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INTERACTIVE EFFECTS OF GENOTYPE, ENVIRONMENT, AND ONTOGENY ON RESISTANCE OF CUCUMBER (*Cucumis sativus*) TO THE GENERALIST HERBIVORE, *Spodoptera exigua*

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Abstract—Host-plant genotype, environment, and ontogeny all play a role in determining plant resistance to herbivory, yet little is known about the nature of the interactions among these factors. We investigated resistance of cucumber plants *Cucumis sativus* to the generalist herbivore *Spodoptera exigua* in a manipulative experiment involving three factors. In particular, we tested the effects of bitter (cucurbitacins present) vs. sweet (cucurbitacins absent) plants (genotype), with or without previous herbivory (environment), and cotyledons vs. true leaves (ontogeny). Contrary to our expectations, *S. exigua* growth was 54% higher on bitter plants than on sweet plants; growth was 63% higher, however, on undamaged plants compared to damaged plants, and 59% higher on true leaves compared to cotyledons. Moreover, all two-way interaction terms between genotype, environment, and ontogeny were significant. For example, *S. exigua* performance was higher on bitter than on sweet plants; however, this effect was strongly influenced by whether the tissue consumed was a cotyledon or true leaf and also whether it had been previously damaged. An examination of leaf nutritional chemistry revealed that some of our results could be explained by genotypic, environmental, and ontogenic differences in foliar nitrogen content. In contrast, the cucurbitacin content of plants did not appear to affect caterpillar growth. Our results provide evidence for the importance of interactions between genotype, environment, and ontogeny in determining herbivory and illustrate the value of manipulative experiments in revealing the complexities of these interactions.

Key Words—Cucurbitacins, genotype by environment interaction, herbivory, nitrogen concentration, plant–insect interactions.

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INTRODUCTION

Determining the mechanisms of plant resistance to insects is a central pursuit in understanding the ecology and evolution of plant–herbivore interactions. Host-plant genotype, environment, and ontogeny are all known to play a role in influencing plant traits such as defense against herbivores (Denno and Mcclure, 1983; Fritz and Simms, 1992; Schlichting and Pigliucci, 1998). Genetically based variation in host-plant chemistry can result in large differences in plant resistance to herbivores (Karban, 1992). In addition, both the biotic and abiotic environment are well known to influence the defensive phenotype of plants (Karban and Baldwin, 1997; Koricheva et al., 1998a,b). Less is known about the influence of plant ontogeny or developmental stage on resistance to herbivory. All individuals pass through a series of ontogenic stages during growth, and recent studies indicate that differences in the developmental phase of plants can strongly influence resistance to herbivory (Kearsley and Whitham, 1989, 1998; Karban and Thaler, 1999; Gianoli, 2002). Although each of these factors has been examined individually, how they interact to determine levels of plant resistance is rarely studied. Understanding the interactions is critical for developing a predictive framework for the influence of plant traits on herbivores in the field, where plants show considerable variation in genotype, environment, and ontogeny.

Here, we use the cultivated cucumber *Cucumis sativus* (Cucurbitaceae) and the generalist herbivore *Spodoptera exigua* (Noctuidae) to determine how plant genotype, environment, and ontogeny interact to influence resistance to herbivory. *Spodoptera exigua* is a generalist herbivore known to feed on plants of at least 20 families, including the Cucurbitaceae (Tietz, 1972). Cucumber varieties vary qualitatively in the presence or absence of defensive cucurbitacins (tetracyclic triterpenoids), as determined by a single diallelic locus (Andeweg and De Bruyn, 1959). Cucurbitacins are bitter tasting, and plants that produce these compounds (hereafter bitter plants) are typically hostile hosts to a wide range of herbivores, including beetles, lepidopteran larvae, cockroaches, mice, and vertebrate grazers (Da Costa and Jones, 1971; Gould, 1978; Metcalf and Lampman, 1989; Tallamy and Krischik, 1989; Tallamy et al., 1997; Agrawal et al., 1999). In contrast, plants that produce no cucurbitacins (hereafter sweet plants) are typically more susceptible to herbivory (but see Chambliss and Jones, 1966; Metcalf et al., 1980; Agrawal et al., 1999). The occurrence of a simply inherited chemical polymorphism provided us with an experimental system for investigating how genotypes that differ in chemical resistance to herbivory are affected by environmental and ontogenic factors.

Several environmental influences have the potential to modify the intensity of herbivory on bitter vs. sweet plants of *C. sativus*. For example, herbivory typically induces responses in plants that negatively impact the preference and performance of subsequently feeding herbivores (Karban and Baldwin, 1997; Agrawal, 1998, 1999). In a comparison of bitter vs. sweet genotypes of *C. sativus,* Agrawal et al. (1999) demonstrated that spider mite herbivory decreased following damage, but this effect was only expressed in bitter plants. To what extent this type of interaction can be modified during plant ontogeny has not been investigated. However, it seems likely that since the chemical composition and hence food quality of plants change during development, these interactions with herbivores should be influenced by ontogeny. Indeed, Agrawal et al. (1999) found that cucurbitacin content varied greatly according to ontogenic stage, with cotyledons containing well over 10 times the cucurbitacins in true leaves. Also, when cotyledons were damaged, cucurbitacins increased by 30%, compared to a 50% increase in true leaves (Agrawal et al., 1999). These cucurbitacins have recently been shown to quantitatively reduce spider mite survival (Balkema-Boomstra et al., 2003).

