

BENEFITS AND COSTS OF INDUCED PLANT DEFENSE FOR *LEPIDIUM VIRGINICUM* (BRASSICACEAE)

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Abstract. Induced responses to herbivores are common and well documented in plants. It has been hypothesized that the evolutionary ecology of induced responses can be understood by studying benefits of induction in the presence of herbivores and costs of induction in the absence of herbivores. Phenotypic benefits and costs of induction would indicate that such plasticity in defense could be adaptive (i.e., that phenotypes matched to their environmental conditions have higher relative fitness than unmatched phenotypes). However, few studies to date have investigated the benefits and costs of induction in the same system. In this study, induced responses of *Lepidium virginicum* to herbivory reduced feeding by generalist noctuid caterpillars in choice and no-choice experiments. Induced plant responses to herbivory were correlated with an increase in the number of trichomes per leaf and an increase in the diversity of the putatively defensive chemical compounds, glucosinolates, present in the foliage of damaged plants compared to undamaged controls. Induction did not affect the feeding behavior of the larvae of the specialist butterfly, *Pieris rapae*.

In field experiments, induction reduced natural colonization of plants by aphids compared to both unmanipulated controls and controls that were damaged (but not induced) by clipping a leaf from the plant using a pair of scissors. Induced plants were more likely to survive in the field than clipped plants, a result that suggests a net fitness benefit of induction when leaf tissue removal was controlled. In experiments conducted in the absence of herbivores, damage induced responses did not reduce the root or shoot biomass of plants grown at low density. At high plant density, induction was associated with both reduced root biomass and increased aboveground growth, suggesting that induction may cause an allocation shift, rather than a loss of total biomass. Induced responses of plants satisfy a necessary component of adaptive plasticity because plants in variable herbivore environments maximize relative fitness by adjusting their defensive phenotype.

Key words: adaptive plasticity; generalists; herbivory; induction; *Lepidium virginicum*; *Noc-tuidae*; *Pieridae*; *Pieris rapae*; plant defense; plant–insect interactions; resistance; specialists.

INTRODUCTION

Induced responses to herbivory appear to be ubiquitous in the plant kingdom. Any change in a plant that occurs following herbivory is an “induced response” (Karban and Myers 1989). These changes include phytochemical induction (Baldwin 1994), increases in physical defenses such as thorns and trichomes (Young 1987, Baur et al. 1991), emission of volatiles that attract predators and parasites of herbivores (Takabayashi and Dicke 1996, De Moraes et al. 1998), reduction in plant nutritional quality for herbivores (Bi et al. 1997), and even increases in extrafloral nectar or ant recruitment in ant–plant systems (Smith et al. 1990, Agrawal 1998a, Agrawal and Rutter 1998). If plant responses to initial damage result in reduced preference and/or performance of subsequent herbivores, this is termed “induced resistance” (Karban and Myers 1989). Induced resistance has been demonstrated in ~100 plant–herbivore systems (Karban and Baldwin 1997). Yet, for

an induced response to be classified as a defense, induction must increase the fitness of plants compared to uninduced controls. Although there are few demonstrations of induced responses increasing plant fitness, evidence for the benefits of induction is accumulating (Karban and Baldwin 1997, Agrawal 1998b, Baldwin 1998).

The dominant hypothesis explaining the evolution of inducible plant defenses suggests that defenses can increase plant fitness in an environment with herbivores, but may be costly due to an allocation trade-off, and result in reduced plant fitness in the absence of herbivores (Herms and Mattson 1992, Zangerl and Bazzaz 1992, Agrawal et al. 1999a). Inducible defenses may economize the plant’s expenditures by allowing it to invest in defense when necessary, and to avoid costly allocations to defense when herbivores are not present. Induction may be particularly effective if the initial herbivory is unpredictable, but subsequent herbivory is likely. More recently, induction has been examined from a more pluralistic perspective, with theory indicating that induction may have many types of benefits and costs (Parker 1992, Simms 1992, Takabayashi and

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Dicke 1996, Karban et al. 1997, Agrawal and Karban 1999).

The vast majority of documented cases of induced resistance to herbivory have been in perennial plants (Karbon and Baldwin 1997). Perennial plants are poor model systems for answering questions about the evolution and consequences of induced responses from the plant's point of view because measuring lifetime plant fitness is difficult. The preponderance of perennials in the study of induced responses may reflect a true biological bias or may have been caused by ecologists initially looking for induced resistance in long-lived plants. Although there have now been >10 annual plant systems for which induced responses have been studied (Karbon and Baldwin 1997; A. Agrawal, *unpublished data*), benefits of induced responses in these systems have rarely been elucidated.

There has been greater interest in documenting the costs of induced responses than benefits to the plant, although only a few studies have examined costs of induction in terms of plant growth, survival, and reproduction (Brown 1988, Baldwin et al. 1990, Karban 1993, Gianoli and Neimeyer 1997, Yano 1997, Zangerl et al. 1997). Of these studies, only a fraction have been successful in detecting allocation costs of induction (Karbon and Baldwin 1997). High density of hosts appears to be an important condition in detecting costs of defense in a broad array of animal and plant systems (e.g., Williams and Jordan 1995, Kraaijeveld and Godfray 1997, Pettersson and Bronmark 1997) and may be a key factor in detecting costs of induced responses to herbivory. Competition at high densities can result in above- and belowground resource limitation and other physiological constraints which may enhance costs of defense (Bazzaz 1996, Karban and Baldwin 1997).

Towards the goal of better understanding the evolutionary ecology of induced defenses, I measured the phenotypic benefits and costs of induction in a short-lived herbaceous plant. By determining the benefits and costs I tested a fundamental assumption of the evolution of phenotypically plastic traits: that such plasticity maximizes relative fitness in variable environments. The adaptive plasticity hypothesis posits that phenotypes matched to their environments will have relatively higher fitness than alternative phenotypes (Bradshaw 1965, Thompson 1991, Gotthard and Nylin 1995, Dudley and Schmitt 1996, DeWitt et al. 1998). In other words, it is predicted that a given phenotype will have an advantage in some environments and a disadvantage in other environments. Phenotypic plasticity allows an organism to maximize the benefits while minimizing the costs. To date, only a few studies have documented such benefits and costs of plastic traits (Kingsolver 1995, Dudley and Schmitt 1996). Costs and benefits of plasticity per se further address questions relating to the evolution and adaptive nature of plasticity (DeWitt et al. 1998).

