

# Trade-offs between the shade-avoidance response and plant resistance to herbivores? Tests with mutant *Cucumis sativus*

R. McGUIRE\* and A. A. AGRAWAL†‡

\*Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada, and

‡Department of Ecology and Evolutionary Biology, Cornell University, Corson Hall, Ithaca, NY 14853-2701, USA

## Summary

1. Plants exhibit adaptations to many stresses, including light competition and herbivory. The expression of these traits may interact negatively, potentially instigating a trade-off.
2. We employed a combination of genetically altered *Cucumis sativus* varieties and phenotypic manipulations to test for trade-offs in field experiments. The different genetic lines of *C. sativus* were altered in their phytochrome-mediated shade responses and the production of terpenoid defence compounds.
3. Cucumber plants constitutively expressing the shade-avoidance response had 93% more herbivory by specialist beetles compared with wild types. The long-hypocotyl mutants also produced leaves with fewer trichomes, greater toughness and a higher carbon to nitrogen ratio (C : N) than wild types. Plants lacking defensive cucurbitacins had 23% longer internodes than the cucurbitacin-producing line.
4. We then manipulated the plant phenotype by artificially imposing neighbours' shade on plants with and without cucurbitacins. As expected, plants responded to shade by growing longer hypocotyls and first internodes, but few trade-offs were found between plant line and shade treatment and, although herbivory levels were very low, there was a trend towards reduced damage on shaded plants.
5. The use of genetically altered plant lines provided strong evidence for the trade-off hypothesis, while phenotypic manipulations did not support the hypothesis.

*Key-words:* *Acalymma vittatum*, cucumber, cucurbitacins, herbivory, long-hypocotyl mutant, phenotypic plasticity

*Functional Ecology* (2005) **19**, 1025–1031  
doi: 10.1111/j.1365-2435.2005.01047.x

## Introduction

Plants typically encounter both competitors and herbivores in their natural environment (Gurevitch *et al.* 2000). Both the shade-avoidance response and defence against herbivores are recognized as important adaptations that mitigate the negative effects of competitors and herbivores, respectively. Shade-avoidance responses include stem elongation, and changes in leaf morphology and expansion that facilitate light capture (Morgan & Smith 1981; Dudley & Schmitt 1996). Defence includes the production of toxic chemicals and physical structures such as trichomes that reduce herbivory (Karban & Baldwin 1997). Both types of adaptation are expressed facultatively and are induced by various environmental cues, and traits associated with their induction have been linked to increased plant fitness (adaptive phenotypic

plasticity: Dudley & Schmitt 1996; Agrawal 1998; Sultan 2000). Given the ubiquity of competition and herbivory, it is likely that these threats occur simultaneously or in close sequence, and that plants are faced with the prospect of responding to multiple challenges.

When two adaptive traits are required, their simultaneous expression may result in positive, negative or neutral effects on the plant's fitness. For example, in the presence of multiple stresses the expression of one factor may inhibit a response to another factor (a trade-off). Such trade-offs may be caused by a variety of mechanisms in both plants and animals (Sih 1980; Thaler *et al.* 1999; Weinig & Delph 2001; Relyea 2002; McPeck 2004; Agrawal 2005). In particular, both models and experimental evidence suggest the existence of trade-offs between plant competition and defence against herbivores (Cipollini 2004; Kurashige & Agrawal 2005). Some models attribute the trade-off in alternative resource allocation to the two biologically necessary processes of growth and defence (Herms & Mattson

1992). Physiological signals involved in the induction process and the subsequent cascade of hormonal changes may also instigate a negative interaction between pathways (Thaler & Bostock 2004). Thus, when both herbivores and competitors are present, the expression of optimal phenotypes may be limited due to resource and physiological constraints on the type, level and location of responses.

Trade-offs in the expression of plant responses to competition and herbivory have recently been reported from phenotypic manipulations. Laboratory and field studies have shown that elongated individuals of the common bean (*Phaseolus vulgaris*) are less tolerant than non-elongated plants of simulated herbivory (Cipollini 1997; Cipollini & Schultz 1999). Other studies have shown reduced induced plant resistance when plants are grown in competitive compared with non-competitive environments (Karban 1993; Cipollini & Bergelson 2001). Kurashige & Agrawal (2005) reported that the induction of stem elongation in response to light competition reduced the expression of plant resistance to herbivory in *Chenopodium album*. In tomato (*Solanum lycopersicon*) and tobacco (*Nicotiana longiflora*) it has recently been found that insect growth was higher on plants treated with a low red to far-red light ratio (R : FR) (C. Ballare, personal communication). The above-mentioned studies utilize phenotypic manipulations to study trade-offs between defence and competitive ability. To generalize the trade-off hypothesis and to rigorously implicate a mechanism of the trade-off, an approach combining the use of genetically altered plants and phenotypic manipulations has been suggested (Thaler & Bostock 2004). Genetically altered plants have the advantage of having fixed phenotypic differences, while phenotypic manipulations have the benefit of realistic triggers of the responses.