In this study, we asked the following questions: (1) Is the performance of *S. exigua* reduced on bitter vs. sweet genotypes? (2) Does previous herbivory by *S. exigua* cause an induced response that reduces the performance of subsequent herbivores of this species? (3) Does *S. exigua* exhibit a preference for feeding on different ontogenic stages (cotyledons vs. true leaves) of *C. sativus,* and how does this influence their performance? We then considered pair-wise interactions between each of these factors to evaluate whether environmental and ontogenic factors influencing food quality might alter the performance of *S. exigua* on bitter vs. sweet plants. Finally, we conducted chemical analyses to determine the carbon and nitrogen concentrations of leaves as influenced by genotype, environment, ontogeny, and their interactions. Examination of these primary nutrients, which can be limiting for herbivores, complements our previous analyses of how genotype, environment, and ontogeny influence defensive cucurbitacins (Agrawal et al., 1999).

METHODS AND MATERIALS

Study System and General Procedures. We conducted all experiments using the near-isogenic varieties Marketmore 76 (bitter) and Marketmore 80 (sweet) of the cultivated cucumber (*C. sativus*) (Gould, 1978; Agrawal et al., 1999, 2002). A single diallelic locus controls the presence or absence of cucurbitacin production in this species, with the dominant allele governing synthesis of cucurbitacins (Andeweg and De Bruyn, 1959). All experiments were conducted during June–August 2002 at the Koffler Scientific Reserve at Jokers Hill, near Newmarket, Ontario, Canada (44°03′ N, 79°29′ W). Cucumber plants were grown from seed, germinated on petri dishes, and grown in 210-ml pots using a Pro-Mix soil (Red Hill, PA) and $\approx$0.3 g of slow-release Nutricote fertilizer (13:13:13::N:P:K) (Vicksburg Chemical, Vicksburg, MS). We typically grew plants in a completely randomized design under field conditions in large enclosures ($2 \times 2 \times 3$ m in size) made of nylon mesh to exclude wild herbivores.

We maintained *S. exigua* in a laboratory colony on an artificial diet (Southland Products, Arkansas). Third to fifth instar larvae were used to damage plants (for induced resistance experiments) and freshly hatched neonates were used for all bioassays of plant resistance. In each bioassay, we typically allowed a single larva to feed for 5 days and used the fresh mass of the caterpillars as our response variable. We conducted bioassays on cut leaves placed in 90-mm Petri dishes lined with moistened filter paper and sealed with Parafilm. In such bioassays, we used a single replicate of cotyledons and true leaves from each plant.

Effects of Genotype, Environment, and Ontogeny on S. exigua. To determine the independent and interactive effects of genotype, environment, and ontogeny on *S. exigua* performance, we conducted two experiments. In the first, we tested whether genotype and ontogeny influenced *S. exigua* performance on *C. sativus* plants grown within enclosures. We used a total of 80 plants distributed equally among the four treatment combinations (bitter vs. sweet and cotyledon vs. true leaf). We measured *S. exigua* growth and mortality on cotyledons or true leaves from bitter and sweet plants using the bioassay procedure described earlier. We assessed differences in mortality using a *G* test and growth using two-way ANOVA.

In the second experiment, we tested for the effects of all three factors (bitter vs. sweet, damaged vs. undamaged, and cotyledon vs. true leaf) and their interactions on *S. exigua* using the protocols described earlier. To condition the damaging caterpillars to cucumber, we placed them in a container with detached cucumber leaves for 24 hr before use. The caterpillars were allowed to damage singly bagged plants for 1 wk and were removed when approximately 10% of total leaf area (estimated visually) was removed per plant. We conducted bioassays 2 days following removal of the caterpillars. We used a total of 160 plants in this experiment, with sample size balanced among the eight treatment combinations. We analyzed data from this experiment using a three-factor ANOVA including all interactions.

To determine if *S. exigua* exhibited a preference for cotyledons or true leaves, we compared the total amount of herbivory on these two developmental stages by third to fifth instar *S. exigua* in the experiment described earlier. In addition, we conducted a separate experiment with a total of 58 *C. sativus* plants that were transplanted into a ploughed field at the cotyledon stage and grown with 1-m spacing between plants. We covered all plants with spun polyester sleeves (Rockingham Opportunities Corporation, Reidsville, NC) to protect them from herbivory. Single third to fifth instar *S. exigua* were placed on plants with at least two true leaves, and caterpillars were replaced if they escaped. For each replicate, preference for cotyledons vs. true leaves was determined by visual estimation of the plant part that had greater leaf area removed. We assessed preference using a *G* test with William's correction; replicates with no herbivory were eliminated from the analysis.

