenge mites were free to move across the entire first true leaf. All mites and eggs were removed by hand after initial attack. (C) Effect of initial spider mite damage to the first true leaf on subsequent mite population growth on the same leaf, one day after the original inoculation. Initial mites and challenge mites were confined by using Tanglefoot enclosures. Initial mites were removed with a miticide.

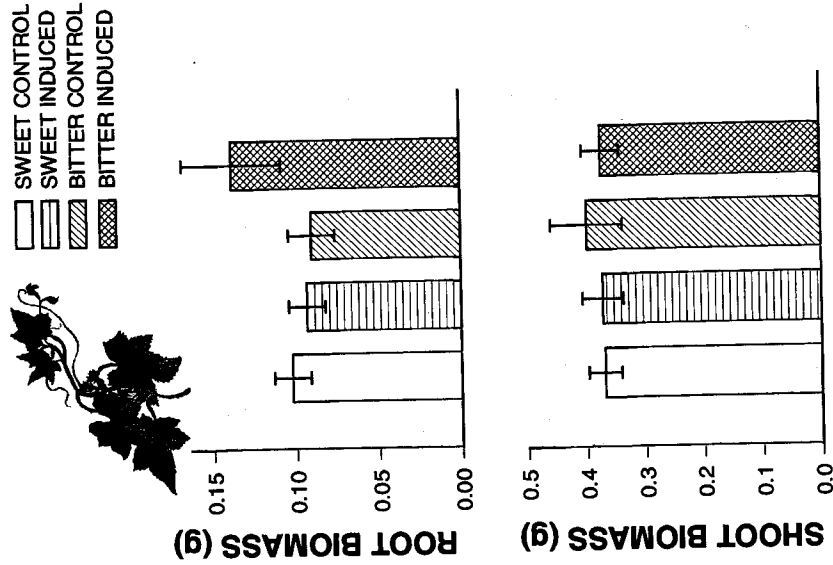


FIG. 5. The influence of plant genotype and induction treatment on allocation to root and shoot biomass (mean \pm SE).

demonstrated in bitter plants was likely due to phytochemical induction of cucurbitacins and probably not due to nutritional effects, the induction of unidentified compounds, or deterioration of host-plant quality. Elsewhere we have demonstrated that when cucurbitacins are added to a benign host plant, mite population growth is reduced compared to that on controls (Agrawal, 1999b). Second, the single locus that appears to be responsible for constitutive production of cucurbitacins in *C. sativus* is also associated with inducible expression of cucurbitacins. Plants with constitutive expression of cucurbitacins are inducible, and plants without constitutive cucurbitacins are not inducible. This result violates theoretical predictions of a negative association between constitutive and inducible resistance in plants (Herms and Mattson, 1992). Zangerl and Beren-

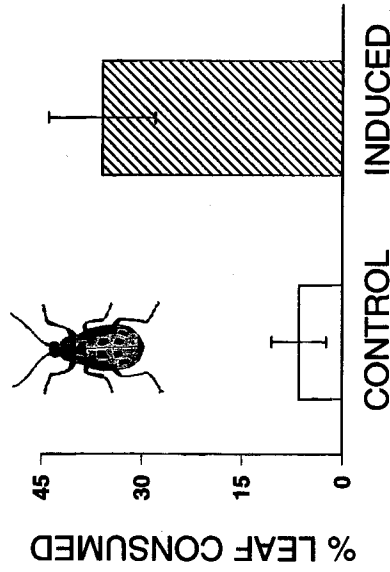


FIG. 6. The effect of induction treatment in the bitter genotype on feeding of a specialist Diabroticite beetle (mean \pm SE).

baum (1990) and Siemens and Mitchell-Olds (1998) also found a positive relationship between constitutive and inducible resistance in wild parsnip and mustard, respectively. Other workers have found no apparent association between constitutive and induced plant resistance to herbivores (Brody and Karban, 1992; Thaler and Karban, 1997).