We employed a combination of genetically altered plants and phenotypic manipulations to examine the trade-off hypothesis as it pertains to a plant's ability to respond to both competition for light, and herbivores. In particular, we define trade-offs in our experiments as the expression of plant traits in relation to competitive ability reducing plant defence against herbivores (and *vice versa*). To do this, we used genetically altered varieties of *Cucumis sativus* that were defective either in their shade-avoidance response or in the production of defence chemicals. We compared lines of plants with a typical wild-type shade response, and a mutant that responds constitutively as if grown in the shade; in addition we compared a line of bitter plants (producing cucurbitacins) with a sweet line (not producing cucurbitacins).

In accordance with the trade-off hypothesis, we predicted that (1) plants constitutively expressing the shade-avoidance response would be more susceptible to herbivory than wild-type plants not responding constitutively as if in the shade; and (2) plants producing constitutive chemical defence should show weaker stem-elongation responses than plants without defence. In addition, we applied a competitive environment

treatment (shading) to the two lines differing in constitutive levels of defence: (3) if a trade-off exists, shade responses should be strongest in the low-defence plant line. Finally, we measured several physical and chemical characteristics of plants to identify plant traits correlated with the observed shade-avoidance response and resistance to herbivores.

## Materials and methods

### STUDY SYSTEM

We used cultivated cucumber plants, *Cucumis sativus* (Cucurbitaceae), which typically produce bitter secondary compounds called cucurbitacins (tetracyclic triterpenoids). These compounds are often deterrent or toxic to herbivores (Tallamy *et al.* 1997); however, they have also been observed to have the opposite effect on some insects (Tallamy & Krischik 1989; Barrett & Agrawal 2004). For example, the chrysomelid beetle *Acalymma vittatum* (Coleoptera: Chrysomelidae: Galerucinae) is well known for its affinity for bitter cucurbits (Metcalf & Lampman 1989; Smyth *et al.* 2002). *Acalymma vittatum*, the major herbivore in our field experiments, is a relatively specialized feeder that is largely restricted to plants in the Cucurbitaceae. Cucurbitacins act as arrestants, reducing movement to other plants, and as phagostimulants, causing compulsive feeding by these beetles (Tallamy & Krischik 1989).

In this study we first employed two lines of cucumber in order to study plasticity to light competition: a wild type, and a mutant line with a constitutive shade-avoidance response (*long hypocotyl*, *lh* mutant: Casal *et al.* 1994). The *lh* mutants are deficient in a phytochrome B, which is required to respond to a lack of shade (Kendrick & Nagatani 1991; Lopez-Juez *et al.* 1990). Although wild-type plants require a low R : FR to elicit stem elongation, elongation occurs constitutively (even in full light) in the *lh* mutant (Casal *et al.* 1994). Neither line produces cucurbitacins, and we thus refer to them as *lh* sweet (*lh-s*) and wild-type sweet (*wt-s*). Compared with wild types, the *lh-s* mutants also have increased apical dominance and tendril production (Lopez Juez *et al.* 1990); higher shoot-to-root ratios (Casal *et al.* 1994); and reduced responses to low R : FR, natural shade and blue light (Ballare *et al.* 1991).

Additionally, we used two near-isogenic varieties of the cucumber, one that produces constitutive and inducible cucurbitacins, Marketmore 76 (bitter); and a second that does not produce any cucurbitacins, Marketmore 80 (sweet) (Gould 1978; Agrawal *et al.* 1999, 2002; Barrett & Agrawal 2004). These varieties are genetically very similar, with the exception of genes that are tightly linked with the one required for cucurbitacin production. The presence of cucurbitacins is controlled by a dominant allele at a single diallelic locus (Andeweg & De Bruyn 1959), and the sweet line has been backcrossed at least four times (T. Wehner, personal communication).

## EXPERIMENTS

Our experiments were conducted from May to August 2004 at the Koffler Scientific Reserve at Jokers Hill ([www.zoo.utoronto.ca/jokershill](http://www.zoo.utoronto.ca/jokershill)), near Newmarket, Ontario, Canada (44°03' N, 79°29' W). Seeds of the four lines were germinated on moist filter paper in Petri dishes sealed with parafilm. Seedlings were potted in 0.05-m pots with ProMix BX soil (Red Hill, PA, USA) and randomized in herbivore-exclusion mesh cages for 2 weeks out of doors. The seedlings were then planted in an open ploughed field (when the first true leaf was expanding), with the varieties randomized within two experimental plots (*lh-s* mutant *vs* *wt-s*, bitter *vs* sweet). Although we initially started with 75 seedlings in each line, mortality after planting and severe initial herbivore damage resulted in lower sample sizes. Our final sample sizes were: *lh-s* mutant,  $n = 47$ ; *wt-s*,  $n = 42$ ; bitter,  $n = 47$ ; sweet,  $n = 43$ .