In this study I first demonstrate that herbivory in-

duces resistance in *Lepidium virginicum* and test for physical and biochemical defense mechanisms that correlate with this resistance. I then test components of the adaptive plasticity hypothesis by addressing the benefits and costs of expressing induced resistance in environments with and without herbivores, respectively. Specifically, I asked the following questions: (1) Do induced responses to herbivory result in reduced consumption of leaf tissue compared to that on uninduced plants, in choice and no-choice tests with a generalist and specialist herbivore? (2) Is induced resistance to herbivory correlated with increased numbers of trichomes per leaf and/or several measures of phytochemical induction of glucosinolates? (3) Do induced responses protect plants from herbivory under field conditions, and does this affect plant performance? (4) In the absence of herbivores, do induced plants reduce allocation to root and shoot growth relative to uninduced controls, and is this allocation affected by plant density?

MATERIALS AND METHODS

Lepidium virginicum (Brassicaceae) is a widely distributed annual or biennial native herb that is found in disturbed areas across North America. It is commonly fed upon by larvae of *Pieris* spp. butterflies, larvae of noctuid moths, aphids, beetles, and grasshoppers (A. M. Shapiro, *personal communication*; A. A. Agrawal, *personal observation*). Seeds were collected from several wild populations of *L. virginicum* in northern California.

Induction experiments: general procedures

Plants were grown from seed in a greenhouse in 0.8 L pots using U.C. greenhouse soil mix (Redi Gro, Sacramento, California, USA). The plants were randomly assigned to control and induced treatments. When the plants had developed 4–5 true leaves, one *Pieris rapae* (Pieridae) larva was allowed to feed on one of the new, but fully expanded true leaves of each induced plant, and was allowed to consume the entire leaf. Caterpillars were confined to clip cages made from the tops of petri dishes (5 cm) attached to either side of a hair clip. Control plants received a clip cage without the caterpillar. Caterpillars consumed the leaf within 48 h.

Induced resistance to feeding by generalist and specialist herbivores

To test if herbivory induced resistance to subsequent herbivory by generalist and specialist herbivores, I conducted a series of choice and no-choice experiments. In the first experiment (no-choice, generalist), 17 plants were used in each treatment. After the initial treatment, plants were allowed to grow for three days. The outside of each pot was then ringed with a thin strip of Tanglefoot (Tanglefoot, Grand Rapids, Michigan, USA) and a first instar larva of the generalist caterpillar, *Spodoptera exigua* (Noctuidae) was placed on each plant.

After five days, each plant was examined for the percentage of leaf area that was consumed by the caterpillar. In the second experiment (choice, generalist), 26 plants (13 pairs) were grown and treated as above. Three days after the plants were treated, one newly expanded leaf from each plant was excised, and control and induced leaves were paired by size and placed in a 90 mm petri dish lined with moistened filter paper. One third instar larva of the mobile generalist caterpillar, *Helicoverpa zea* (Noctuidae) was added to each petri dish and it was sealed with parafilm. After 24 h, the percentage of leaf damage on each leaf was recorded using an acetate grid. *S. exigua* and *H. zea* were obtained from the U.S.D.A. (Stoneville, Mississippi, USA) and raised on artificial diet until they were used.

The above described no-choice and choice experiments were also conducted with *P. rapae*, a specialist herbivore of the Brassicaceae, with the following sample sizes: no-choice, specialist: 16 plants in each treatment; choice, specialist: 34 pairs. In addition, weight gain of *P. rapae* larvae was measured in the no-choice experiment. *P. rapae* were maintained in a colony at the University of California–Davis from wild-collected individuals.

Effects of induction in the no-choice tests were analyzed using *t* tests on arcsine-square-root transformed data of the percent leaf area consumed. Effects of induction in the choice tests were analyzed using paired *t* tests on similarly transformed data. Untransformed data are presented in the figures.

Phytochemical and physical defense measurements

To test for phytochemical correlates of induced resistance, I assayed glucosinolates in caterpillar damaged and control plants. I grew 18 plants and induced nine of them with *P. rapae* caterpillars as in the above experiments. The entire aboveground components of the plants were harvested four days after treatments were imposed and immediately frozen in liquid nitrogen. Samples were then lyophilized and kept in a 0°C freezer until analyzed. The analytical procedure was modified from published procedures for determination of trimethylsilyl glucosinolate derivatives with capillary gas chromatography (GC) and flame ionization detection (FID) (Brown and Mora 1995). The procedure starts with a methanolic extraction of ~0.2 g of lyophilized, ground plant material with addition of the internal standard (1 mMol of benzyl glucosinolate), followed by separation of glucosinolates using an ion exchange column containing Sephadex DEAE (Pharmacia Biotech, Uppsala, Sweden). After removing impurities, glucosinolates were desulfated using desulfatase enzyme, and transferred into GC autosampler vials, where they were derivatized with a silylation mixture. Silylated samples were analyzed by capillary gas chromatography using a HP-5 (30 m, 0.25 mm ID, 0.25 μm film) (Hewlett-Packard, Wilmington, Delaware, USA), injector temperature 290°C, FID temper-

ature 305°C, using the following oven temperature program: 260°C for 7 min, 8°C for 1 min, and 300°C for 10 min.

Glucosinolate peaks were identified by retention times. Early peaks with retention times <8 m are usually sugar impurities, followed with peaks of silylated glucosinolates. Only peaks of glucosinolates >1% of total glucosinolate peak area were evaluated. Retention indices (ratio: peak retention time/standard (benzyl glucosinolate) retention time) were used for identification of known glucosinolates. Peak areas were normalized to the standard peak area and to the sample size using the following formula: [(peak area-1000)/(peak area of internal standard-sample weight)]. No additional FID response factors were used.

To estimate effects of leaf damage on glucosinolates, I report several measures of chemical induction: (1) total glucosinolate concentrations, (2) concentrations of allyl glucosinolate (sinigrin), the dominant glucosinolate in *L. virginicum* (*personal observation*), (3) concentrations of indole glucosinolates (glucobrassicins), which appear to be the dominant class of inducible glucosinolates in other brassicaceous plants (Koritsas et al. 1991, Bodnaryk 1992, 1994, Doughty et al. 1995), and (4) the number of different glucosinolates (chemical diversity). These phytochemical factors were compared between control and induced plants using *t* tests; the α value for the first three measurements was corrected for multiple tests using the Bonferroni correction. The fourth measure, diversity of glucosinolate peaks, was considered independent because it was not a measure of concentration of compounds. Concentrations of total glucosinolates and sinigrin were natural log transformed for the analysis to equalize scedasticity.

