INDUCED RESPONSES TO HERBIVORY IN WILD RADISH: EFFECTS ON SEVERAL HERBIVORES AND PLANT FITNESS

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Abstract. Induced responses to herbivory are changes in plant quality following initial damage by herbivores. These changes can negatively affect subsequent herbivores. I studied induced responses in annual wild radish plants, *Raphanus raphanistrum* and *R. sativus* (Brassicaceae), which included increased density and total number of setose trichomes on newly formed leaves of previously damaged plants compared to undamaged controls. I also characterized the effects of induced responses on the preference and performance of several herbivores and the net consequences for plant performance in the field.

Wild radish plants damaged by caterpillars or sprayed with a natural plant response elicitor, jasmonic acid, supported reduced growth of generalist noctuid larvae compared to unmanipulated control plants. Induced responses did not affect the growth of specialist *Pieris rapae* larvae. In choice and no-choice experiments, induction reduced the feeding by noctuid larvae but did not reduce gross growth efficiency, an indicator of insect growth per unit of plant material consumed. Finally, colonization of plants by leafminers was lower on plants that received previous damage, compared to controls. In field experiments conducted over 2 yr, herbivory by naturally occurring grasshoppers was lower on induced plants than on both unmanipulated controls and leaf damage controls (plants with leaf area removed without the associated induced response). Plant fitness, as estimated by fruit and seed production, was enhanced for induced plants compared to controls. These results demonstrate that induced responses to herbivory can reduce the preference and performance of a variety of herbivores and ultimately increase plant fitness in natural environments. In order for a plant trait to be characterized as a defense, it must increase plant fitness in environments with herbivory. Although much attention has been given to detecting costs of plant defenses, this is one of the first systems for which fitness benefits of induced resistance have been demonstrated.

Key words: Brassicaceae; defense; fitness; glucosinolates; herbivory; induced resistance; jasmonic acid; plant–insect interactions; trichomes.

INTRODUCTION

Plants have evolved defenses against their predators and parasites that do not involve fleeing areas with high risk of attack. Because plant attackers are not always predictable in time and space, and defenses are thought to impose a cost, it is believed that plants use damage as a cue to induce resistance against subsequent herbivores. Such induced responses appear to be common among a very wide array of plants (Karban and Baldwin 1997). Animals, such as mussels (Reimer and Tedengren 1996), bryozoans (Harvell 1990), frogs (McCollum and Van Buskirk 1996), and ants (at the colony level) (Passera et al. 1996), also have well-developed inducible response systems. To understand the evolutionary ecology of inducible response systems, and whether they are examples of adaptive plasticity (sensu Dudley and Schmitt 1996), it is important to measure the fitness consequences (benefits and costs) of induction for the inducing organism.

Karban and Myers (1989) divided induced responses to herbivory into three categories: responses, resistance, and defense. (1) Induced responses are changes in the plant that occur after herbivory. Such changes may be incidental, and range from shifts in water content (Faeth 1992), leaf toughness (Kudo 1996), and nitrogen uptake (Jaramillo and Detling 1988), to changes in secondary chemistry (Baldwin 1994, Karban and Baldwin 1997), trichome density (Baur et al. 1991; A. A. Agrawal, *unpublished manuscript*), and traits attractive to predaceous arthropods (Smith et al. 1990, Shimoda et al. 1997, Agrawal 1998*a*, De Moraes et al. 1998, Agrawal and Rutter 1998). (2) Induced resistance is a change in the plant that reduces the preference or performance of subsequently attacking herbivores. Induced resistance can be caused by a variety of nonexclusive biochemical and physical resistance mechanisms. For example, nicotine production is induced in wild tobacco plants and has been causally linked to reduced performance of tobacco herbivores (Baldwin 1991). Similarly, spines of African *Acacia* trees have been shown to increase in length following

giraffe herbivory (Young 1987); plants with larger spines experience reduced herbivory compared to plants with smaller spines (Milewski et al. 1991). (3) Induced defense is a term reserved for cases where induction results in a fitness benefit to induced plants compared to plants that were not induced. Evidence for induced defense is limited, and similarly, most plant traits thought to be defensive are not studied from the plant's perspective (Hartley and Jones 1997). Although induced resistance has been reported from over 100 plant–herbivore systems, and induced responses to herbivory are thought to be adaptive (Green and Ryan 1972, Haukioja and Neuvonen 1985, Baldwin 1988, Wold and Marquis 1997), there is little information available on the effects of induction on plant fitness (Karban and Baldwin 1997).

Negative consequences of induction for herbivores do not necessarily lead to benefits for the plant. For example, slower growing larvae on induced plants may consume more plant tissue than larvae on control plants (sensu Slansky and Feeny 1977). In addition, many plants tolerate herbivory or can compensate for losses to herbivores (Paige 1992, Trumble et al. 1993, Rosenthal and Kotanen 1994, Agrawal et al. 1999b, Strauss and Agrawal 1999). It is necessary to demonstrate fitness benefits to the plant to show that induction is an adaptive plant defense and a strategy that may be favored by natural selection.

I link these three levels (plant responses, resistance, and defense) in the study of induced responses to herbivory in annual wild radish plants. My goal was to examine induced plant responses in the context of the adaptive plasticity hypothesis (Dudley and Schmitt 1996) with special regard to the benefits of induction for plant fitness. Specifically, I asked: (1) Does herbivory induce changes in the density and total number of foliar trichomes, which are putative defenses against herbivores? (2) Does herbivory or application of a natural plant response elicitor, jasmonic acid, induce local and/or systemic resistance affecting herbivores' preference and performance in choice and no-choice experiments? (3) In the field, do induced responses protect plants from subsequent herbivory and increase plant fitness?