Effect of Genotype on Generalist Weevil Preference and Spider Mite Performance. Because of our nonintuitive results of *S. exigua* having higher performance on bitter plants (see results), we tested how two other generalist herbivores were influenced by the presence of cucurbitacins. In particular, we investigated whether the feeding preference of weevils or the fecundity of spider mites differed between bitter and sweet plants of *C. sativus*. We used a naturally occurring weevil (*Sciaphilus asperatus*) from our study site and spider mites (*Tetranychus urticae*) obtained from a laboratory colony maintained on cultivated cotton plants. Both of these herbivores have broad host ranges and feed on plants in many families. In the feeding preference experiment, we recorded whether weevils fed on bitter ($N = 26$) or sweet ($N = 16$) plants grown within a single enclosure ($2 \times 2 \times 3$ m). Plants with two to three true leaves were randomized within the enclosure, and greater than 20 weevils were foraging inside. After 2 wk, we recorded whether weevils had imposed visible damage on each plant. We determined the effects of genotype on herbivore preference (damaged vs. undamaged) using a G test.

For the separate experiment using spider mites, we conducted two bioassay trials to test if plant genotype had an effect on mite fecundity. Previous experiments have established strong negative effects of cucurbitacins on spider mites (Agrawal et al., 1999). We inoculated cotyledons of nearly equal numbers of bitter and sweet plants with three adult female spider mites ($N = 29$ for trial 1 and $N = 47$ for trial 2). We counted the number of eggs on each plant after 1 wk and tested for the effect of plant genotype and trial on mite fecundity using a factorial ANOVA.

Plant Chemistry: Analysis of Carbon and Nitrogen Content of Leaves. To determine whether the nutritional status of leaves may be influenced by plant genotype, environment, and ontogeny, we examined the carbon and nitrogen concentrations of leaves. In particular, we were interested in whether the positive nutritional impact of nitrogen modified herbivore performance and thereby counteracted the influence of other factors such as cucurbitacins. We investigated the effects of all three factors and their interactions on leaf chemistry by growing and treating 60 plants as above, equally distributed among the eight treatment combinations (bitter vs. sweet, damaged vs. undamaged, and cotyledon vs. first true leaf). We separated true leaves and cotyledons and stored the samples in a −20°C freezer. Total foliar carbon and nitrogen concentrations were analyzed using a Perkin-Elmer CHNS Elemental analyzer with autosampler at the Analest facility, University of Toronto. We analyzed data from this experiment using a three-factor ANOVA including all interactions.

RESULTS

Plant Genotype and Performance of S. exigua. In contrast to our prediction, performance of *S. exigua* on bitter plants was generally higher than on sweet plants. The mortality of neonates was one-third lower ($G = 21.81$, $P < 0.001$), and the growth of survivors was 54% higher (separate variance $t = 2.52$, $df = 41.9$, $P = 0.016$) when feeding on bitter compared to sweet plants (Figure 1). In this first experiment, ontogeny had no effect on *S. exigua* performance ($P > 0.100$).

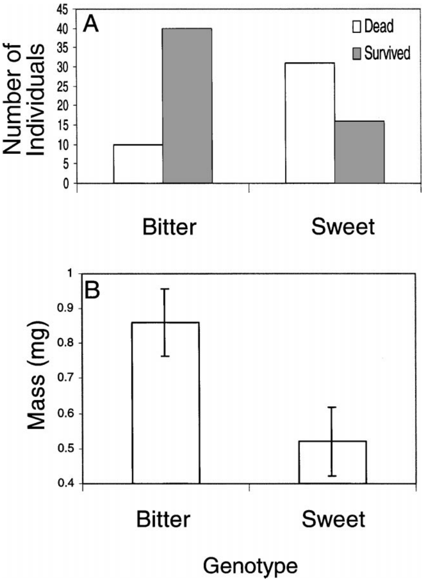

FIG. 1. The influence of plant genotype (leaves from bitter or sweet plants) on (A) mortality and (B) mass of surviving *Spodoptera exigua* (mean ± SE).

Interactive Effects of Genotype, Environment, and Ontogeny on S. exigua *Performance.* Genotype, environment, and ontogeny all influenced *S. exigua* performance in the experiment involving all three factors (Table 1, Figures 2 and 3). In common with the preceding experiment, *S. exigua* grew 16% faster on bitter than on sweet plants (Table 1, Figure 2). As predicted, growth was 63% higher on undamaged plants compared to damaged plants, providing evidence for induced resistance to herbivory (Table 1, Figures 2 and 3). However, in contrast to the

TABLE 1. ANALYSIS OF VARIANCE FOR THE EFFECTS OF GENOTYPE (BITTER OR SWEET), ENVIRONMENT (INDUCED OR CONTROL) AND ONTOGENY (COTYLEDON OR TRUE LEAF) ON *Spodoptera exigua* GROWTH

Source	SS	*df*	MS	*F*	*P*
Plant genotype (G)	0.407	1	0.407	5.860	0.016
Herbivore induction (I)	1.978	1	1.978	28.479	<0.001
Ontogeny (O)	11.285	1	11.285	162.510	<0.001
G × I	0.258	1	0.258	3.720	0.055
G × O	0.498	1	0.498	7.178	0.008
I × O	0.359	1	0.359	5.165	0.024
G × I × O	0.063	1	0.063	0.900	0.343
Error	19.860	286	0.069		