Putatively defensive leaf trichomes were counted as an additional correlate of induced resistance. Trichomes on *L. virginicum* are most common around the perimeter of the serrated leaves and often form small clumps at the tips of the serrations. Twenty-four plants were grown and induced as above ($n = 12$ each of control and induced plants) at the four-leaf stage, and trichomes were counted ten days later on the newly expanding eighth true leaf. I also counted the number of serrations on the leaves. Trichome number and number of serrations of induced and control plants were compared using *t* tests.

Effects of induced resistance on plant performance: a field experiment

To test for effects of induced resistance on plant protection in the field, I conducted an experiment in an old plowed field at the University of California Student Experimental Farm, Davis, California, USA. Plants were placed in a lath house for one week before transplanting; 300 greenhouse grown seedlings were transplanted to the field from plug trays at the two-true-leaf stage. Plants were randomly assigned to one

of three treatments: (1) unmanipulated controls, (2) induced, (3) leaf damage controls. Induced plants were treated as in the above experiments with a caged *P. rapae* caterpillar when the plants had 4–5 true leaves. At the same stage, leaf damage control plants had one leaf clipped off using a pair of scissors. One half of the leaf damage control plants were clipped at the initiation of the caterpillar induction treatment, and the other half were clipped two days later when the caterpillars were finished feeding. Such clipping resulted in an equal amount of leaf tissue removed as in the induced treatment, but without the associated induced plant response. Induced plant responses are thought to be minimized by clipping with scissors because of the absence of herbivore saliva and the greatly reduced area of actual leaf tissue that is damaged (rather than removed) (Haukioja and Neuvonen 1985, Mattson and Palmer 1988, Bodnaryk 1992, Mattiacci et al. 1995). My previous experiments with other plants in the Brassicaceae indicate that clipping does not induce resistance (Agrawal 1998b, 1999; unpublished data). Because of initial transplanting mortality, the sample sizes were reduced to 88 control plants, 78 induced plants, and 82 leaf damage control plants. Herbivores were removed from all plants by hand until the treatments were fully imposed. This effectively created treatments where plants were denied their normal early season induction cue (controls and leaf damage controls), while plants in the induced treatment were given an imposed uniform induction cue. Two weeks after the plants had been treated, I surveyed each plant for herbivores and leaf damage. Plant mortality at the end of the growing season was measured as an indicator of plant fitness.

At this field site, green peach aphids, *Myzus persicae* (Aphidae), were the only abundant herbivore early in the season. The numbers of naturally occurring winged and non-winged aphids were compared on the three treatments using a multivariate analysis of variance (MANOVA) and planned contrasts between: (1) control and induced plants, and (2) control and leaf damage controls. Total number of aphids was not used as a response variable in the MANOVA, although it is presented in the figure and was analyzed using a univariate ANOVA. Mortality was compared between treatments using a Pearson Chi-square test (2×3 table) with a priori contrasts as above. In addition, in this analysis a comparison of clipped and induced plants indicates the fitness consequences of induction per se, while controlling for leaf area removed associated with the induction treatment. Contrasts were conducted using 2×2 tables. Contrasts were conducted without adjusting the *P* values because they were constructed from a priori hypotheses.

Effects of damage induced resistance and plant allocation to roots and shoots

To measure allocation shifts associated with induced responses to herbivory, I conducted two experiments

TABLE 1. Analysis (*t* tests) of the effects of induced responses to herbivory of *Lepidium virginicum* on the leaf area consumed by generalist and specialist herbivores in choice and no-choice experiments.

Experiment	df	<i>t</i>	<i>P</i>
Generalist, choice	12	2.594	0.023
Generalist, no-choice	32	3.030	0.005
Specialist, choice	33	0.871	0.390
Specialist, no-choice	30	0.189	0.851

Note: Data were arcsine square-root transformed prior to analysis.

in the greenhouse in the absence of herbivores. In the first experiment, I grew 72 plants individually in 1.5 L pots and divided them into two treatments: controls and induced plants. At the 4–5 leaf stage, one leaf on each induced plant was consumed by one *P. rapae* larva. After one month of growth, the experiment was terminated. The roots were washed free of soil and then the above- and belowground parts of each plant were dried for one week in a 50°C drying oven.

In the second experiment, I manipulated induction at a higher density of plants. In each of 48 pots I germinated three seeds and randomly divided the pots into control and induced treatments. In the induced pots, one of the three plants was induced as above at the 4–5 leaf stage; plants in control pots were left unmanipulated. After one month of growth the plants were cleaned and dried as above and separated into three categories: (1) undamaged plants from induced pots, (2) induced plants from induced pots, and (3) undamaged plants from control pots. In both of the experiments, 0.02 g was added to the measured shoot biomass of each of the damaged plants because this was the mean dry weight of the true leaf consumed by the caterpillars on induced plants. Additionally, the analyses were conducted in the absence of the 0.02 g weight addition to the induced plants and the results were identical.

Treatment differences were evaluated using a MANOVA on root and shoot biomass allocation (total biomass was not included in the MANOVA). For the high-density experiment, additional preplanned contrasts were conducted as follows: (1) damaged vs. undamaged plants—both from induced pots, (2) undamaged plants from induced pots vs. undamaged plants from control pot, and (3) mean total biomass from the damaged pot vs. mean total biomass from the control pot.

RESULTS

Induced responses to herbivory significantly reduced the amount of feeding (leaf area consumed) by generalist noctuid larvae in both choice and no-choice tests (Table 1, Fig. 1a). Induced responses did not affect feeding by specialist *P. rapae* larvae in either choice or no-choice experiments (Table 1, Fig. 1b). In the no-choice experiment, weight gain of *P. rapae* larvae was unaffected by the induction treatment (mean \pm SE, con-

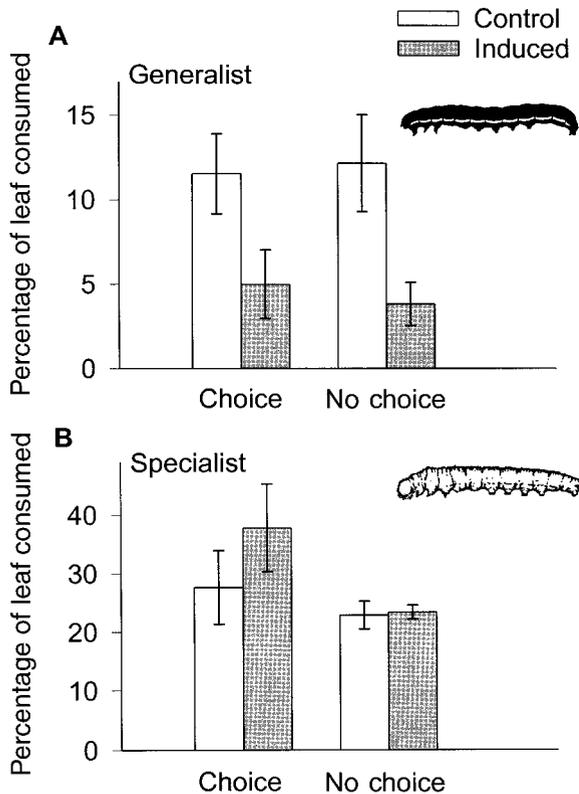


FIG. 1. Percentage leaf damage by (A) generalist (Noctuidae) and (B) specialist (Pieridae) caterpillars on control and induced plants in choice and no-choice experiments. Bars are ± 1 SE.

trol: 9.994 ± 1.032 mgs, induced: 9.575 ± 0.269 mgs; $t = 0.393$, $df = 30$, $P = 0.697$).