METHODS

Study organisms and general methods

Wild radish plants, *Raphanus raphanistrum* and *R. sativus* (Brassicaceae), are cosmopolitan herbaceous annuals common to disturbed sites. My experiments on induced responses to herbivory in wild radish are divided into three categories: responses, resistance, and defense. *Pieris rapae* (Lepidoptera: Pieridae) larvae were used to induce responses in wild radish plants. *P. rapae* is a specialist herbivore that feeds only on plants in the Brassicaceae. All *P. rapae* larvae used in these experiments were obtained from a colony of recently
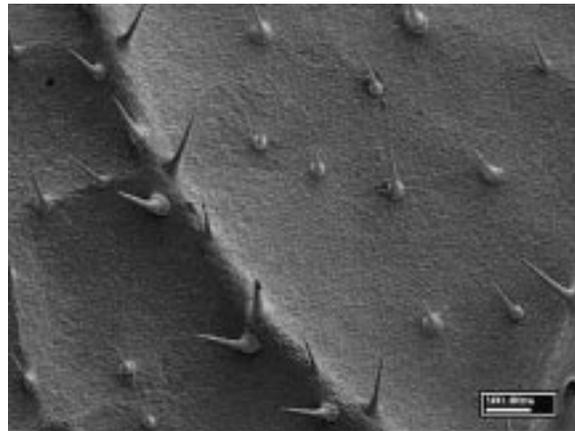

FIG. 1. Scanning electron micrograph of the setose trichomes of *Raphanus sativus*. The horizontal white bar represents 500 μm.

field-collected individuals maintained in the greenhouse. The following general procedure was used to study induction. Plants were randomly divided into control and induced groups. One *P. rapae* larva was confined to one leaf of the induced plants using a clip cage, and the larva was allowed to feed on the entire leaf. Control plants received a clip cage without the caterpillar. Clip cages were made from the tops of ventilated Petri dishes (5 cm) attached to either side of a hair clip. The cage structure was supported by a wooden stake, so as not to weigh down the leaves.

Induced responses to herbivory

In previous experiments I documented that foliar herbivory to wild radish resulted in systemic increases in concentrations of indole glucosinolates, putative defense compounds (Agrawal et al. 1999b). In the current study I investigated the effects of herbivory on production of setose trichomes, an additional putative defense of wild radish plants (Fig. 1). Ågren and Schemske (1993) showed that closely related *Brassica rapa* plants selected for high densities of setose trichomes had a reduced leaf area consumed by *P. rapae* larvae, compared to plants selected for low densities of trichomes.

Raphanus sativus plants were grown in a greenhouse in 0.8-L pots using University of California greenhouse soil mix. Thirty-six plants were randomly assigned to control or induced groups. When the plants had developed two fully expanded true leaves, one *P. rapae* larva was caged on one of the first true leaves of each induced plant. Caterpillars consumed the leaf within 48 h. After 3 wk, when all plants had eight true leaves and were beginning to flower, the plants were harvested for trichome measurements. From each plant, the third, fifth, and seventh leaves were removed, and the area of each leaf was measured using a digital portable area meter (Li-Cor 3000, Lambda Instruments Corporation, Lincoln, Nebraska). Leaves from both treatments were

at the same developmental stage and were fully expanded, except for the seventh leaves, which were still expanding. I removed a leaf disc (1.8 cm^2) from the apex of each leaf using a number 10 cork borer, and counted the number of trichomes on the top and bottom of each leaf disc.

Two aspects of the number of trichomes on each leaf may be relevant to subsequent insect herbivory. First, an increased density of trichomes may create a less favorable environment for herbivores (Ågren and Schemske 1993). However, the density of trichomes may simply reflect changes in leaf size. For example, on two leaves with an equal number of trichomes, there will be a higher density of trichomes on the smaller leaf. Therefore, I estimated both the density and the total numbers of trichomes on each leaf. Densities are presented as trichomes per square centimeter, and total trichomes were calculated by multiplying trichomes per square centimeter by the area of the leaf. A multivariate analysis of variance (MANOVA) was conducted on leaves at each developmental stage (position) to test for the effects of treatment (induction) on leaf size and trichome density. A separate ANOVA was conducted to detect effects of treatment on total trichome number for each leaf. A sequential Bonferroni correction was used to adjust the $\alpha_{0.05}$ values for the different analyses of different leaf positions (Rice 1989). Total trichome number was not included in the MANOVA because this variable was calculated by multiplying leaf size by trichome density.

Induced resistance to herbivory

Two experiments were conducted with *R. raphanistrum* to test for induced resistance to a generalist noctuid herbivore, *Spodoptera exigua*. *S. exigua* eggs were obtained from the USDA (Stoneville, Mississippi). In both experiments plants were grown in the greenhouse as described above and randomly assigned to induction and control treatments. In these experiments, the fourth true leaf was used as the treatment leaf, and was exposed to *P. rapae* herbivory as described above. In the first experiment, an additional treatment was imposed in which I sprayed each plant liberally with 0.5 mmol/L jasmonic acid (control $n = 9$, caterpillar induced $n = 8$, jasmonic acid $n = 9$). Approximately 1 mL of solution was delivered to each plant. Jasmonic acid is naturally found in plants and can be used as an elicitor to stimulate plant responses to herbivory (Bodnaryk and Rymerson 1994, Doughty et al. 1995, Baldwin 1996, Thaler et al. 1996).