Surveys of herbivory were initiated after the first true leaves expanded, and were conducted weekly for the next 3 weeks. Each leaf was rated for herbivore damage by visually separating the leaves into quarters, and recording the percentage herbivory to the nearest 25%; separate measures were taken for all leaves including cotyledons. The length of the hypocotyls and first internodes were also taken on each individual plant after they had fully expanded. One-way repeated-measures ANOVAS were employed to test for varietal differences in herbivory and internode length; separate analyses were conducted to compare *lh-s* mutant *vs* *wt-s* and bitter *vs* sweet. All analyses were conducted with SYSTAT (ver. 9).

In addition, we measured traits of the *lh-s* mutant and *wt-s* to characterize differences between the two lines that may be relevant for herbivory (differences between the bitter and sweet varieties have been well characterized: Agrawal *et al.* 1999, 2002; Barrett & Agrawal 2004). We grew approximately 10 plants of each line in a glasshouse, and measured trichome density, water content, specific leaf area (SLA), leaf toughness, carbon to nitrogen ratio (C : N), and the area of the second true leaf. We assessed trichome density by counting the tops and bottoms of leaf discs (28 mm<sup>2</sup>) under a dissection microscope. Leaf discs were taken from the tips of the first true leaves. Percentage water content was measured by taking the wet and dry mass of the leaf disc, and SLA was calculated as the area of the leaf disc divided by the dry mass. We measured leaf toughness of the first true leaf with a force-gauge penetrometer (Type 516, Chatillon Corp., NY, USA) that measures the grams of force needed to penetrate a surface (Agrawal 2004). Two measures were taken per leaf and averaged as a single data point. Leaf C : N was estimated from total concentrations measured by microcombustion of 5 mg dried ground leaf material (separate measures of cotyledon and first true leaf) in an Elemental Combustion System 4010, CHNS-O analyser (Costech Analytical Technologies, Valencia, CA, USA). Two C : N samples were taken from each replicate and

the data were averaged. Differences between the *lh-s* mutant and *wt-s* lines were first assessed together by MANOVA and were then followed by univariate *t*-tests.

## SHADE MANIPULATION

To test for differences in induction of the shade-avoidance response in the altered defence lines (bitter *vs* sweet), we conducted an additional set of experiments. Bitter and sweet lines were germinated as above, and 25 plants from each line received one of two light treatments simulating a competitive or non-competitive environment; the potted plants were placed out of doors on a gravel bed. The light environment was altered using translucent plastic theatrical gels. The control plants received a colourless gel (0.005 Dura-Lar, Grafix Plastics, Cleveland, OH, USA); this filter reduces photosynthetic photon flux density (PPFD) by 10% but does not alter the R : FR ratio compared with ambient light (Kurashige & Agrawal 2005). To simulate the presence of light competitors, a green filter was used (#4430 filter, Rosco, Markham, Ontario, Canada). The #4430 filter matches spectrophotometric leaf absorbance spectra, and reduces PPFD by 38% and reduced R : FR by 45% relative to ambient light (for details see Kurashige & Agrawal 2005). Cones made of these gels were fitted to the pots and were constructed with a 1-cm opening on top to allow for gas exchange. Our previous research showed no effect of using these gels on leaf temperature (Kurashige & Agrawal 2005). After production of the first true leaf, the height of hypocotyls and first internode were measured for all plants.

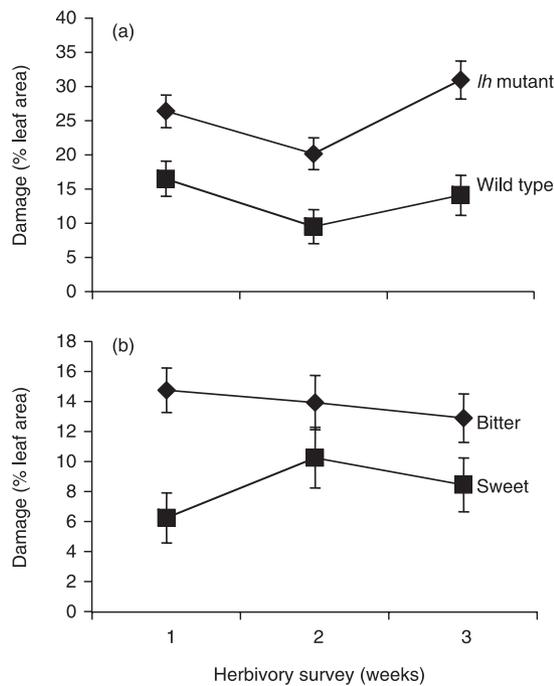
A subset of plants ( $n = 5-8$  from each treatment) were harvested destructively to measure the density of trichomes, leaf toughness and C : N as indicators of plant quality for herbivores. The remaining plants ( $n = 17$  for each of the four treatments) were planted out in the field (pots were buried) and assessed for beetles (*A. vittatum*) and herbivory over the next week; the light-altering cones were removed from plants after they were moved to the field.