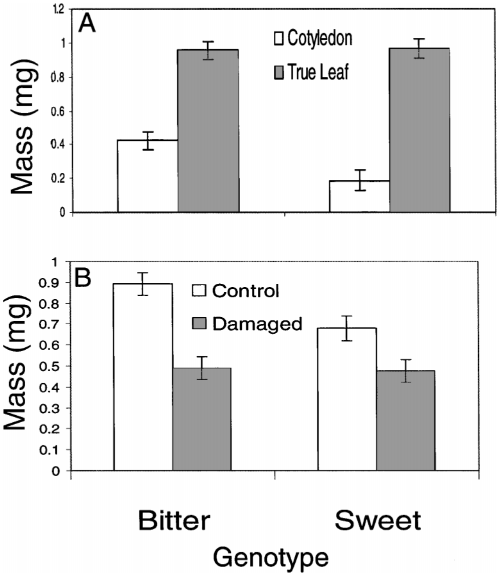

FIG. 2. The influence of plant genotype (leaves from bitter or sweet plants), ontogenic position (cotyledon or true leaf), and induction treatment (with or without previous damage by *Spodoptera exigua*) on mass of neonate *S. exigua* (least-squares means ± SE). Significant interactions are depicted in the figures: (A) the effect of genotype and ontogenic position on *S. exigua* growth; (B) the effect of leaf genotype and induction treatment on *S. exigua* growth.

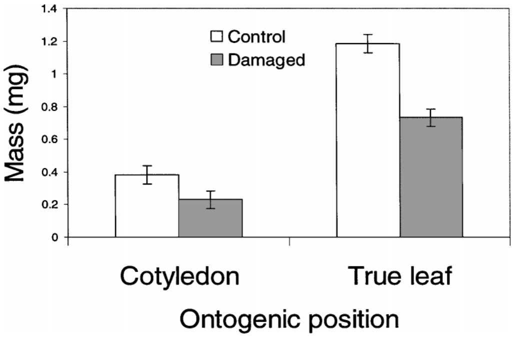

FIG. 3. The interactive effect of ontogenic position (cotyledon or true leaf) and induction treatment (with or without previous damaging by *Spodoptera exigua*) on growth of neonate *S. exigua* (least squares means ± SE).

preceding experiment, we detected a significant difference in performance of *S. exigua* neonates on the two developmental stages of *C. sativus* (Table 1). Caterpillar growth on true leaves was 59% higher than on cotyledons. It is unclear what caused this differential result between the two trials.

All three of the two-way interactions were significant (Table 1) indicating complex relations among genotype, environment, and ontogeny. For example, we found that *S. exigua* performance was higher on bitter vs. sweet plants; however, this effect was strongly influenced by whether the tissue consumed was a cotyledon or true leaf, and also whether or not it had been previously damaged (Figure 2). In particular, the increased performance seen on bitter plants was only apparent on cotyledons and undamaged plants. Similarly, ontogenic stage significantly affected *S. exigua* growth, but this response differed between previously damaged vs. undamaged plants (Figure 3). The increased performance on true leaves compared to cotyledons was much greater on control plants than on previously damaged plants.

Spodoptera exigua herbivory on bitter plants grown in the field was 10 times greater on cotyledons than on true leaves (Figure 4, adjusted $G = 58.253$, $P < 0.001$). A similar pattern of herbivory was evident on bitter plants in the enclosure (adjusted $G = 7.819$, $P = 0.005$) but not on sweet plants (Figure 4, adjusted $G = 0.116$, $P = 0.733$).

Plant Genotype and Weevils and Spider Mites. Results from two other generalist herbivores feeding on *C. sativus* differed from those described for *S. exigua*. Weevils from a natural population at our study site showed a clear preference for sweet plants (70% damaged) over bitter plants (11% damaged) ($G = 14.996$, $P < 0.001$). Spider mites also performed better on sweet plants than bitter plants, with higher fecundity during the 1-wk observation period (mean ± SE eggs, Bitter: 58.745 ± 3.741, Sweet: 71.546 ± 3.632; Genotype $F(1, 72) = 6.027$, $P = 0.017$;

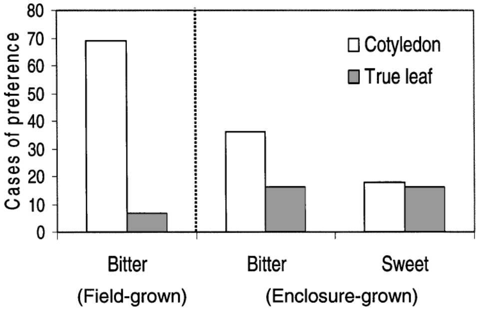

FIG. 4. Preference of *Spodoptera exigua* on bitter and sweet plants for feeding on cotyledons vs. true leaves.