Jasmonic acid (JA) is produced as part of the octadecanoid phytochemical pathway and increases in plants following wounding and natural herbivory (Albrecht et al. 1993, McCloud and Baldwin 1997). Its increase in response to herbivory is thought to be one of the initial stages of the octadecanoid cascade of biochemical events relevant to induced resistance, and this response pathway is highly conserved among plant families (Bergey et al. 1996, Karban and Baldwin 1997). Direct application or consumption of jasmonic acid by herbivores does not affect their performance (Avdiushko et al. 1997; J. S. Thaler, *personal communication*).

Three days after treatment, the newest fully expanded leaf was removed from each plant (in all treatments) with a razor blade and placed in a 90-mm Petri dish with moistened filter paper. A first-instar neonate of *S. exigua* was added to each Petri dish and the dish was sealed. Caterpillars were weighed wet after 48 h and the effects of induction on mass gain were analyzed using a one-way ANOVA. Initial masses were not taken because all caterpillars were freshly hatched neonate larvae, and it was assumed that they started at equal masses. Masses were natural $\log(x + 1)$ transformed to equalize scedasticity. I also performed two preplanned contrasts between (1) control vs. caterpillar-damaged plants, and (2) caterpillar-damaged plants vs. plants sprayed with jasmonic acid.

The second experiment was conducted to test if leaves that expanded after the damage event would still show induced resistance. Plants were grown and subjected to herbivory by *P. rapae* as described above, but the subsequent challenge was made 1 wk after treatment on a newly formed leaf not present at the time of treatment. Second-instar *S. exigua* larvae were caged onto the intact assay leaf using a clip cage and were allowed to feed for 3 d. The assay leaves were at the same developmental stage across treatments. The caterpillars were weighed before and after use to calculate mass gain. Mass gain values were natural $\log(x + 1)$ transformed to equalize scedasticity and compared between control and induced plants using a *t* test.

I conducted several additional experiments with *R. sativus* to characterize induced resistance to herbivory. In the first experiment, plants were grown and assigned to control and induced treatments as above ($n = 23$ control, and $n = 27$ induced plants). Three d after treatment the newest fully expanded leaf was removed from each plant with a razor blade and placed in a 90-mm Petri dish with moistened filter paper. A first-instar neonate of *S. exigua* was added to each Petri dish and it was sealed for 3 d. Caterpillars were weighed and the leaf area consumed was estimated using an acetate grid. In addition, the gross growth efficiency (GGE) was calculated as: mass gained per leaf area consumed (Waldbauer 1968). Because GGE is an estimate of caterpillar growth per unit area of plant consumed, it was used to reflect toxic or antinutritive effects of induction. Effects of induction on these variables were analyzed using MANOVA.

Two choice tests were conducted to test for effects of induced responses on herbivory by more mobile herbivores. In the first experiment, a natural outbreak of leaf-mining flies, *Liriomyza* sp., (Diptera: Agromyzidae) was present in the greenhouse, and 39 plants were introduced (20 control, 19 induced) for 1 wk. The

plants were arranged randomly with respect to treatment over two greenhouse benches. After allowing another week for the flies to oviposit and the eggs to hatch, I counted the number of successfully initiated mines on each plant. The number of mines on control and induced plants was compared using a *t* test. In the second choice test, I used naturally occurring *R. sativus* plants growing in a population near the Orchard Park vicinity of the University of California at Davis campus, and a mobile noctuid caterpillar, *Helicoverpa zea. H. zea* eggs were obtained from the USDA (Stoneville, Mississippi). I randomly assigned 13 of 26 plants to be damaged by a caged *P. rapae* larva, while the other 13 remained as controls. Four days after treatments were imposed, I removed a single undamaged leaf from each of the 26 plants and paired a control leaf with a similarly-sized leaf from an induced plant. Paired leaves were placed in a 90-mm petri dish with moistened filter paper and a third-instar *H. zea.* The percentage of area consumed of each leaf was estimated after 48 h using an acetate grid. A nonparametric sign test was employed to detect effects of induction on the feeding choice of the caterpillars. The leaf with a greater percentage of area consumed was classified as the preferred leaf, and replicates in which an equal amount of leaf area was removed were omitted from the analysis (Zar 1996).

Finally, I conducted a no-choice experiment to test for effects of induced responses to herbivory in *R. sativus* on growth of a specialist herbivore, *P. rapae.* Plants were grown and treated with control and induced treatments as above, and one first-instar neonate caterpillar was placed on leaves in petri dishes ($n = 12$ control, and $n = 7$ induced plants). Mass gain after 3 d was compared using a *t* test.

Induced defense against herbivory

I conducted field experiments over 2 yr to detect fitness consequences of induced responses to herbivory for annual wild radish plants. The experiments were conducted at the Blodgett Forest Research Station, near Georgetown, California, in the Sierra Nevada mountain range (1300 m). The research plot was a plowed, disturbed site where several weedy brassicaceous plants had been growing for several years. In both years of the experiment, cotyledonary plants were transplanted to the field from seeds germinated in greenhouse plug trays. In 1996, only 42 experimental *R. raphanistrum* plants were available after transplant because of mortality from early-season drought. In 1997, 148 *R. sativus* plants were used.