Induced resistance to spider mites in *C. sativus* is spatially dynamic within individual plants (Figure 3). Resistance was induced systemically throughout the plant when cotyledons were damaged. Damage to the cotyledons led to more resistant first true leaves compared to undamaged controls, even though the first true leaf was not fully formed when the damage took place. Similarly, we found systemic effects of damaging the first true leaf, which resulted in a more resistant second true leaf compared to undamaged controls. Finally, induced resistance to spider mites is a robust plant response that did not collapse or lose its effectiveness when faced with outbreak conditions where up to 50 mites infested the leaves. In other systems, such as the pine-bark beetle association, large numbers of attackers have been shown to overwhelm the induced host-plant defenses (reviewed by Tallamy, 1986).

Localized responses to leaf damage appeared to be more complex than systemic responses. In two types of experiments set up to detect localized induced resistance, initial damage actually increased susceptibility to subsequent attack (after short-term induced resistance), greatly increasing mite population growth on the previously damaged leaf (Figure 4). This susceptibility was found on plants that were initially damaged six days earlier. In addition, cucurbitacin content of damaged leaves was two times higher than in control leaves. This result is contrary to our predictions of cucurbitacins increasing resistance to the spi-

der mites. On plants that were damaged in the past 24 hr, we did find induced resistance locally. These results suggest that local induced responses can result in increased resistance or susceptibility, depending on the timing of subsequent attack. Similar results were recently reported by Underwood (1998), in which damaged soybean plants initially became more resistant to beetles; however, this resistance decayed and eventually led to induced susceptibility. Although some cucurbit responses to herbivory yield rapid increases in cucurbitacins followed by subsequent relaxation of cucurbitacins (Tallamy and McCloud, 1991), our phytochemical analysis suggests that cucurbitacins were higher in locally damaged leaves, even those that were more susceptible to herbivores. Thus, even though there was increased local allocation to defense, the localized herbivory may have caused the damaged leaf to become a nutrient sink (e.g., Inbar et al., 1995), increasing the nutritive value of the leaf to the herbivores, and leaving the leaf more susceptible to herbivores several days after attack.

In addition to the direct effects of induced plant responses to herbivory, damage to some plants results in the release of volatiles that attract natural enemies of herbivores (Takabayashi and Dicke, 1996; Shimoda et al., 1997; Agrawal, 1998b; De Moraes et al., 1998; Thaler, 1999). Young *C. sativus* plants that were infested with spider mites released a blend of volatiles that attracted the predaceous mite, *Phytoseiulus persimilis* (Takabayashi et al., 1994). Taken together, our study and that of Takabayashi et al. indicate that when *C. sativus* is damaged by spider mites, the plants not only increase defense directly by increasing compounds that reduce herbivore population growth, but also indirectly by attracting natural enemies. At this stage, it is not clear whether sweet plants (lacking cucurbitacins and induced resistance) would also produce the blend of volatiles that attract natural enemies of herbivores. Recent evidence from systems involving beet army worms, corn, and tomato plants indicated that induced emission of natural enemy attractants is physiologically linked to the broadly conserved octadecanoid pathway that regulates phytochemical induction of resistance (Alborn et al., 1997; Thaler, 1999). If the octadecanoid pathway is defective in sweet *C. sativus* plants, then they may also lack induction of volatiles that attract predaceous arthropods.

Why all plants do not constitutively express high levels of plant resistance is still debated (Fritz and Simms, 1992; Mole, 1994; Karban and Baldwin, 1997; Agrawal and Karban, 1999). The current paradigm of allocation based trade-offs has not been strongly supported by experiments that have measured costs of constitutive or inducible defenses (Parker, 1992; Simms, 1992; Karban, 1993; Bergelson and Purrington, 1996; Karban and Baldwin, 1997). Under the conditions of this study, we detected no energetic cost of constitutive or inducible resistance in *C. sativus*. Root and shoot biomass accumulation was not affected by genotypic (bitter or sweet) or induced expression of cucurbitacins and the correlated resistance to spider mites. Cucurbitacins are carbon-based molecules

lacking potentially expensive nitrogen and may even serve the plant in temporary carbon storage. Multiple roles for plant defenses may mask their costs.