The effects of plant line (bitter or sweet) and phototreatment (shade or control) were assessed for all response variables by MANOVA and ANOVA. In our analyses of this two-by-two factorial experiment, trade-offs between competitive and defensive traits are tested by the interaction terms of the ANOVAS. A significant interaction would indicate that the bitter and sweet plant lines have differential responses to shade. Two-way MANOVA was employed first on the plant measures (trichomes, leaf toughness, C : N) before proceeding to the univariate analyses.

## Results

COMPARISON OF SHADE MUTANT (*lh-s*) AND WILD TYPE

We confirmed the constitutive shade response of the *lh-s* mutants, as the long-hypocotyl plants had nearly



**Fig. 1.** Influence of cucumber line, (a) shade mutant *vs* wild type; (b) bitter *vs* sweet, on percentage leaf area damaged by herbivores on true leaves over three census dates. Both the shade mutant (*lh-s*) *vs* wild type in (a) are sweet. Least squares means and standard errors are shown.

threefold longer first internodes than wild-type plants in full sun [mean length (mm)  $\pm$  SE: wild type,  $8.0 \pm 0.18$ ; shade mutant,  $23.3 \pm 0.17$ ,  $F_{1,82} = 36.900$ ,  $P < 0.001$ ]. *Acalymma vittatum* was the most common insect on plants and was responsible for the bulk of herbivory observed. On average, *lh-s* plants had 93% more herbivory than the wild types on true leaves (Fig. 1a; Table 1), although there was no difference on cotyledons ( $F_{1,81} = 0.129$ ,  $P = 0.721$ ). In addition, the first true leaves of *lh-s* plants had 16% fewer trichomes, were 110% tougher, and had a 29% higher C : N ratio than wild types (MANOVA: Wilks's  $\lambda = 0.096$ ,  $F_{7,10} = 13.447$ ,  $P < 0.001$ ; see Table 3). Water content, SLA and leaf area did not differ between plant lines (Table 2).

#### COMPARISON OF BITTER AND SWEET PLANTS

Sweet plants had 69% more damage on their cotyledons than bitter plants (first census, mean percentage damage

**Table 1.** Repeated-measures ANOVA for effects of plant line (shade mutant *vs* wild type) on herbivory over three census dates

Source	SS	df	MS	F	P	G-G
Plant line	0.962	1	0.962	18.179	<0.001	
Error	4.288	81	0.053			
Census	0.287	2	0.143	9.636	<0.001	<0.001
Census $\times$ line	0.060	2	0.030	2.028	0.135	0.140
Error	2.410	162	0.015			

Probabilities adjusted for sphericity are presented using the Greenhouse-Geisser (G-G) correction factor.

$\pm$  SE: sweet,  $19 \pm 2$ ; bitter,  $11 \pm 2$ ,  $F_{1,94} = 6.256$ ,  $P = 0.014$ ), but this trend was reversed for true leaves, with bitter plants having 67% more damage over three censuses (Fig. 1b; Table 3). Sweet plants had 23% longer internodes than bitter plants [first internode, mean length (mm)  $\pm$  SE: sweet,  $101 \pm 7$ ; bitter,  $82 \pm 6$ ,  $F_{1,92} = 6.491$ ,  $P = 0.037$ ].

#### SHADE MANIPULATION

We first conducted an overall MANOVA analysis on the effects of plant line and shade treatment on the hypocotyl and first internode lengths. In this analysis both main effects and the interaction term were highly significant: plant line,  $\lambda = 0.644$ ,  $F_{2,81} = 22.428$ ,  $P < 0.001$ ; treatment,  $\lambda = 0.454$ ,  $F_{2,81} = 48.792$ ,  $P < 0.001$ ; interaction term,  $\lambda = 0.883$ ,  $F_{2,81} = 5.375$ ,  $P = 0.006$ . Breaking this down to the univariate analyses, our shading treatment induced stem elongation in both bitter and sweet plants, with shaded plants having 112% longer hypocotyls ( $F_{1,82} = 65.921$ ,  $P < 0.001$ ) and 39% longer first internodes ( $F_{1,82} = 7.956$ ,  $P = 0.006$ ) than controls (Fig. 2). Sweet plants in the control (full sun and clear filter) treatment had 54% longer hypocotyls than bitter plants ( $F_{1,82} = 22.894$ ,  $P < 0.001$ ), but this trend was not significant for the first internode ( $F_{1,82} = 0.665$ ,  $P < 0.417$ ). There were no interactions between plant line and shading treatment (hypocotyl,  $F_{1,82} = 2.685$ ,  $P = 0.105$ ; first internode,  $F_{1,82} = 0.238$ ,  $P = 0.627$ ). Thus, although the multivariate analyses provided some evidence for an interaction and trade-off, this was not evident in the univariate analyses.