In both years, plants were equally divided into three treatments: induced plants, leaf damage controls, and overall (unmanipulated) controls. The plants were treated just before the time that grasshoppers (the main herbivore at this site) begin to severely damage the plants. I induced plants by caging a caterpillar larva (*P. rapae*) on one leaf at the four-leaf stage and allow-

ing it to consume the entire leaf. Leaf damage controls had one leaf clipped off at the petiole with a scissors. Such clipping resulted in an amount of leaf tissue removed equal to that in the induced treatment, but without the associated induced plant response (Haukioja and Neuvonen 1985, Hartley and Lawton 1987, Mattson and Palmer 1988, Baldwin 1990, Bodnaryk 1992, Mattiacci et al. 1995, Alborn et al. 1997, Agrawal et al. 1999b). Induced plant responses are thought to be minimized by clipping damage because of the absence of herbivore saliva, the greatly reduced area of actual leaf tissue that is damaged, and quick nature of the removal. The leaf damage control treatment was used to factor out effects of leaf removal on herbivores and plant fitness. In other words, leaf removal associated with induction causes changes in plant size, which may be perceived by herbivores, as well as an energetic drain on the plant. Because the induction treatment had the same amount of leaf tissue removal as the leaf damage control treatment (without induction), I was able to detect the independent effects of leaf removal and induction on plant fitness. It should be noted that the pattern of leaf removal in wild radish plants has been shown to affect plant fitness (Mauricio et al. 1993).

During each experiment, plants were censused three times for the percentage of leaf area consumed by naturally occurring herbivores. The primary herbivore at this site was the crackling forest grasshopper, *Trimerotropis suffusa* (A. A. Agrawal, *personal observation*; M. A. Salser, *personal communication*). At the end of the season I counted the total number of fruits and seeds produced by each plant and weighed the total fruit mass, to use as indicators of female plant reproductive fitness. Arcsine square-root transformed percentage data (herbivory) were compared using a two-factor repeated-measures ANOVA, with treatment and trial (year) as the main effects. The repeated measures were the censuses of herbivory on the plants three times in each trial. Fitness measures were analyzed using a two-factor (treatment and trial) MANOVA with fruits, fruit mass, and seed number as the response variables. I also performed two preplanned contrasts on the herbivory and fitness data: (1) overall controls vs. induced plants, and (2) overall controls vs. leaf damage controls.

RESULTS

Induced responses to herbivory

Herbivory to the first true leaf affected the leaf size and density of trichomes on subsequently formed leaves (Table 1, Figs. 1 and 2). The third newly formed leaf was larger on induced plants compared to controls. There was a trend for the third true leaf to have higher densities of trichomes on induced plants compared to controls and a marginal effect indicating the fifth true leaf to be larger on induced plants compared to controls. Leaf size and trichome density on the seventh

TABLE 1. Multivariate analysis of variance (MANOVA) and univariate analyses for effects of induced responses to herbivory on size and trichome density of *Raphanus sativus* leaves.

Variable	Wilks' λ	df	F	P
MANOVA (leaf 3)	0.801	2, 37	4.585	0.017†
Size		1, 38	6.349	0.016
Density		1, 38	2.544	0.119
MANOVA (leaf 5)	0.906	2, 37	1.922	0.161†
Size		1, 38	2.995	0.092
Density		1, 38	0.014	0.907
MANOVA (leaf 7)	0.997	2, 31	0.048	0.953†
Size		1, 32	0.098	0.756
Density		1, 32	0.048	0.828

† Sequential Bonferroni corrected $\alpha_{0.05} = 0.017$ (leaf 3), 0.025 (leaf 5), and 0.05 (leaf 7).

true leaf were not affected by treatments. Induction caused an increase in the total number of trichomes on the third ($F_{1,38} = 7.559$, $P = 0.009$, adjusted $\alpha_{0.05} = 0.017$) and marginally on the fifth ($F_{1,38} = 4.377$, $P = 0.043$, adjusted $\alpha_{0.05} = 0.025$) true leaf, but not the seventh true leaf ($F_{1,32} = 0.024$, $P = 0.878$, adjusted $\alpha_{0.05} = 0.05$).

Induced resistance to herbivory

Caterpillar herbivory or exposure to the natural plant hormone jasmonic acid induced resistance in *R. raphanistrum* plants when I assayed growth of *S. exigua,* (Fig. 3, experiment 1: $F_{2,23} = 29.743$, $P < 0.001$; contrast: control vs. caterpillar damaged: $F_{1,23} = 20.176$, $P < 0.001$). The jasmonic acid dose used (0.5 mmol/L) induced greater resistance than did herbivory to the fourth true leaf (Fig. 3, contrast: caterpillar damaged vs. jasmonic acid: $F_{1,23} = 8.650$, $P = 0.007$). Induced resistance persisted in newly formed leaves of damaged plants, reducing the mass of caterpillars feeding on induced plants compared to uninduced controls (Fig. 3, experiment 2; $t = 5.194$, df $= 14.8$, $P < 0.001$).

Leaf herbivory to *R. sativus* increased resistance to generalist *S. exigua* larvae (Fig. 4, Table 2). Induction appeared primarily to reduce the amount of leaf area consumed (and subsequent gain in mass), but did not affect gross growth efficiency (Table 2). I did not detect any effect of induced responses on the growth of specialist *P. rapae* larvae (mean ± SE: controls $= 0.867 ± 0.168$ g; induced $= 1.086 ± 0.352$ g; $t = 0.635$, df $= 17$, $P = 0.534$).