TABLE 2. ANALYSIS OF VARIANCE FOR THE EFFECTS OF GENOTYPE (BITTER OR SWEET), ENVIRONMENT (INDUCED OR CONTROL) AND ONTOGENY (COTYLEDON OR TRUE LEAF) ON NITROGEN CONTENT IN *Cucumis sativus*

Source	SS	*df*	MS	*F*	*P*
Plant genotype (G)	5.396	1	5.396	14.145	<0.001
Herbivore induction (I)	2.653	1	2.653	6.955	<0.001
Ontogeny (O)	21.357	1	21.357	55.985	<0.001
G × I	1.475	1	1.475	3.867	0.052
G × O	0.200	1	0.200	0.524	0.471
I × O	0.297	1	0.297	0.779	0.380
G × I × O	0.035	1	0.035	0.092	0.762
Error	33.570	1	0.381		

Trial $F(1, 72) = 63.903$, $P < 0.001$; Genotype × Trial, $F(1, 72) = 0.226$, $P = 0.636$).

Plant Chemistry: Analysis of Carbon and Nitrogen Content of Leaves. Genotype, environment, and ontogeny all significantly influenced nitrogen concentration (Table 2, Figure 5), but only ontogeny had any effect on carbon concentration (least-squares means ± SE percentage carbon, cotyledons: 38.9 ± 0.3, true leaves: 43.9 ± 0.3, $F(1, 88) = 8.987$, $P < 0.001$, all other P values in the fully factorial analysis >0.100). Bitter plants showed 12% higher nitrogen concentration than the sweet genotype. Nitrogen concentration was 8% higher on damaged compared to undamaged plants. In addition, true leaves contained 22% higher nitrogen concentration and 11% higher carbon concentration than cotyledons (Figure 6). Finally,

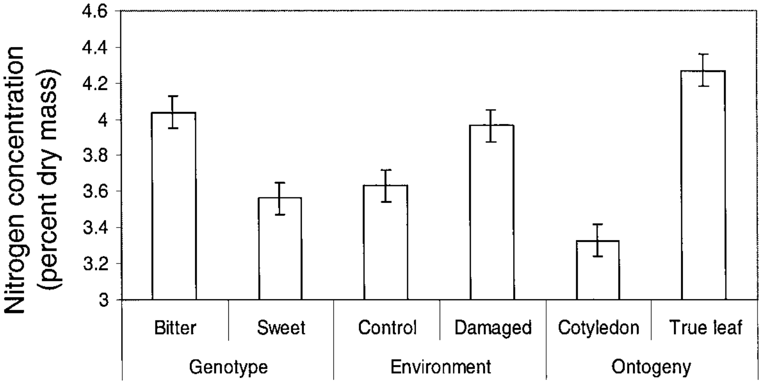

FIG. 5. Nitrogen concentration of leaves as affected by plant genotype (bitter or sweet), ontogenic position (cotyledon or true leaf), and induction treatment (with or without previous damaging by *Spodoptera exigua*). Overall main effects are reported in the figure (averaging over other factors, as calculated by least squares means ± SE).

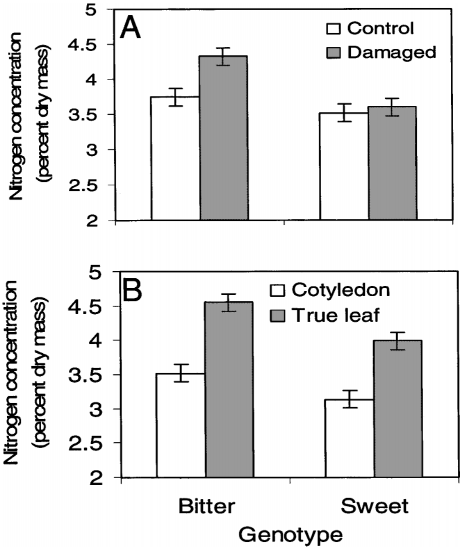

FIG. 6. The effect of cucumber genotype (bitter or sweet) and (A) damage state or (B) ontogenic position (cotyledon or true leaf) on nitrogen content (least squares means ± SE).

there was a significant interaction between the effects of genotype and previous damage on nitrogen concentration (Figure 6). Damaged bitter plants showed 17% higher nitrogen concentration than damaged sweet plants; however, this difference was minimal (6%) in undamaged plants.

DISCUSSION

The role of genotype, environment, and ontogeny in determining plant and animal phenotypes is widely recognized (Schlichting and Pigliucci, 1998). Yet, experimental investigations of herbivory rarely capture this ecological realism. The results of our study implicate complex interactions among all three factors on generalist herbivore feeding. This finding has important implications for understanding the variation in intensity of herbivory that is commonly observed in the field. Later we discuss how factors influencing host-plant chemistry may have played a decisive role in the complexity of our findings.

Why Does S. exigua *Perform Better on Bitter vs. Sweet Plants?* Contrary to our predictions, *S. exigua* exhibited higher performance on the bitter than on the sweet genotype of *C. sativus*. In experiments with adult *S. exigua*, Tallamy et al. (1997) found that cucurbitacins deterred oviposition. We expected that larval *S. exigua*, like other generalist herbivores, would also be deterred by the presence of cucurbitacins in bitter plants. Indeed, both of the other generalist herbivores (weevils and spider mites) either preferred or performed better on sweet compared to bitter plants. This suggests that the response of *S. exigua* larvae on *C. sativus* may be atypical for a generalist (Tallamy et al., 1997). Insights into the potential explanation for this unexpected response can be obtained by examining the interactions between genotype and the other two factors investigated.