Plant line and treatment each had a significant effect in the MANOVA analysis on leaf traits (plant line:  $\lambda =$

**Table 2.** Characterization of the *lh-s* mutant and wt-s cucumber line for traits relevant to plant resistance to herbivory

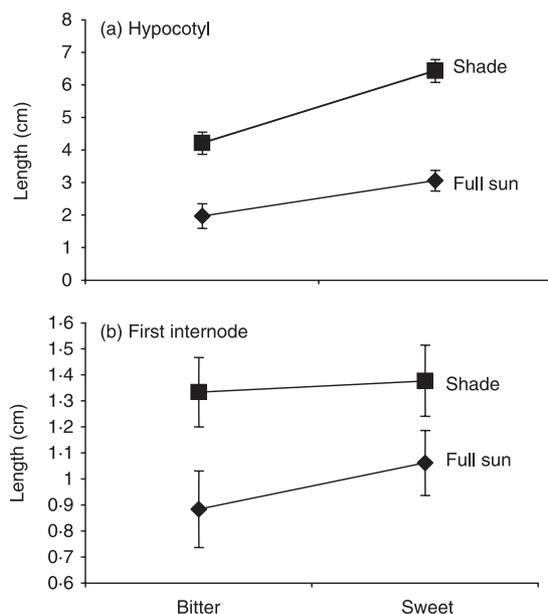
Trait	<i>lh-s</i> mutant	Wild type	<i>t</i>	<i>P</i>
Trichomes (per 28 mm <sup>2</sup> )	$136.2 \pm 5.8$	$161.6 \pm 10.7$	2.201	0.043
Water content (%)	$90.8 \pm 0.7$	$92.0 \pm 0.6$	1.202	0.247
Specific leaf area (mm <sup>2</sup> mg <sup>-1</sup> )	$99.0 \pm 6.2$	$88.7 \pm 9.1$	0.963	0.350
Leaf toughness (g)	$14.5 \pm 2.2$	$6.9 \pm 1.2$	2.874	0.011
C : N ratio, cotyledon	$10.9 \pm 0.3$	8.3	5.715	< 0.001
C : N ratio, first true leaf	$9.4 \pm 0.1$	$7.3 \pm 0.2$	9.943	< 0.001
Area of second true leaf (cm <sup>2</sup> )	$44.8 \pm 3.2$	$40.3 \pm 7.2$	0.626	0.540

Means, standard errors and results of *t*-tests are shown. In all cases, total  $n = 18$ .

**Table 3.** Repeated-measures ANOVA for effects of plant line (bitter vs sweet) on herbivory over three census dates

Source	SS	df	MS	F	P	G-G
Plant line	0.214	1	0.214	7.104	0.009	
Error	2.767	92	0.030			
Census	0.014	2	0.007	1.187	0.308	0.306
Census × line	0.031	2	0.016	2.594	0.077	0.081
Error	1.109	184	0.006			

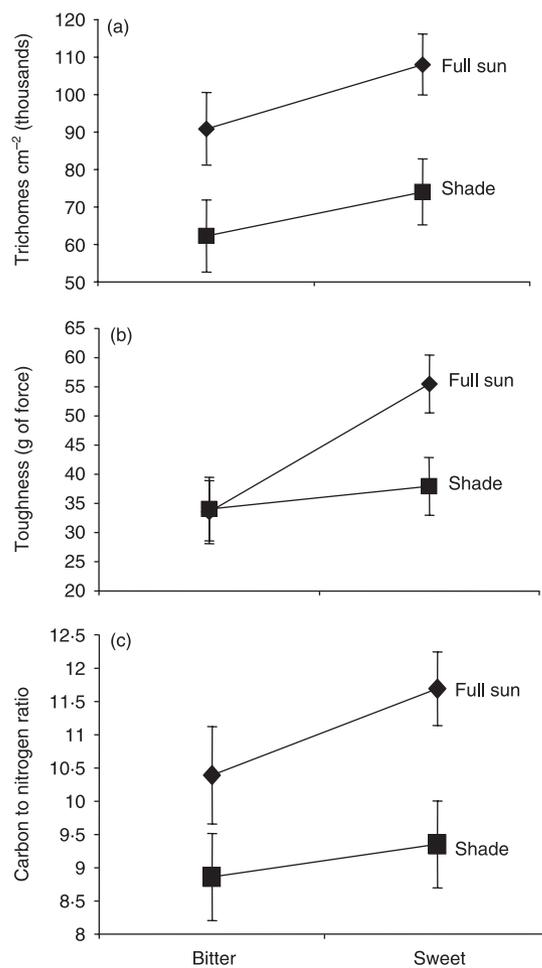
Probabilities adjusted for sphericity are presented using the Greenhouse–Geisser (G–G) correction factor.



**Fig. 2.** Influence of cucumber line (bitter vs sweet) and shade treatment (clear vs green gel filter) on the length of (a) hypocotyls; (b) first internodes. Least squares means and standard errors are shown.