In choice experiments, induction reduced the number of generalist leaf mines successfully initiated by agromizid flies (Fig. 5a, $t = 2.232$, df $= 37$, $P = 0.032$). When a mobile generalist caterpillar, *H. zea,* was offered paired leaves from control and induced *R. sativus* plants, the caterpillars preferred control leaves in 8 out of 9 replicates where there was a difference in the percent leaf area consumed (Fig. 5b, $P = 0.039$, sign test). Overall, caterpillars fed nearly twice as much on leaves
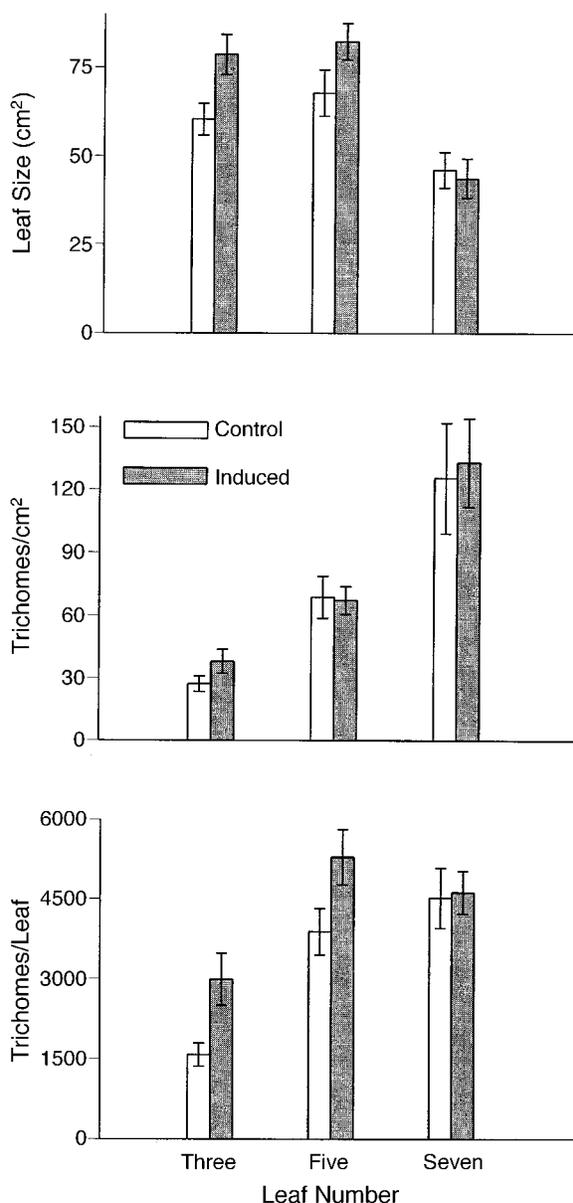

FIG. 2. The effect of herbivory to the first true leaf of *Raphanus sativus* on leaf size, density, and total number of trichomes on leaves produced subsequently to damage. Bars are means ± 1 SE.

from control plants ($70.6 ± 6.9\%$) compared to leaves from induced plants ($35.4 ± 6.2\%$).

Induced defense against herbivory

In field experiments, induced responses to early-season caterpillar herbivory decreased subsequent herbivory by grasshoppers in both years of study (Fig. 6, Table 3, MANOVA contrast: control vs. induced: Wilks' λ $= 0.923$, df $= 3, 142$, $F = 3.953$, $P = 0.010$). Experimental clipping damage with a scissors removed an equal amount of leaf area as the induction treatment,
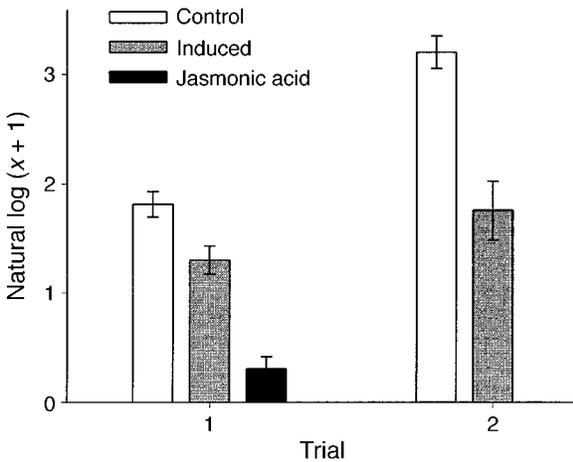
FIG. 3. The effect of leaf herbivory (induction) and application of jasmonic acid to *Raphanus raphanistrum* on larval growth (*x*) of the generalist caterpillar, *Spodoptera exigua*. In experiment 1, the bioassay was conducted on the opposite leaf to the one treated; in experiment 2, a newly formed leaf was bioassayed. Bars are means ± 1 SE. Original data were in milligrams.

but did not affect the amount of subsequent herbivory experienced by the plants (Fig. 6, Table 3, MANOVA contrast: overall control vs. leaf damage control: Wilks' $\lambda = 0.983$, df = 3, 142, $F = 0.808$, $P = 0.492$). These effects of induced responses on herbivory resulted in lifetime fitness differences between plants. Induced plants outperformed overall controls and leaf damage controls in both years of this study (Fig. 7, Table 4). Results for univariate analyses were consistent with the MANOVA for each of the fitness components measured: fruit number, fruit mass, and seed number ($P < 0.015$ in all cases).