Why then might variation in plant resistance be maintained? In this study, we found evidence that induced plant resistance to spider mites was associated with increased feeding by specialist diabroticite beetles. Similar results have been obtained by O. Cheeseman (personal communication), who found that diabroticites were attracted to and fed more on damaged *Cucurbita pepo* plants compared with undamaged control plants. Interestingly, diabroticites will often ignore cucurbits without cucurbitacins (Tallamy and McCloud, 1991). Thus, at the phenotypic level, induction of cucurbitacins is a double-edged sword, defending plants against some herbivores while attracting others. At the genotypic level, resistance to mites associated with susceptibility to diabroticite beetles (and vice versa) has been known as one of the classic examples in trade-offs in plant defense (Chambliss and Jones, 1996; Da Costa and Jones, 1971).

Trade-offs between resistance and susceptibility to generalist and specialist herbivores are not likely to be specific to the Cucurbitaceae. We are currently investigating similar trade-offs between brassicaceous plants and their specialist and generalist lepidopteran herbivores. Interactions between plants in several other families and specialist and generalist herbivores appear to mirror these patterns (Matsuda, 1988; Wink, 1988; Linhart, 1991; Pasteels and Rowell-Rahier, 1992; Vaughn and Hoy, 1993; Landau et al., 1994; Matsuki and MacLean, 1994; Loughrin et al., 1995, 1996; Alados et al., 1996; Jiang et al., 1997; van Dam and Hare, 1998).

Other constraints and selection pressures may also favor constitutive and induced plant variation in resistance traits (reviewed by Agrawal and Karban, 1999). For example, plants may not be able to maximize defense against the many different types of attackers. In *C. sativus*, resistance induced by pathogens is not effective against herbivores (Ajlan and Potter, 1991; Apriyanto and Potter, 1990). These authors found that inoculation of plants with various pathogens led to induction of resistance against subsequent attack by several pathogens. However, these induced responses did not affect performance or preference of a variety of arthropod herbivores including spider mites, aphids, noctuid larvae, and whiteflies. Reciprocal initial attack by arthropods did not affect plant resistance to the pathogens. Evidence from other systems indicates that these independent plant defense responses may be controlled by separate pathways, which may actually inhibit each other (Doares et al., 1995; Thaler et al., 1999). Inducible defenses may be favored to maximize a plant's ability to defend itself against various attackers, since resistance to all attackers at once may not be physiologically possible. It is also possible that molecules such as cucurbitacins serve multiple functions, and selection on one function may be tempered by selection on other functions (Tallamy and Krischik, 1989).

There may be other costs and benefits of variation in plant resistance traits

(Mole, 1994; Karban and Baldwin, 1997; Agrawal and Karban, 1999; Agrawal et al., 1999b,c). For example, Kirschik and Tallamy (unpublished) have demonstrated that cucurbitacins enhance the fitness of plants exposed to ultraviolet light (UV-B irradiance). However, expression of compounds implicated in defense may be constrained by physiological problems, such as autotoxicity. Plant resistance against herbivores may also interfere in interactions with mutualists such as seed dispersers and pollinators (Stephenson, 1982; Willmer and Stone, 1997). Many cucurbits have cucurbitacins in their flowers (Andersen and Metcalf, 1987). *Cucumis sativus* fruits with cucurbitacins were visited significantly less often by wasps and honeybees than fruits without cucurbitacins (Da Costa and Jones, 1971). Variation in plant resistance traits may maximize defense in additional ways that we discuss in detail elsewhere (Agrawal and Karban, 1999). It is clear from this and other plant-herbivore studies that to fully understand variable defenses we will have to look beyond the allocation trade-off model.

In *C. sativus*, there appears to be strong genetic variation for constitutive and induced variation in plant resistance to spider mite herbivory. This variation was not correlated with allocations to plant growth. However, high levels of resistance to spider mites associated with high levels of cucurbitacins were correlated with susceptibility to attack by specialist diabroticite beetles. Such ecological trade-offs are likely to be more common than previously thought and may play a role in the maintenance of constitutive and induced variation in plant resistance traits.

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