The benefit that *S. exigua* gained on bitter plants was only evident when larvae were feeding on either cotyledons or undamaged plants. In contrast, the difference in performance on the two genotypes was significantly less when larvae fed on true leaves or on damaged plants. This difference in when bitterness affected *S. exigua* was associated with contrasting nitrogen levels in plants. Our chemical analysis (Figures 5 and 6) indicated that cotyledons and undamaged plants possessed lower levels of nitrogen than true leaves and damaged plants, respectively. Moreover, we also found that overall bitter plants had significantly higher nitrogen content than sweet plants. Therefore, since nitrogen is almost certainly a limiting nutrient for *S. exigua* (Al-Zubaidi and Capinera, 1983; Broadway and Duffey, 1988), it is plausible that larvae performed better on bitter plants because of the additional nitrogen. In addition, our bioassays were conducted with newly hatched first instars, which may be particularly sensitive to low nitrogen concentrations in leaves (Zalucki et al., 2002).

Spodoptera exigua only performed better on bitter plants in treatments in which the bitter genotype was paired with low nitrogen states (undamaged plants and cotyledons). This finding suggests that in these combinations, higher nitrogen in bitter plants was important as a source for enhanced performance of the caterpillars (Figure 6). Conversely, there was no enhancement of performance on bitter vs. sweet plants where larvae fed on damaged bitter plants or their true leaves, presumably because nitrogen demand was met by factors other than genotype. These contrasting responses suggest that the detrimental effects of cucurbitacins in the bitter genotype can be countered by increased performance, resulting from high nitrogen, but only when other factors limit this essential nutrient. This explanation implies that the effectiveness of chemical defenses may be modified depending on the nitrogen composition of the plants employing them (Broadway and Duffey, 1988).

The underlying causes for nitrogen differences among the treatments are unknown. It may be argued that since nitrogen concentrations are higher in bitter plants and in induced bitter plants (but not in sweet plants, Figure 6), the cucurbitacins are responsible for elevating nitrogen concentrations. However, cucurbitacins may have a pleiotropic effect influencing other traits affecting nitrogen production. Alternatively, the genes that are tightly linked to the gene that controls the production of cucurbitacins may influence other traits affecting nitrogen production.

A potential explanation for the higher nitrogen concentrations present in damaged plants could be that previously damaged plants induced nitrogen-rich compounds in addition to the carbon-based cucurbitacins. Although herbivory has been reported to both increase and decrease nitrogen concentrations, depending on the plant–herbivore system (Murray et al., 1996; Ruohomaki et al., 1996; Danell et al., 1997; Karban and Baldwin, 1997), cucumbers in particular increase photosynthetic efficiency following herbivory (Thomson et al., 2003). Such increases in photosynthetic efficiency are frequently due to increases in foliar Rubisco levels, which are rich in nitrogen (Lambers et al., 1998). The effect of ontogeny on nitrogen concentration is more difficult to explain. Karban and Thaler (1999) showed that cotton cotyledons have an increased rate of photosynthesis compared to true leaves, and suggested that this may be responsible for the higher level of herbivory that was observed. It is possible that some process or attribute correlated with photosynthesis (i.e., chlorophyll concentrations) is accountable for altering the nitrogen concentrations of different plants or plant parts.

Induced Responses and Ontogeny Modify Herbivore Performance. As expected, the larvae of *S. exigua* feeding on undamaged plants performed better than those on previously damaged plants. This finding is consistent with induced defense theory, which predicts that following an attack, defenses are mounted that serve to increase the resistance of plants to further herbivory (Karban and Baldwin, 1997; Agrawal, 1998). In our case, we assume that the induced compounds involved elevated cucurbitacins, as was demonstrated in an earlier study with the same varieties (Agrawal et al., 1999). Since only bitter plants of *C. sativus* produce cucurbitacin, we expected no difference in the performance of *S. exigua* on damaged vs. undamaged plants of the sweet genotype. However, the presence of a small decrease in *S. exigua* performance on damaged sweet plants in relation to undamaged plants suggests that there may be other detrimental induced responses in *C. sativus*. Indeed, in an analysis of the head-space volatiles produced by damaged bitter and sweet plants, we found several induced compounds in sweet plants (Agrawal et al., 2002). The greater strength of induced resistance in bitter plants than sweet plants presumably results from an interaction of several factors including changes in cucurbitacins, nitrogen, and other unknown factors.