DISCUSSION

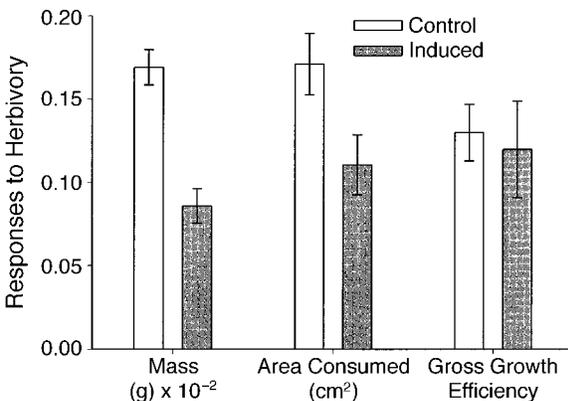Foliar herbivory in wild radish plants resulted in induced biochemical and physical responses, increased

FIG. 4. The effect of induced responses to herbivory of *Raphanus sativus* on mass gain, leaf area consumed, and gross growth efficiency (mass gain per area consumed) of *Spodoptera exigua*. Bars are means ± 1 SE.

TABLE 2. Multivariate analysis of variance (MANOVA) and univariate analyses for effects of induced responses to herbivory on gain in mass, leaf area consumed, and gross growth efficiency (GGE) of *Spodoptera exigua* on *Raphanus sativus*.

Source	Wilks' λ	df	MS	F	P
MANOVA	0.591	3, 46		10.591	<0.001
Mass gain		1	8.598	31.259	<0.001
Error		48	0.275		
Area consumed		1	453.636	5.461	0.024
Error		48	83.062		
GGE		1	0.001	0.077	0.783
Error		48	0.015		

resistance to several herbivores, and increased seed set compared to uninduced control plants. Investigators rarely examine plant responses to herbivory at these three scales. Measures of the changes in the plant following herbivory provide information about potential mechanisms responsible for resistance to herbivores and are the most common measure of induction. Demonstrations of induced resistance are less common, and are necessary to infer the mode of the subsequent effects on plant performance. Effects of induction on plant fitness are almost completely lacking (Karban 1986, 1993, Brown 1988, Baldwin et al. 1990, Karban
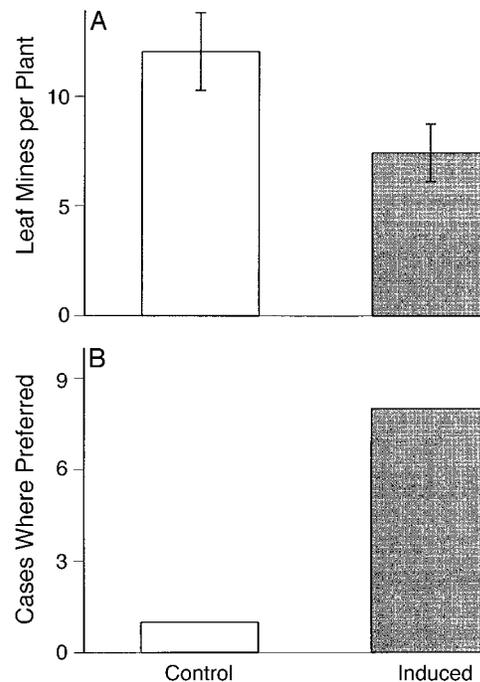

FIG. 5. The effect of induced responses to herbivory of *Raphanus sativus* on choice in two herbivores. (A) The number of successfully initiated agromizid fly leaf mines on control and induced plants. (B) The number of replicates where *Helicoverpa zea* preferred to feed on control leaves or induced leaves when offered a paired control and induced leaf. There was no difference in four replicates. Bars are means ± 1 SE.
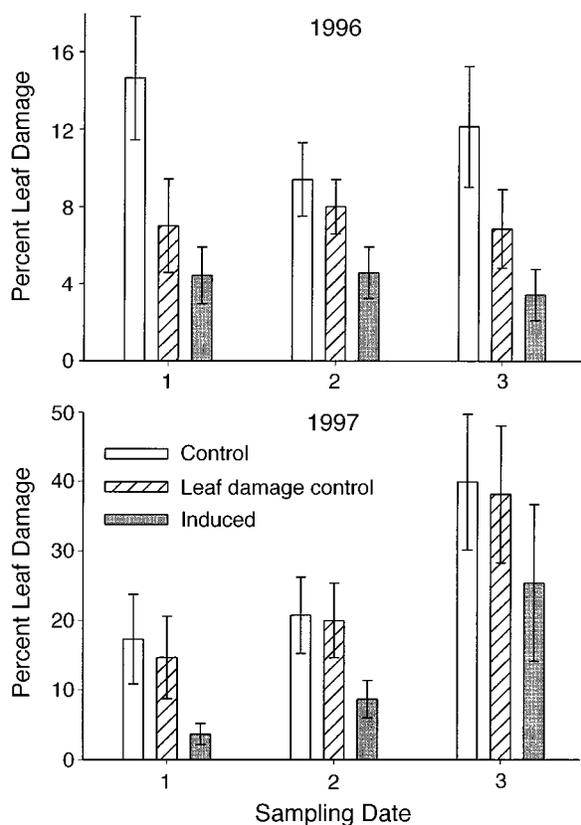
FIG. 6. The effect of induced responses to herbivory of *Raphanus raphanistrum* (1996) and *R. sativus* (1997) on herbivory by naturally occurring grasshoppers (*Trimerotropis suffusa*) on three sampling dates (separated by 2 wk each). Bars are means ± 1 SE.
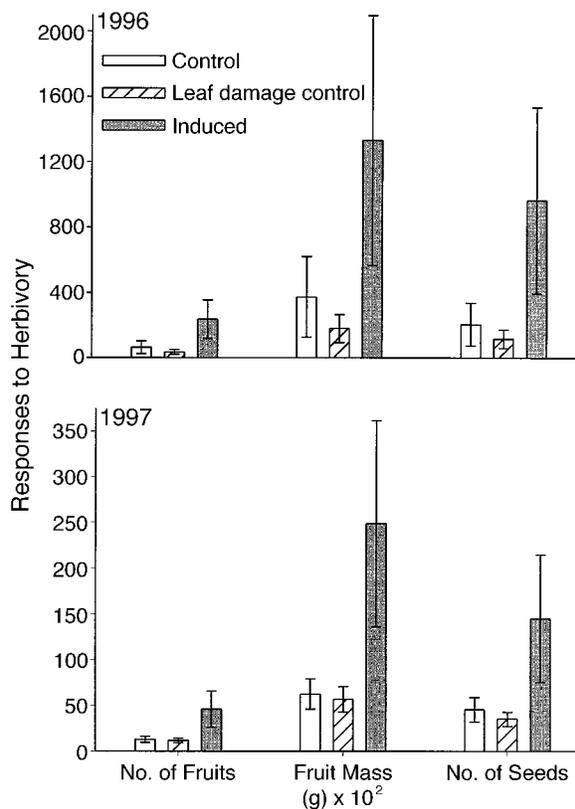