Leaf damage did not affect foliar concentrations of total glucosinolates, indole glucosinolates, or the most abundant glucosinolate, sinigrin (Table 2). However, the diversity of glucosinolates was increased by nearly 50% in induced plants compared to controls (Table 2, Fig. 2). The number of leaf trichomes was increased by $>64\%$ on induced plants compared to controls ($t = 5.613$, $df = 22$, $P < 0.001$; Fig. 2). Although many of the clumps of trichomes (3–5 trichomes in each clump) were at the tips of the leaf serrations, there was no difference in the number of leaf serrations on control and induced plants (mean \pm SE, control: $14.250 \pm$

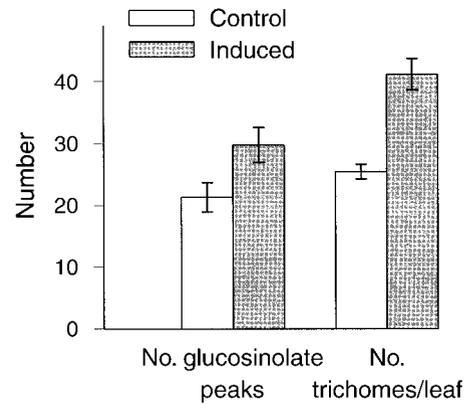


FIG. 2. Two correlates of induced resistance to herbivory in *Lepidium virginicum*. Bars are ± 1 SE.

0.479, induced: 13.417 ± 0.529 ; $t = 1.168$, $df = 22$, $P = 0.255$).

In the field experiment to measure the net benefits or costs of induction, aphids colonized the plants early in the season. Treatments had a significant impact on the number of winged and nonwinged aphids found on plants (MANOVA, Wilks' lambda = 0.945, $df = 4$, 488, $F = 3.526$, $P = 0.008$; Fig. 3a). Winged aphids were likely to be colonizing adults, while nonwinged aphids were mostly asexually produced adults and immatures on the plant. Induced resistance significantly reduced the number of aphids on plants compared to controls (MANOVA contrast, Wilks' lambda = 0.972, $df = 2$, 244, $F = 3.531$, $P = 0.031$; Fig. 3a). Clipping plants with a pair of scissors did not affect the number of aphids when compared to controls (MANOVA contrast, Wilks' lambda = 0.988, $df = 2$, 244, $F = 1.452$, $P = 0.236$).

The treatments significantly affected survival of the plants, an important component of plant fitness ($df = 2$, $\chi^2 = 15.220$, $P < 0.001$; Fig. 3b). Although survival of clipped plants was lower than that of controls ($df = 1$, $\chi^2 = 15.152$, $P < 0.001$) and survival of induced plants was marginally lower than that of controls ($df = 1$, $\chi^2 = 3.521$, $P = 0.061$), induced plants were more likely to survive than clipped plants ($df = 1$, $\chi^2 = 3.936$, $P = 0.047$). This mortality was, in part, due to infestation by darkling beetles, *Blapstinus* sp. (Tene-

TABLE 2. Analysis of phytochemicals involved in induced resistance to herbivory in *Lepidium virginicum* and effects of leaf damage on several measures of phytochemical induction.

Experiment	Mean \pm 1 SE (control)	Mean \pm 1 SE (induced)	df	T	P
Total glucosinolates†	2110 (+1727)(-851)	881 (+935)(-733)	16	1.018	0.324‡
Indole glucosinolates	8.875 ± 3.373	8.554 ± 2.796	16	0.073	0.943‡
Allyl glucosinolate†	1456 (+138)(-5.912)	328 (+221)(-32.5)	16	0.363	0.721‡
Diversity of glucosinolates	21.333 ± 2.392	29.778 ± 2.813	16	2.287	0.036

Note: Glucosinolate concentrations are reported in $\mu\text{M/g}$.

† Natural log transformed for the analysis to equalize scedasticity (back-transformed means and SE are presented).

‡ Bonferroni corrected; $\alpha_{0.05} = 0.0125$.

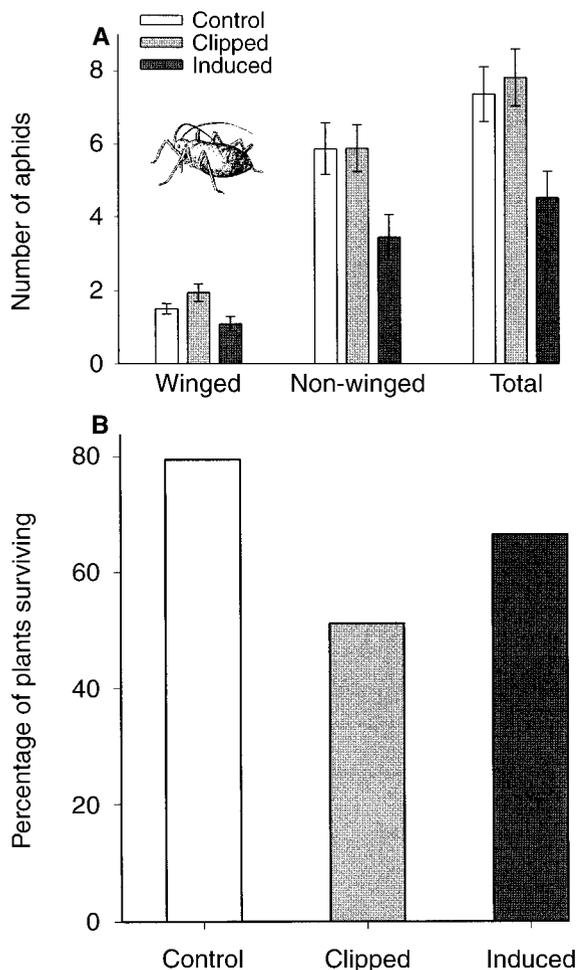


FIG. 3. Benefits of induced resistance demonstrated in a field experiment. (A) Effects of induction on winged and non-winged aphids (bars are ± 1 SE), and (B) percentage of plants surviving.

brionidae), which severely damaged the plants later in the season.