0.520,  $F_{3,13} = 4.004$ ,  $P = 0.032$ ; treatment:  $\lambda = 0.517$ ,  $F_{3,13} = 4.047$ ,  $P = 0.031$ ), but there was no interaction ( $\lambda = 0.881$ ,  $F_{3,13} = 0.587$ ,  $P = 0.634$ ). Shade treatment reduced trichome densities among both bitter and sweet plants by 32% ( $F_{1,19} = 11.899$ ,  $P = 0.003$ ; Fig. 3a). Trichome densities were not strongly different for the two plant lines ( $F_{1,19} = 2.530$ ,  $P = 0.128$ ), and there was no interaction between plant line and shade treatment. Sweet plants produced 38% tougher leaves than bitter plants ( $F_{1,18} = 6.210$ ,  $P = 0.023$ ) (Fig. 3b). The interaction between plant line and shading treatment was not detectable statistically for effects on leaf toughness ( $F_{1,18} = 3.015$ ,  $P = 0.1$ ; Fig. 3b). The only effect on the C : N ratio of leaves was an 18% decrease caused by shading treatment ( $F_{1,17} = 8.829$ ,  $P = 0.009$ ; Fig. 3c); there was no effect of plant line or interaction.

Herbivory levels (percentage damage) on plants that were put out into the field were consistently low: shade treatment had the effect of decreasing damage by 63% on cotyledons ( $F_{1,59} = 27.757$ ,  $P = 0.007$ ) but not on the first true leaves ( $F_{1,59} = 0.385$ ,  $P = 0.537$ ; Fig. 4).

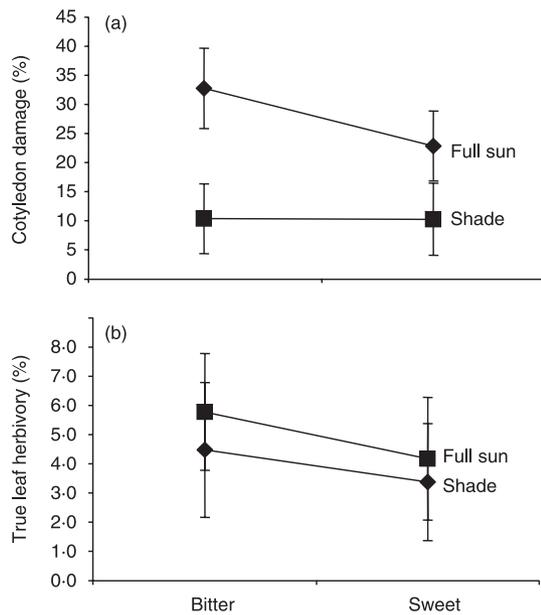


**Fig. 3.** Influence of cucumber line (bitter vs sweet) and shade treatment (clear vs green gel filter) on (a) trichome density; (b) leaf toughness (grams of force required to break leaf tissue); (c) carbon to nitrogen ratio of cucumber leaves. Least squares means and standard errors are shown.

This herbivory was received in the field, as beetles did not enter the cones to feed on plants. Plant line and its interaction with shade treatment were not significant for either leaf position (in both cases  $P > 0.4$ ). Because herbivory levels were low, and our data contained many zero values, we confirmed these analyses with non-parametric Kruskal–Wallis tests; the one significant effect remained of shade reducing damage on cotyledons.

## Discussion

Several lines of physiological evidence as well as allocation theory have suggested that trade-offs should exist between plant responses to herbivory and light competition (Cipollini 2004; Agrawal 2005). In our genetic manipulations, we found evidence consistent with the trade-off hypothesis: plants producing chemical defences had shorter internodes than plants lacking defences, and plants with constitutive stem elongation had greater levels of herbivory than non-elongating plants. Nonetheless, the results from our phenotypic manipulations using light filters were inconsistent with



**Fig. 4.** Influence of cucumber line (bitter vs sweet) and shade treatment (clear vs green gel filter) on percentage leaf herbivory on (a) cotyledons; (b) first true leaves. Least squares means and standard errors are shown.

the trade-off hypothesis, suggesting that trade-offs may be more likely to occur when traits are expressed constitutively. For example, although plants in the shade did elongate their stems, elongation did not differ strongly between bitter and sweet plants. Contrary to the prediction of higher herbivory on shaded plants, feeding damage was lower (cotyledons) or similar (true leaves) compared with controls. Given that light-manipulated plants were exposed to herbivores in the field following removal of their light filters, it is possible that the phenotypic differences between control and shaded plants dissipated quickly. This possibility highlights one limitation of the phenotypic approach: the difficulty of maintaining phenotypic expressions in a common environment.

In order to understand mechanistically how plant responses to one environmental stress may have mediated effects on other challenges, we measured several plant traits thought to be important in resistance to herbivory. Compared with plants in full sun, shaded plants are typically thought to have leaves that are thinner and less tough, with fewer trichomes, and with higher SLA and N content – all factors that should increase herbivory (Young & Smith 1980; Morgan & Smith 1981; Jansen & Stamp 1997). Mutant cucumber plants altered to express the shade-avoidance response did have fewer trichomes, but were not different from wild types in many traits, and contrary to predictions had a higher C : N. In our phenotypic manipulation, shade also induced a reduction in trichomes, but caused an opposite effect on C : N (18% reduction as predicted).