FIG. 7. The net effect of induced responses to herbivory of *Raphanus raphanistrum* (1996) and *R. sativus* (1997) on lifetime fitness components in an environment with naturally occurring herbivores. Bars are means ± 1 SE.

and Baldwin 1997, Baldwin 1998). The lack of evidence for fitness benefits of induced responses is particularly surprising because such demonstrations are needed to consider induced responses as defensive. The current study is one of the few demonstrations of such fitness benefits (Agrawal 1998b, Baldwin 1998).

Induced responses that take the form of increases in physical defenses have been reported much less often than biochemical responses (see McNaughton and Tarrants 1983, Young 1987, Baur et al. 1991, Myers and

Bazely 1991, Gowda 1997, Young and Okello 1998; A.A. Agrawal, *unpublished manuscript*). Herbivory to the first true leaf of wild radish induced an increase in the densities of trichomes on the third true leaf. Such a response may potentially be mediated by a change in leaf size (i.e., smaller leaves may have higher densities of trichomes). For example, Obeso (1997) showed that browsed branches of holly trees produced leaves with increased spinescence. However, this effect was largely due to a decrease in the size of leaves on browsed branches. In the current study, although tri-

TABLE 3. Repeated-measures ANOVA for the effects of induced responses on subsequent herbivory on wild radish in field plots. Probabilities corrected for sphericity are provided using the Greenhouse-Geisser correction (G-G).

Variable	df	MS	F	P	G-G
Treatment	2	2306.593	5.977	0.003	
Trial	1	11 294.178	29.267	<0.001	
Treatment × trial	2	70.295	0.182	0.834	
Error	144	385.907			
Date	2	2915.096	16.116	<0.001	<0.001
Date × treatment	4	30.824	0.170	0.916	0.922
Date × trial	2	3608.894	19.952	<0.001	<0.001
Date × treatment × trial	4	23.058	0.127	0.943	0.949
Error	288	180.880			

TABLE 4. Multivariate analysis of variance (MANOVA) for the effects of induced responses to herbivory on plant fitness of wild radish. Fitness components measured were fruit number, fruit mass, and total seeds produced (see Fig. 7).

Source	Wilks' λ	df	F	P
Treatment	0.854	6, 264	3.607	0.002
Trial	0.894	3, 132	5.236	0.002
Treatment × Trial	0.890	6, 264	2.651	0.016
Treatment contrast: control vs. induced	0.897	3, 132	5.045	0.002
Treatment contrast: control vs. clipped	0.992	3, 132	0.356	0.785

chome densities increased, the mean size of the third true leaf of damaged plants was also larger than that of undamaged plants. Thus, although an increase in leaf size could have reduced the density of trichomes due to a "dilution effect," it did not, because of an overall increase in trichome production. For the fifth true leaf, the densities of trichomes were the same for damaged and control plants. However, total numbers of trichomes were marginally increased on damaged plants, indicating that induced plants produced larger leaves. It is not clear why induced plants produced larger leaves, although this may be a compensatory response to the herbivory and loss of photosynthetic area (Strauss and Agrawal 1999).

Although others have shown that densities of setose trichomes can reduce herbivory (Baur et al. 1991, Ågren and Schemske 1993, Fernandes 1994), I did not specifically test this hypothesis in my study. However, I did show that leaf herbivory resulted in decreased herbivore growth on undamaged leaves of damaged plants, which was correlated with an increase in trichomes. I detected induced resistance on leaves that were present at the time of initial damage and on leaves that were produced following damage. Because leaves present at the time of damage could not induce trichomes, this result indicates that the observed induced resistance was not entirely due to induction of trichomes, and probably was related to phytochemical induction. Induced resistance affected the leaf area consumed, and hence the growth of larvae, but did not have a direct negative impact on gross growth efficiency, an indicator of growth per unit of leaf area consumed (Waldbauer 1968). In other words, induced resistance did not appear to be due to poisonous or antinutritive effects, but rather due to deterrent effects. In choice experiments, leaf-mining flies and noctuid larvae preferred uninduced control plants over induced plants. Therefore, induced responses in wild radish affect a diverse array of herbivores including noctuids, grasshoppers, and leaf-mining flies, as well as aphids, flea beetles, and earwigs (Agrawal 1998b). Although further studies are needed, induced responses to herbivory in wild radish do not appear to affect specialist herbivores, such as larvae of *P. rapae*.