To estimate allocation shifts associated with induced responses I measured effects of induction on root and shoot biomass accumulation in the absence of herbivores. In the low-density experiments, induction did not statistically affect biomass accumulation, although the trends were for lower biomass of damage-induced plants (Wilks' lambda = 0.967, df = 2, 69, $F = 1.185$, $P = 0.312$; Fig. 4a). In the high-density experiments, induction significantly reduced root biomass allocation compared to uninduced controls in the same pot (Table 3, Fig. 4b). Control plants in pots with an induced plant gained the most root and shoot biomass of all treatments. This high level of biomass accumulation of control plants came at the expense of biomass accumulation in induced plants for roots, but not for shoots. The mean total biomass of plants in the pot with induced plants (averaging biomass of induced and uninduced

plants) was greater than that of plants in control pots (Table 3, Fig. 4b). This result is largely driven by increased aboveground growth in the induced pot; the ranking of aboveground growth from lowest to highest is: plants in control pot, induced plants in induced pot, control plants in induced pot (Fig. 4b).

DISCUSSION

Induced responses to herbivory in *Lepidium virginicum* influenced feeding by generalist herbivores (noctuid caterpillars) when plants were offered in choice and no-choice environments. The diversity of glucosinolate peaks was increased in damaged plants compared to controls, although I did not find an overall increase in the concentrations of glucosinolates. Biochemical diversity is likely an important component of defense against herbivores, and is thought to enhance resistance to herbivores, even when total phytochemical concentrations do not change (McKey 1979, Berenbaum and Zangerl 1993, 1996, Slansky 1993, Castellanos and Espinosa-Garcia 1997, Lindig-Cisneros et

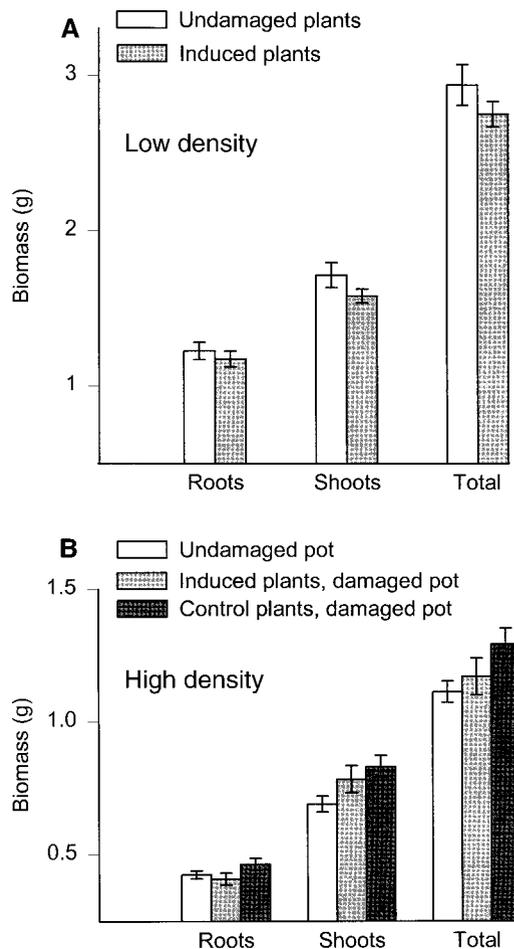


FIG. 4. The effects of induction on root and shoot biomass accumulation in (A) low and (B) high plant density experiments. Bars are ± 1 SE.

TABLE 3. Multivariate analysis of variance and univariate ANOVAs for effects of induction in high-density experiments on root, shoot, and total biomass accumulation.

Source	Wilks' λ	df	F	P
MANOVA	0.843	4, 136	3.025	0.020
Roots		2, 69	2.106	0.129
Shoots		2, 69	2.928	0.060
Total		2, 69	2.563	0.084
Contrast 1 (MANOVA)	0.937	2, 68	2.302	0.108
Roots		1, 69	3.988	0.050
Shoots		1, 69	0.644	0.425
Total		1, 69	2.290	0.135
Contrast 2 (MANOVA)	0.923	2, 68	2.848	0.065
Roots		1, 69	1.984	0.163
Shoots		1, 69	5.656	0.020
Total		1, 69	4.907	0.030
Contrast 3 (MANOVA)	0.900	2, 68	3.775	0.028
Roots		1, 69	0.224	0.637
Shoots		1, 69	5.212	0.026
Total		1, 69	2.837	0.097

Notes: Contrast 1 compares induced plants and uninduced plants in the same pot. Contrast 2 compares undamaged plants in the induced pot and undamaged plants in the control pot. Contrast 3 compares the average biomass in the induced pot to the average biomass of the control pot.

al. 1997). The increase in the number of trichomes per leaf, an often cited putative plant defense (Björkman and Anderson 1990, Ågren and Schemske 1993, Fernandez 1994), was also correlated with induced resistance in *L. virginicum*. Although induction of mechanical defenses is far less commonly reported than phytochemical induction, induction of trichomes, thorns, and spines has now been reported in several systems (Young 1987, Baur et al. 1996, Gowda 1997, Agrawal 1999).

Contrary to the findings for a generalist herbivore, a specialist caterpillar (*P. rapae*), whose diet is restricted to plants in the Brassicaceae, was not affected by induced responses in *L. virginicum*. Although induction has been demonstrated to negatively affect herbivores in many systems, in other cases, especially those involving specialized herbivores, herbivores may not be affected by induction, or may even benefit from induction (Karban and Baldwin 1997, Agrawal and Karban 1999, Agrawal et al. 1999b). *L. virginicum* contains glucosinolates (mustard oil glycosides) which likely defend the plant against generalist herbivores (Chew 1988, Louda and Mole 1992). These same chemicals serve as feeding stimulants for specialized herbivores, and may also induce egg laying by adults (Reed et al. 1989, Haug and Renwick 1994, Giannouaris and Mithen 1995). Several accounts suggest that damaged plants in the Brassicaceae are more susceptible to oviposition and feeding by specialist herbivores such as diamondback moths, pierid butterflies, flea beetles, and cabbage root flies (Vaughn and Hoy 1993, Baur et al. 1996, Riggin-Bucci and Gould 1996; P. K. Kwapong, *personal communication*; A. A. Agrawal, *unpublished data*).