Our herbivory observations were thus not completely consistent with our characterization of plant traits. For example, *lh-s* plants had fewer trichomes than wt-

s, and this is concordant with them also having higher herbivory levels. Nevertheless, *lh-s* leaves were also tougher and had less N than the wild types, which we would have predicted to reduce herbivore pressure. It is possible that the main herbivore, *A. vittatum*, was not affected by either leaf toughness or N content, and responded primarily to trichome density. Alternatively, given the lower quality of tough, low-N leaves, the beetles may have responded by eating more to compensate for this poorer quality food. Plant attractiveness to herbivores may have also been determined by factors we did not measure, such as volatiles released following herbivory (Agrawal *et al.* 2002). Defensive cucurbitacins, however, did not drive these patterns, because both lines (*lh-s* and wild type) lack the chemicals.

It was expected that bitter plants would have more damage overall, as *A. vittatum* is attracted to cucurbitacins. In our shade-manipulation experiment, bitter plants were observed to suffer more herbivory than sweet plants except on shaded cotyledons. Although sweet plants do not produce cucurbitacins, they may invest in other types of defence, and thus a strong trade-off may not be observed (Agrawal *et al.* 2002). Sweet plants tended to have more trichomes, tougher leaves and higher C : N ratios than bitter plants. To compensate for the lack of cucurbitacins, sweet plants may invest in other defence traits. In a previous study we found no difference in the growth rate of bitter and sweet plants (Agrawal *et al.* 1999). Sweet plants respond to shade by reducing the number of trichomes, toughness and C : N ratio. Bitter plants respond to shade by reducing trichomes and C : N ratio, but leaf toughness remains similar. In order to observe a clear trade-off, if one is indeed present, other defence characteristics and resistance to other herbivores should be assayed.

Thaler & Bostock (2004) describe the advantages and limitations of both phenotypic and genetic approaches to manipulating plant responses to stress in testing for trade-offs. Phenotypic manipulations are typically quantitative in nature, can be conducted in one genetic background, and the plants can be raised in one environment until treatment. However, especially when phenotypic treatments initiate a hormonal cascade, treatments may cause multiple factors to change, not just those of interest, and maintaining phenotypic differences after treatment can be difficult. With genetic manipulations for traits controlled by just one or two genes, the factors of interest can be altered qualitatively, resulting in a response or no response. However, any pleiotropic effects of genes involved in the altered response will also be changed. The disadvantage of such a ‘cleaner’ manipulation is the fact that wild populations of plants with such altered gene expression probably do not persist in nature.

In our study we found different results between our genetic and phenotypic manipulations. While our phenotypic experiments did not result in a trade-off, our genetic cucumber lines strongly suggested a trade-off between plant responses to herbivores and to light

competitors. In particular, these results indicate that the trade-off associated with constitutive deficiency of phytochrome B is apparently not comparable with the effect of induced downregulation of this phytochrome pathway.

### Acknowledgements

We thank Carlos Ballare for providing seeds of the *lh* mutant, and Carlos Ballare, James Cresswell, Marc Johnson, Michael Stastny, Jennifer Thaler and anonymous reviewers for comments on the manuscript. Our research (www.herbivory.com) is supported by the NSF DEB-0519730 and NSERC of Canada.

### References

- Agrawal, A.A. (1998) Induced responses to herbivory and increased plant performance. *Science* **279**, 1201–1202.
- Agrawal, A.A. (2004) Plant defense and density dependence in the population growth of herbivores. *American Naturalist* **164**, 113–120.
- Agrawal, A.A. (2005) Future directions in the study of induced plant responses to herbivory. *Entomologia Experimentalis et Applicata* **115**, 97–105.
- Agrawal, A.A., Gorski, P.M. & Tallamy, D.W. (1999) Polymorphism in plant defense against herbivory: constitutive and induced resistance in *Cucumis sativus*. *Journal of Chemical Ecology* **25**, 2285–2304.
- Agrawal, A.A., Janssen, A., Bruin, J., Posthumus, M.A. & Sabelis, M.W. (2002) An ecological cost of plant defence: attractiveness of bigger cucumber plants to natural enemies of herbivores. *Ecology Letters* **5**, 377–385.
- Andeweg, J. & De Bruyn, J. (1959) Breeding of non-bitter cucumbers. *Euphytica* **8**, 13–20.
- Ballare, C.L., Casal, J.J. & Kendrick, R.E. (1991) Response of light grown wild type and long-hypocotyl mutant cucumber seedlings to natural and simulated shade light. *Photochemistry and Photobiology* **54**, 819–826.
- Barrett, R.D.H. & Agrawal, A.A. (2004) Interactive effects of genotype, environment, and ontogeny on resistance of cucumber (*Cucumis sativus*) to the generalist herbivore *Spodoptera exigua*. *Journal of Chemical Ecology* **30**, 37–51.
- Casal, J.J., Ballare, C.L., Tourn, M. & Sanchez, R.A. (1994) Anatomy, growth, and survival of a long-hypocotyl mutant of *Cucumis sativus* deficient in phytochrome B. *Annals of Botany* **73**, 569–575.
- Cipollini, D. (1997) Gibberellic acid treatment reduces the tolerance of field-grown common bean to leaf removal. *Journal of Plant Growth Regulation* **16**, 123–127.
- Cipollini, D. (2004) Stretching the limits of plasticity: can a plant defend against both competitors and herbivores? *Ecology* **85**, 28–37.
- Cipollini, D.F. & Bergelson, J. (2001) Plant density and nutrient availability constrain constitutive and wound-induced expression of trypsin inhibitors in *Brassica napus*. *Journal of Chemical Ecology* **27**, 593–610.
- Cipollini, D. & Schultz, J.C. (1999) Exploring cost constraints on stem elongation in plants using phenotypic manipulation. *American Naturalist* **153**, 236–242.
- Dudley, S.A. & Schmitt, J. (1996) Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist* **147**, 445–465.