Induced responses to herbivory may increase plant fitness via a variety of mechanisms (Agrawal and Karban 1999). First, induction may directly reduce preference or performance of herbivores. Induction of de-

fenses against herbivores is also often accompanied by induction of volatiles that attract predaceous or parasitic arthropods to the plant (Pare and Tumlinson 1996, Takabayashi and Dicke 1996, Shimoda et al. 1997, De Moraes et al. 1998). In addition, a reduction in food quality also may cause omnivores to reduce plant feeding and increase predation on herbivores of the plant (Agrawal et al. 1999a). Two benefits of induction that have been less well explored are (1) increased competitive ability of induced plants against uninduced neighboring plants (e.g., Sadras 1997), and (2) increased tolerance to subsequent herbivory of induced plants compared to controls (e.g., Wittmann and Schoenbeck 1996).

Importance of studying plant defense

The interactions between herbivores and plants have long been viewed as antagonistic, with each participant having the ability to affect selection on the other (Dethier 1954, Painter 1958, Fraenkel 1959, Ehrlich and Raven 1964, Fritz and Simms 1992, but see McNaughton et al. 1997). In particular, if herbivores decrease plant fitness and plants possess genetic variation for traits that reduce herbivory and increase plant fitness, then herbivores may select for more resistant plants. Evolutionary ecologists have recognized this, and there have been many recent studies documenting the fitness consequences of herbivory for plants (reviewed by Marquis 1992, Bigger and Marvier 1998). However, fewer studies have shown that variation in plant traits that affect herbivores also have fitness consequences for the plant. Recent quantitative genetic studies with short-lived plants provide the best evidence that plant resistance traits have important consequences for plant fitness (Berenbaum et al. 1986, Simms and Rausher 1987, 1989, Mauricio and Rausher 1997). For example, Simms and Rausher (1989) found that morning glory plants exhibited genetic variation for resistance to corn earworms, which decreased plant fitness. These herbivores were found to impose natural selection for increased resistance. Thus, although rarely demonstrated empirically, resistance traits can have positive effects on plant fitness by reducing herbivory.

Induced resistance in plants has been largely studied from the herbivore's perspective. Plant resistance mechanisms may benefit plants through a variety of mechanisms, and demonstrating effects on plant fitness is key to documenting that they truly serve a defensive

function. For wild radish, induced responses to herbivory are correlated with a net fitness benefit in environments with herbivory. This effect on fitness has two important implications. First, inducible resistance appears to be an example of adaptive plasticity in plants. Adaptive plasticity is defined as the higher relative fitness individuals have when they express one phenotype rather than another in a particular environment (Bradshaw 1965, Thompson 1991, Gotthard and Nylin 1995, Fox et al. 1997). For induced resistance in wild radish, not only is the induced phenotype associated with higher relative fitness in environments with herbivory (Agrawal 1998b), but the induced phenotype is associated with reduced fitness in environments lacking herbivory (i.e., fitness cost of expressing the ''wrong'' phenotype) (Agrawal et al. 1999b). These results demonstrating benefits and costs of induction are important because phenotypic plasticity is thought to evolve as a mechanism for organisms to express adaptive phenotypes in variable environments. Few studies have documented adaptive plasticity (reviewed in Dudley and Schmitt 1996).

The second implication of this study concerns the evolution of inducible defenses against herbivory, and of phenotypically plastic traits in general. In order for such traits to evolve by natural selection, there must be heritable variation that affects fitness. I did not attempt to detect genetic variation for induction. In a previous study, however, I detected a significant family by treatment interaction for effects of induction in wild radish on resistance to aphids ($F_{24, 852} = 1.555$, $P = 0.044$, data from Agrawal 1998b), indicating that there may be genetic variation for induction. Similarly, a significant family by induction interaction was detected for seed production of wild radish plants, again indicating that there may be genetic variation for induction or its effects on fitness (Agrawal et al. 1999b). Few other studies have detected genetic variation for induction. Studies by Zangerl and Berenbaum (1990) and van Dam and Vreiling (1994) detected genetic variation for chemical induction, although these studies did not characterize variation in induced resistance to herbivores. No study to date has asked whether genetic variation for induction is associated with differential fitness. If, however, the observed phenotypic correlations between induction and fitness (Agrawal 1998b, Agrawal et al. 1999b; A. A. Agrawal, *unpublished manuscript*) are reasonable estimates of their genetic counterparts, as is the case in many situations (Cheverud 1988), then induction may have evolved as a defense strategy because of benefits in the presence of herbivores and cost savings in the absence of herbivores. Given that there is likely to be genetic variation for induced resistance, future studies that can detect such variation and determine its fitness consequences will be important for directly measuring natural selection on inducible plant defenses.

The current study provides evidence for fitness benefits of induction in environments with herbivory, demonstrating that induced responses can truly serve a defensive function. Inducible defenses are an example of adaptive plasticity because the induced phenotype realizes the higher fitness in an environment with strong herbivory, whereas the uninduced phenotype realizes the higher fitness in an environment with low herbivory.

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