One hypothesis for the maintenance of variation in

constitutive defense chemicals is that resistance against generalist herbivores and attraction of specialist herbivores may result in balancing selection (Linhart 1991, van der Meijden 1996). Mithen et al. (1995) report a particularly compelling example of this in wild populations of *Brassica oleracea*. In populations with high levels of herbivory by generalists, glucosinolate levels are high. However, in populations where specialist pierid butterflies are the main herbivore, genetically determined glucosinolate levels are quite low. An ecological trade-off between defense against generalists and susceptibility to specialist herbivores has been proposed as a mechanism favoring the evolution of inducible defenses (Carroll and Hoffman 1980, Adler and Karban 1994, Karban et al. 1997, Agrawal and Karban 1999). In other words, it may benefit the plant to wait until it can perceive the herbivore environment before deploying the appropriate defense. Carroll and Hoffman's (1980) classic study demonstrated this double edged nature of induction in *Cucurbita moschata*.

Benefits of induced defenses

In the field experiments, generalist aphids and darkling beetles were the dominant herbivores. Controlled herbivory early in the season induced resistance against colonization by aphids. Resistance against herbivory was not induced by leaf clipping, suggesting that reduction in plant size and leaf tissue removal per se did not affect plant resistance. In addition, it is likely that clipped plants were not induced because the actual amount of leaf tissue that was damaged by clipping was very low, and the absence of herbivore saliva may have minimized the induced responses. These factors have been demonstrated to be important for induced responses to herbivory in other species of the Brassicaceae (Bodnaryk 1992, Mattiacci et al. 1995).

Fitness benefits of induction were detected in the field experiment. Survival of induced plants was higher than that of clipped plants (with the same amount of leaf tissue removed), suggesting a net benefit of induction. However, control plants were more likely to survive than plants in either the clipped or induced treatments. My interpretation of this suggests two points: (1) early season leaf tissue removal is costly to the plant, and (2) the benefits of induction did not outweigh the costs of early season leaf tissue removal and/or potential costs of induction itself.

Few other studies have attempted to detect consequences of induction for plant performance. In experiments with cultivated and wild cotton, Karban (1986 and 1993, respectively) was unable to detect fitness benefits of induction. In field experiments with annual wild radish plants, plants that were experimentally induced early in the season, as in the current experiment, outperformed overall controls and leaf damage controls (Agrawal 1998b, 1999). Future studies should examine other fitness benefits of induction in addition to reduced herbivory (Agrawal and Karban 1999). It is important

to note the disparity in the number of studies that have attempted to detect costs vs. benefits of inducible resistance. Costs of induction have been studied in many systems where fitness benefits of induction have not been looked for or demonstrated (e.g., Brown 1988, Gianoli and Niemeyer 1997, Zangerl et al. 1997).

Costs of induced defenses

I found that induction did not affect plant biomass when plants were grown at low densities, suggesting that the generally accepted allocation arguments may be environment dependent. Density has been shown to be an important factor in detecting costs of herbicide resistance in plants (Williams and Jordan 1995), parasitoid resistance in flies (Kraaijeveld and Godfray 1997), and induction of a morphological defense in fish (Pettersson and Bronmark 1997). I suspect that allocation shifts due to induced responses to herbivory may be more easily detected at higher competition regimes because of decreased nutrient and light availability. At high densities, although induced plants accumulated less biomass than undamaged neighbors, plants in an environment with some damage (i.e., the mean biomass of damaged and undamaged plants in the induced pot) accumulated more biomass overall than did plants in an environment without herbivory (Fig. 4). More specifically, induced plants had higher aboveground biomass than did control plants in the control pot. These results are a bit counterintuitive and suggest that although induction may be costly at some level, it also stimulates compensatory growth in *L. virginicum*. Thus, the net effects of induction may be negligible in the absence of herbivores, even in highly competitive environments. Induced plants did not reduce total allocation to growth, but rather shifted the allocation away from root growth to aboveground shoot growth. Zangerl et al. (1997) found that induced responses in wild parsnip reduced belowground allocation to root biomass, but that aboveground biomass was unaffected by induction.

Allocation costs of phytochemical defense have, in general, been difficult to detect. In this system, induction of resistance was correlated with a diversification of the putative biochemical defenses in the apparent absence of increasing total concentrations of these compounds. In addition, trichomes were found to be inducible, with increasing numbers on the new growth of damaged plants; however, costs of trichome production in other brassicaceous plants have not been detected, even in statistically powerful selection experiments (Ågren and Schemske 1993).

Induced defenses as adaptive plasticity

The hallmark of adaptive plasticity is individuals having higher relative fitness when expressing particular phenotypes in particular environments (Bradshaw 1965, Thompson 1991, Gotthard and Nylin 1995, Kingsolver 1995, Schmitt et al. 1995, Dudley and

Schmitt 1996, DeWitt et al. 1998). A more restrictive definition of adaptive plasticity involves comparing the fitness of plastic genotypes with genotypes that are not plastic (M. D. Rausher, *personal communication*). In the latter case, demonstrating adaptive plasticity would require that the relative fitness of genotypes with induced responses is higher than for genotypes that do not exhibit induced responses in environments with herbivory. If costs of plasticity per se are high, then potential benefits of induced responses may be swamped out by costs associated with the machinery required to be plastic. In the current study I conducted phenotypic manipulations without examining genetic variation in plasticity.

For induced resistance in *Lepidium virginicum*, the induced phenotype is associated with higher relative fitness in environments with herbivory (only when leaf tissue removal is controlled). In addition, the induced phenotype may be associated with reduced fitness in environments lacking herbivory (i.e., fitness cost of expressing the wrong phenotype), although I was not able to detect this. Demonstrating such phenotypic benefits and costs are important because phenotypic plasticity is thought to evolve as a mechanism for organisms to express adaptive phenotypes in variable environments. Benefits and costs of induction in environments with and without herbivory, respectively, confirms that inducible resistance can be an adaptive trait across variable environments. In only two other systems to date have plant defensive characters been documented as adaptively plastic (Agrawal 1998b, 1999, Baldwin 1998, Agrawal et al. 1999a).

Further experiments on fitness benefits and costs of induced plant responses to herbivory in individual systems will help us to better understand the evolutionary ecology of plant defense. Studies incorporating a multifaceted approach, investigating alternative benefits and costs will be especially important in understanding the relative roles of different selection pressures and constraints on the evolution of plant defense (Agrawal and Karban 1999). The current study contributes to this goal by demonstrating that induced responses to herbivory can correlate with increased chemical defense diversity and increases in physical defenses such as trichomes. Induction deterred herbivory by noctuids and aphids, and increased the probability of plant survival. However, induction may only be effective against particular herbivores. In addition, although allocation costs may exist, and may be exacerbated under higher competitive regimes, they may be obscured by compensatory growth, and may be minimized by particular plant strategies. In conclusion, induced responses to herbivory in *L. virginicum* enhance plant performance in the field and provide support for adaptive plasticity in plant defense.