Our results indicate that induced response may also be modified by plant ontogeny. *Spodoptera exigua* performance on undamaged plants was significantly higher than damaged plants when larvae fed on true leaves. In contrast, there was little difference in larval performance when larvae fed on cotyledons, irrespective of whether plants were damaged or not (Figure 3). This result may be associated with the interaction between nitrogen content, ontogeny, and the mechanisms of induced resistance. In a study on plant phase change and resistance to herbivory, Karban and Thaler (1999) demonstrated that population growth of spider mites on cotton is greater on cotyledons than on true leaves, and that this difference is likely due to some process correlated with high photosynthetic rates in cotyledons. Cucurbitacin content also varies with ontogenic stage (Agrawal et al., 1999). The extent to which *S. exigua* performed better on undamaged plants appears to be moderated by whether damaged or undamaged plants are combined with a high (true leaves) or low (cotyledons) nitrogen factor. We speculate that since undamaged plants have lower nitrogen content than damaged plants, the positive advantage these plants confer to *S. exigua* from the absence of induced defenses is offset by their lack of nitrogen. This may explain why, if nitrogen deficiency is removed through combination with a high nitrogen factor (i.e., true leaves or the bitter genotype), the undamaged plants confer an increased advantage over damaged plants.

Our results suggest that plants in the field are likely to provide diverse food choices for the same species of generalist herbivore depending on their developmental status and prior history of herbivory. Interestingly, in preference tests, *S. exigua* overwhelmingly chose to feed on cotyledons over true leaves, even though their performance is lower on this ontogenic stage. Although this is counterintuitive, previous studies have also found that individual preference and performance are not always linked in herbivory (Waldbauer and Friedman, 1991; Karban and Agrawal, 2002). For example, *Smynthurodes betae* galling aphids did not display any preference for optimal galling sites on leaves (Burstein and Wool, 1993).

Interactions Between Genotype, Environment, and Ontogeny Maintain Variation in Food Quality. Our results have highlighted the complex interactions that can occur among genotype, environment, and ontogeny in governing generalist herbivore performance. There was no single combination of the three factors in our experiments that optimized larval feeding on *Cucumis sativus*. This implies that in natural populations where there is considerable environmental heterogeneity and variation in developmental status of plants, herbivores are likely confronted with a large range of food choices resulting from the changing nutritional profiles of plant material. This pervasive spatial and temporal variation within and between plants may make it quite difficult for herbivores to optimally choose their foods and may ultimately be a resistance mechanism itself (Denno and Mcclure, 1983; Whitham, 1983; Karban and Baldwin, 1997; Agrawal and Karban, 1999; Orians and Jones, 2001; Orians et al., 2002).

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REFERENCES

AGRAWAL, A. A. 1998. Induced responses to herbivory and increased plant performance. *Science* 279:1201–1202.

AGRAWAL, A. A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. *Ecology* 80:1713–1723.

AGRAWAL, A. A., GORSKI, P. M., and TALLAMY, D. W. 1999. Polymorphism in plant defense against herbivory: Constitutive and induced resistance in *Cucumis sativus*. *J. Chem. Ecol.* 25:2285–2304.

AGRAWAL, A. A., JANSSEN, A., BRUIN, J., POSTHUMUS, M. A., and SABELIS, M. W. 2002. An ecological cost of plant defence: attractiveness of bitter cucumber plants to natural enemies of herbivores. *Ecol. Lett.* 5:377–385.

AGRAWAL, A. A. and KARBAN, R. 1999. Why induced defenses may be favored over constitutive strategies in plants, pp. 45–61, *in* R. Tollrian and C. D. Harvell (eds.). The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, NJ.

AL-ZUBAIDI, F. S. and CAPINERA, J. L. 1983. Utilization of food and nitrogen by the beet armyworm, *Spodoptera exigua* (Huebner) (Lepidoptera: Noctuidae), in relation to food type and dietary nitrogen levels. *Environ. Entomol.* 13:1604–1608.

ANDEWEG, J. and DE BRUYN, J. 1959. Breeding of non-bitter cucumbers. *Euphytica* 8:13–20.

BALKEMA-BOOMSTRA, A. G., ZIJLSTRA, S., VERSTAPPEN, F. W. A., INGGAMER, H., MERCKE, P. E., JONGSMA, M. A., and BOUWMEESTER, H. J. 2003. Role of cucurbitacin C in resistance to spider mite (*Tetranychus urticae*) in cucumber (*Cucumis sativus* L.). *J. Chem. Ecol.* 29:225–235.

BROADWAY, R. M. and DUFFEY, S. S. 1988. The effect of plant protein quality on insect digestive physiology and the toxicity of plant proteinase inhibitors. *J. Insect Physiol.* 34:1111–1117.

BURSTEIN, M. and WOOL, D. 1993. Gall aphids do not select optimal galling sites (*Smynthurodes betae*, Pemphigidae). *Ecol. Entomol.* 18:155–164.

CHAMBLISS, O. L. and JONES, C. M. 1966. Cucurbitacins: Specific insect attractants in Cucurbitaceae. *Science* 153:1392–1393.

DA COSTA, C. P. and JONES, C. M. 1971. Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in *Cucumis sativus* L. *Science* 172:1145–1146.

DANELL, K., HAUKIOJA, E., and HUSS-DANELL, K. 1997. Morphological and chemical responses of mountain birch leaves and shoots to winter browsing along a gradient of plant productivity. *Ecoscience* 4:296–303.