- Gould, F. (1978) Resistance of cucumber varieties to *Tetranychus urticae*: genetic and environmental determinants. *Journal of Economic Entomology* **71**, 680–683.
- Gurevitch, J., Morrison, J.A. & Hedges, L.V. (2000) The interaction between competition and predation: a meta-analysis of field experiments. *American Naturalist* **155**, 435–453.
- Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**, 283–335.
- Jansen, M.P.T. & Stamp, N.E. (1997) Effects of light availability on host plant chemistry and the consequences for behavior and growth of an insect herbivore. *Entomologia Experimentalis et Applicata* **82**, 319–333.
- Karban, R. (1993) Induced resistance and plant-density of a native shrub, *Gossypium thurberi*, affect its herbivores. *Ecology* **74**, 1–8.
- Karban, R. & Baldwin, I.T. (1997) *Induced Responses to Herbivory*. University of Chicago Press, Chicago, IL, USA.
- Kendrick, R.E. & Nagatani, A. (1991) Phytochrome mutants. *Plant Journal* **1**, 133–139.
- Kurashige, N. & Agrawal, A.A. (2005) Phenotypic plasticity in *Chenopodium album*. *American Journal of Botany* **92**, 21–26.
- Lopez-Juez, E., Buurmeijer, W.F., Heeringa, G.H., Kendrick, R.E. & Wesseliuss, J.C. (1990) Response of light-grown wild-type and long hypocotyl mutant cucumber plants to end-of-day far-red light. *Photochemistry and Photobiology* **85**, 350–354.
- McPeck, M.A. (2004) The growth/predation risk trade-off: so what is the mechanism? *American Naturalist* **163**, 88–111.
- Metcalfe, R.L. & Lampman, R.L. (1989) The chemical ecology of dipterocarpaceae and Cucurbitaceae. *Experientia* **45**, 240–247.
- Morgan, D.C. & Smith (1981) Non-photosynthetic responses to light quality. *Encyclopedia of Plant Physiology, New Series* (O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler, eds), pp. 109–134. Springer-Verlag, Berlin.
- Relyea, R.A. (2002) Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs* **72**, 523–540.
- Sih, A. (1980) Optimal behavior: can foragers balance two conflicting demands? *Science* **2**, 1041–1043.
- Smyth, R.R., Tallamy, D.W., Renwick, J.A.A. & Hoffmann, M.P. (2002) Effects of age, sex, and dietary history on response to cucurbitacin in *Acalymma vittatum*. *Entomologia Experimentalis et Applicata* **104**, 69–78.
- Sultan, S.E. (2000) Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* **5**, 537–542.
- Tallamy, D.W. & Krischik, V.A. (1989) Variation and function of cucurbitacins in *Cucurbita*: an examination of current hypothesis. *American Naturalist* **133**, 766–786.
- Tallamy, D.W., Stull, J., Ehresman, N.P., Gorski, P.M. & Mason, C.E. (1997) Cucurbitacins as feeding and oviposition deterrents to insects. *Environmental Entomology* **26**, 678–683.
- Thaler, J.S. & Bostock, R.M. (2004) Interactions between abscisic acid-mediated responses and plant resistance to pathogens and insects. *Ecology* **85**, 48–58.
- Thaler, J.S., Fidantsef, A.L., Duffey, S.S. & Bostock, R.M. (1999) Trade-offs in plant defense against pathogens and herbivores: a field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology* **25**, 1597–1609.
- Weinig, C. & Delph, L.F. (2001) Phenotypic plasticity early in life constrains developmental responses later. *Evolution* **55**, 930–936.
- Young, D.R. & Smith, W.K. (1980) Influence of sunlight on photosynthesis, water relations, and leaf structure in the understory species *Arnica cordifolia*. *Ecology* **61**, 1380–1390.

Received 25 April 2005; revised 17 July 2005; accepted 2 August 2005