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LITERATURE CITED

- Adler, F. R., and R. Karban. 1994. Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. *American Naturalist* **144**: 813–832.
- Agrawal, A. A. 1998a. Leaf damage and associated cues induced aggressive ant recruitment in a neotropical ant-plant. *Ecology* **79**:2100–2112.
- Agrawal, A. A. 1998b. Induced responses to herbivory and increase 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**:1731–1723.
- Agrawal, A. A., and R. Karban. 1999. Why induced defenses may be favored over constitutive strategies in plants. Pages 45–61 in R. Tollrian and D. Harvell, editors. *Ecology and evolution of inducible defenses*. Princeton University Press, Princeton, New Jersey, USA.
- Agrawal, A. A., and M. T. Rutter. 1998. Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos* **83**:227–236.
- Agrawal, A. A., S. Y. Strauss, and M. J. Stout. 1999a. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* **53**:1093–1104.
- Agrawal, A. A., D. W. Tallamy, and P. M. Gorski. 1999b. Polymorphism in plant defense against herbivory: constitutive and induced resistance in *Cucumis sativus*. *Journal of Chemical Ecology* **25**:2285–2304.
- Ågren, J., and D. W. Schemske. 1993. The cost of defense against herbivores: an experimental study of trichome production in *Brassica rapa*. *American Naturalist* **141**:338–350.
- Baldwin, I. T. 1994. Chemical changes rapidly induced by folivory. Pages 1–23 in E. Bernays, editor. *Insect plant interactions*. V. Chemical Rubber Company Press, New York, New York, USA.
- Baldwin, I. T. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences (USA)* **95**: 8113–8118.
- Baldwin, I. T., C. L. Sims, and S. E. Kean. 1990. The reproductive consequences associated with inducible alkaloidal responses in wild tobacco. *Ecology* **71**:252–262.
- Baur, R., S. Binder, and G. Benz. 1991. Nonglandular leaf trichomes as short-term inducible defense of the gray alder, *Alnus incana* (L.), against the chrysomelid beetle, *Agelastica alni* L. *Oecologia* (Berlin) **87**:219–226.
- Baur, R., V. Kostal, B. Patrian, and E. Staedler. 1996. Preference for plants damaged by conspecific larvae in ovipositing cabbage root flies: influence of stimuli from leaf surface and roots. *Entomologia Experimentalis et Applicata* **81**:353–364.
- Bazzaz, F. A. 1996. *Plants in changing environments: linking physiological, population, and community ecology*. Cambridge University Press, Cambridge, UK.
- Berenbaum, M. R., and A. R. Zangerl. 1993. Furanocoumarin metabolism in *Papilio polyxenes*: biochemistry, genetic variability, and ecological significance. *Oecologia* **95**:370–375.
- Berenbaum, M. R., and A. R. Zangerl. 1996. Phytochemical diversity: adaptation or random variation? Pages 1–24 in J. T. Romeo, J. A. Saunders, and P. Barbosa, editors. *Phytochemical diversity and redundancy in ecological interactions*. Plenum, New York, New York, USA.
- Bi, J. L., J. B. Murphy, and G. W. Felton. 1997. Antinutritive and oxidative components as mechanisms of induced resistance in cotton to *Helicoverpa zea*. *Journal of Chemical Ecology* **23**:97–117.
- Björkman, C., and D. B. Anderson. 1990. Trade-off among antiherbivore defenses in a South American blackberry (*Rubus bogotensis*). *Oecologia* (Berlin) **85**:247–249.
- Bodnaryk, R. P. 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. *Phytochemistry* (Oxford) **31**:2671–2677.
- Bodnaryk, R. P. 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. *Phytochemistry* (Oxford) **35**:301–305.
- Bradshaw, A. D. 1965. The evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* **13**:115–155.
- Brown, D. G. 1988. The cost of plant defense: an experimental analysis with inducible proteinase inhibitors in tomato. *Oecologia* (Berlin) **76**:467–470.
- Brown, P. D., and M. J. Morra. 1995. Glucosinolate-containing plant tissues as bioherbicides. *Journal of Agricultural and Food Chemistry* **43**:3070–3074.
- Carroll, C. R., and C. A. Hoffman. 1980. Chemical feeding deterrent mobilized in response to insect herbivory and counteradaptation by *Epilachna tredecimnotata*. *Science* **209**:414–416.
- Castellanos, I., and F. J. Espinosa-Garcia. 1997. Plant secondary metabolite diversity as a resistance trait against insects: a test with *Sitophilus granarius* (Coleoptera: Curculionidae) and seed secondary metabolites. *Biochemical Systematics and Ecology* **25**:591–602.
- Chew, F. S. 1988. Searching for defensive chemistry in the Cruciferae, do glucosinolates always control interactions of Cruciferae with their potential herbivores and symbionts? No! Pages 81–112 in K. C. Spencer, editor. *Chemical mediation of coevolution*. Academic Press, San Diego, California, USA.
- De Moraes, C. M., W. J. Lewis, P. W. Pare, H. T. Alborn, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* (London) **393**:570–573.
- DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution* **13**:77–81.
- Doughty, K. J., G. A. Kiddle, B. J. Pye, R. M. Wallsgrove, and J. A. Pickett. 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. *Phytochemistry* (Oxford) **38**:347–350.
- Dudley, S. A., and J. Schmitt. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist* **147**:445–465.
- Fernandes, G. W. 1994. Plant mechanical defenses against insect herbivory. *Revista Brasileira de Entomologia* **38**: 421–433.
- Giamoustaris, A., and R. Mithen. 1995. The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests. *Annals of Applied Biology* **126**: 347–363.
- Gianoli, E., and H. M. Niemeyer. 1997. Lack of costs of herbivory-induced defenses in a wild wheat: integration of