DENNO, R. F. and MCCLURE, M. S. (eds.) 1983. Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

FRITZ, R. S. and SIMMS, E. L. (eds.) 1992. Plant Resistance to Herbivores and Pathogens. University of Chicago Press, Chicago.

GIANOLI, E. 2002. A phenotypic trade-off between constitutive defenses and induced responses in wheat seedlings. *Ecoscience* 9:482–488.

GOULD, F. 1978. Resistance of cucumber varieties to *Tetranychus urticae*: Genetic and environmental determinants. *J. Econ. Entomol.* 71:680–683.

KARBAN, R. 1992. Plant variation: Its effects on populations of herbivorous insects, pp. 195–215, *in* R. S. Fritz and E. L. Simms (eds.). Plant Resistance to Herbivores and Pathogens. Ecology, Evolution, and Genetics. University of Chicago Press, Chicago.

KARBAN, R. and AGRAWAL, A. A. 2002. Herbivore offense. *Annu. Rev. Ecol. Syst.* 33:641–664.

KARBAN, R. and BALDWIN, I. T. 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago.

KARBAN, R. and THALER, J. S. 1999. Plant phase change and resistance to herbivory. *Ecology* 80:510–517.

KEARSLEY, M. J. C. and WHITHAM, T. G. 1989. Developmental changes in resistance to herbivory: Implications for individuals and populations. *Ecology (Tempe)* 70:422–434.

KEARSLEY, M. J. C. and WHITHAM, T. G. 1998. The developmental stream of cottonwoods affects ramet growth and resistance to galling aphids. *Ecology* 79:178–191.

KORICHEVA, J., LARSSON, S., and HAUKIOJA, E. 1998a. Insect performance on experimentally stressed woody plants: A meta-analysis. *Annu. Rev. Entomol.* 43:195–216.

KORICHEVA, J., LARSSON, S., HAUKIOJA, E., and KEINANEN, M. 1998b. Regulation of woody plant secondary metabolism by resource availability: Hypothesis testing by means of meta-analysis. *Oikos* 83:212–226.

LAMBERS, H., CHAPIN, F. S., and PONS, T. L. 1998. Plant Physiological Ecology. Springer-Verlag, New York.

METCALF, R. L. and LAMPMAN, R. L. 1989. The chemical ecology of diabroticites and Cucurbitaceae. *Experientia* 45:240–247.

METCALF, R. L., METCALF, R. A., and RHODES, A. M. 1980. Cucurbitacins as kairomones for diabroticite beetles. *Proc. Natl. Acad. Sci. U.S.A.* 77:3769–3772.

MURRAY, P. J., HATCH, D. J., and CLIQUET, J. B. 1996. Impact of insect root herbivory on the growth and nitrogen and carbon contents of white clover (*Trifolium repens*) seedlings. *Can. J. Bot.* 74:1591–1595.

ORIANS, C. M., ARDON, M., and MOHAMMAD, B. A. 2002. Vascular architecture and patchy nutrient availability generate within-plant heterogeneity in plant traits important to herbivores. *Am. J. Bot.* 89:270–278.

ORIANS, C. M. and JONES, C. G. 2001. Plants as resource mosaics: A functional model for predicting patterns of within-plant resource heterogeneity to consumers based on vascular architecture and local environmental variability. *Oikos* 94:493–504.

RUOHOMAKI, K., CHAPIN, F. S., III, HAUKIOJA, E., NEUVONEN, S., and SUOMELA, J. 1996. Delayed inducible resistance in mountain birch in response to fertilization and shade. *Ecology* 77:2302–2311.

SCHLICHTING, C. D. and PIGLIUCCI, M. 1998. Phenotypic Evolution: A Reaction Norm Approach. Sinauer Associates, Sunderland, MA.

TALLAMY, D. W. and KRISCHIK, V. A. 1989. Variation and function of cucurbitacins in Cucurbita: An examination of current hypotheses. *Am. Nat.* 133:766–786.

TALLAMY, D. W., STULL, J., EHRESMAN, N. P., GORSKI, P. M., and MASON, C. E. 1997. Cucurbitacins as feeding and oviposition deterrents to insects. *Environ. Entomol.* 26:678–683.

THOMSON, V. P., CUNNINGHAM, S. A., BALL, M. C., and NICOTRA, A. B. 2003. Compensation for herbivory by *Cucumis sativus* through increased photosynthetic capacity and efficiency. *Oecologia* 134:167–175.

TIETZ, H. M. 1972. An Index to the Described Life Histories, Early Stages and Hosts of the Macrolepidotera of the Continental United States and Canada, Vols. I/II. A.C. Allyn. Sarasota, FL.

WALDBAUER, G. P. and FRIEDMAN, S. 1991. Self-selection of optimal diets by insects. *Annu. Rev. Entomol.* 36:43–63.

WHITHAM, T. G. 1983. Host manipulation of parasites: Within-plant variation as a defense against rapidly evolving pests, pp. 15–41, *in* R. F. Denno and M. S. McClure (eds.). Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

ZALUCKI, M. P., CLARKE, A. R., and MALCOLM, S. B. 2002. Ecology and behavior of first instar larval Lepidoptera. *Annu. Rev. Entomol.* 47:361–393.