- physiological and ecological approaches. *Oikos* **80**:269–275.
- Gotthard, K., and S. Nylin. 1995. Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* **74**:3–17.
- Gowda, J. H. 1997. Physical and chemical response to juvenile *Acacia tortilis* trees to browsing: experimental evidence. *Functional Ecology* **11**:106–111.
- Haukioja, E., and S. Neuvonen. 1985. Induced long-term resistance of birch foliage against defoliators: defensive or incidental? *Ecology* **66**:1303–1308.
- Hermes, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**:283–335.
- Huang, X., and J. A. A. Renwick. 1994. Relative activities of glucosinolates as oviposition stimulants for *Pieris rapae* and *P. napi* oleracea. *Journal of Chemical Ecology* **20**:1025–1037.
- Karban, R. 1986. Induced resistance against spider mites in cotton: field verification. *Entomologia Experimentalis et Applicata* **42**:239–242.
- Karban, R. 1993. Costs and benefits of induced resistance and plant density for a native shrub, *Gossypium thurberi*. *Ecology* **74**:9–19.
- Karban, R., A. A. Agrawal, and M. Mangel. 1997. The benefits of induced defenses against herbivores. *Ecology* **78**:1351–1355.
- Karban, R., and I. T. Baldwin. 1997. Induced responses to herbivory. University of Chicago Press, Chicago, Illinois, USA.
- Karban, R., and J. H. Myers. 1989. Induced plant responses to herbivory. *Annual Review of Ecology and Systematics* **20**:331–348.
- Kingsolver, J. G. 1995. Fitness consequences of seasonal polyphenism in western white butterflies. *Evolution* **49**:942–954.
- Koritsas, V. M., J. A. Lewis, and G. R. Fenwick. 1991. Glucosinolate responses of oilseed rape, mustard and kale to mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala*). *Annals of Applied Biology* **118**:209–222.
- Kraaijeveld, A. R., and H. C. J. Godfray. 1997. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature (London)* **389**:278–280.
- Lindig-Cisneros, R., B. Benrey, and F. J. Espinosa-Garcia. 1997. Phytoalexins, resistance traits, and domestication status in *Phaseolus coccineus* and *Phaseolus lunatus*. *Journal of Chemical Ecology* **23**:1997–2011.
- Linhart, Y. B. 1991. Disease, parasitism and herbivory: multidimensional challenges in plant evolution. *Trends in Ecology and Evolution* **6**:392–396.
- Louda, S., and S. Mole. 1992. Glucosinolates chemistry and ecology. Pages 123–164 in G. A. Rosenthal and M. R. Berenbaum, editors. *Herbivores: their interactions with secondary plant metabolites*. Volume I: the chemical participants. Second edition. Academic Press, San Diego, California, USA.
- Mattiacci, L., M. Dicke, and M. A. Posthumus. 1995. Beta-glucosidase: An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proceedings of the National Academy of Sciences (USA)* **92**:2036–2040.
- Mattson, W. J., and S. R. Palmer. 1988. Changes in levels of foliar minerals and phenolics in trembling aspen *Populus tremuloides* in response to artificial defoliation. Pages 157–170 in W. J. Mattson, J. Leveux, and C. Bernard-Dagan, editors. *Mechanisms of woody plant defenses against insects: search for pattern*. Springer-Verlag, Berlin, Germany.
- McKey, D. 1979. The distribution of secondary compounds within plants. Pages 56–134 in G. A. Rosenthal and D. H. Janzen, editors. *Herbivores: their interaction with secondary plant metabolites*. Academic, New York, New York, USA.
- Mithen, R., A. F. Raybould, and A. Giamoustaris. 1995. Divergent selection for secondary metabolites between wild populations of *Brassica oleracea* and its implications for plant-herbivore interactions. *Heredity* **75**:472–484.
- Parker, M. A. 1992. Constraints on the evolution of resistance to pests and pathogens. Pages 181–197 in P. G. Ayres, editor. *Pests and pathogens: plant responses to foliar attack*. Bios Scientific, Oxford, UK.
- Pettersson, L. B., and C. Bronmark. 1997. Density-dependent costs of an inducible morphological defense in crucian carp. *Ecology* **78**:1805–1815.
- Reed, D. W., K. A. Pivnick, and E. W. Underhill. 1989. Identification of chemical oviposition stimulants for the diamondback moth, *Plutella xylostella*, present in three species of Brassicaceae. *Entomologia Experimentalis et Applicata* **53**:277–286.
- Riggin-Bucci, T. M., and F. Gould. 1996. Effects of surfactants, *Bacillus thuringiensis* formulations, and plant damage on oviposition by diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* **89**:891–897.
- Schmitt, J., A. C. McCormac, and H. Smith. 1995. A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *American Naturalist* **146**:937–953.
- Simms, E. L. 1992. Costs of plant resistance to herbivores. Pages 392–425 in R. S. Fritz and E. L. Simms, editors. *Plant resistance to herbivores and pathogens*. University of Chicago Press, Chicago, Illinois, USA.
- Slansky, F., Jr. 1993. Xanthine toxicity to caterpillars synergized by allopurinol, a xanthine dehydrogenase-oxidase inhibitor. *Journal of Chemical Ecology* **19**:2635–2650.
- Smith, L. L., J. Lanza, and G. C. Smith. 1990. Amino acid concentrations in extrafloral nectar of *Impatiens sultani* increase after simulated herbivory. *Ecology* **71**:107–115.
- Takabayashi, J., and M. Dicke. 1996. Plant-carnivore mutualism through herbivore-induced carnivore attractants. *Trends in Plant Science* **1**:109–113.
- Thompson, J. D. 1991. Phenotypic plasticity as a component of evolutionary change. *Trends in Ecology and Evolution* **6**:246–249.
- van der Meijden, E. 1996. Plant defence, an evolutionary dilemma: contrasting effects of (specialist and generalist) herbivores and natural enemies. *Entomologia Experimentalis et Applicata* **80**:307–310.
- Vaughn, T. T., and C. W. Hoy. 1993. Effects of leaf age injury morphology and cultivars on feeding behavior of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Environmental Entomology* **22**:418–424.
- Williams, M. M., and N. Jordan. 1995. The fitness cost of triazine resistance in jimsonweed (*Datura stramonium* L.). *American Midland Naturalist* **133**:131–137.
- Yano, S. 1997. Silique burst of *Cardamine scutata* (Cruciferae) as a physical inducible defense against seed predatory caterpillars. *Researches on Population Ecology (Kyoto)* **39**:95–100.
- Young, T. P. 1987. Increased thorn length in *Acacia depranobium*—an induced response to browsing. *Oecologia (Berlin)* **71**:436–438.
- Zangerl, A. R., A. M. Arntz, and M. R. Berenbaum. 1997. Physiological price of an induced chemical defense: photosynthesis, respiration, biosynthesis, and growth. *Oecologia* **109**:433–441.
- Zangerl, A. R., and F. A. Bazzaz. 1992. Theory and pattern in plant defense allocation. Pages 363–391 in R. S. Fritz and E. L. Simms, editors. *Plant resistance to herbivores and pathogens*. University of Chicago Press, Chicago, Illinois, USA.