INDUCED RESISTANCE IN Ipomoea purpurea

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Abstract. A series of experiments indicate that induced changes occur in the foliage of the annual morning glory Ipomoea purpurea when subjected to prior damage. These changes reduce the growth rate, consumption rate, and growth efficiency of larvae of the generalist lepidopteran Spodoptera eridania. They also alter the pattern of damage caused by specialist flea beetles, Chaetocnema confinisi, and by generalist insect herbivores in the field. No effects of induction were detected on specialist tortoise beetles, Deloyaala guttata and Metria abcolor. Although total damage by both flea beetles and generalist insects did not differ between induced and noninduced plants, this result does not necessarily imply that the effects on these insects were nonadaptive. The observed effects of induced response are consistent with both “diffuse” and “pairwise” views of the selection imposed by herbivores on plant resistance. Finally, the existence of induced responses suggests that previous conclusions by Simms and Rausher that resistance to flea beetles and generalist insects is not costly in I. purpurea may be premature.

Key words: Chaetocnema; costs of resistance; Deloyaala; diffuse selection; herbivory; induced resistance; inducible defenses; Ipomoea; pairwise selection; plant defenses.

INTRODUCTION

Over the past 15 yr, a large number of studies have indicated that many plants, when subjected to damage by herbivores, undergo physiological changes in foliage and other tissues (see review by Karban and Myers 1989). Because these “induced responses” often reduce the quality of foliage as a substrate for herbivore growth, survival, and/or reproduction, it has been suggested that these changes are adaptive responses that have evolved to reduce the detrimental impact of herbivores on plant fitness (Rhoades 1983, Haukojoa and Neuvonen 1985, Baldwin 1988a, Tallamy and Krishik 1989, Zangerl 1990, Krishik et al. 1991). As pointed out by Fowler and Lawton (1985), however, this suggestion can be valid only if induced responses actually reduce the amount of damage experienced by a plant, and there is presently little evidence that induced changes are protective in this fashion (but see Karban 1986). One objective of the investigation reported here was thus to determine, for one insect–plant system, whether induced changes occur and whether they confer this type of resistance.

A second objective was to determine whether induced changes influence different herbivores in different ways or to different degrees. Krishik et al. (1991) have argued that most induced responses are broadband, generalized defenses that are effective against most herbivores and pathogens. If correct, this suggestion would imply that the evolution of induced responses would be influenced by “diffuse” selection pressures (sensu Janzen 1980, Fox 1981) imposed jointly by a variety of herbivore and pathogen species. By contrast, limited genetic evidence (Rausher 1992) suggests that a plant species may often possess several distinct resistance mechanisms that are specific to different enemies, and there is no reason to believe induced resistance mechanisms should not have similar characteristics. This evidence would imply that evolution of induced responses would tend to be pairwise (=“stepwise” sensu Fox 1981), rather than diffuse.

These two views can be distinguished because they make different predictions. The “diffuse” view predicts that when herbivores are found that are not affected by an induced response, they should primarily be specialists because for specialists, adaptations that overcome induced defenses are more likely to be a prerequisite for continued association with their host, and thus for continued existence (for a similar argument regarding constitutive defenses, see Feeny 1976, Rhoades and Cates 1976). By contrast, the “pairwise” view predicts that generalists should often be unaffected by induced responses because many such responses are directed specifically at other (specialist) herbivores. Unfortunately, for most systems in which induced changes have been identified, their effects have been assayed on only one or a small number of herbivores. There is thus very little current evidence that allows an evaluation of these two views.

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glory *Ipomoea purpurea*. Although the cost of resistance is a central parameter in many models of the evolution of resistance to herbivores (Lomnicki 1974, Feeny 1976, Rhoaodes and Cates 1976, Rhoaodes 1979, Krischik and Denno 1983, Coley et al. 1985, Fagerström 1989), Simms and Rausher (1987, 1989) detected no costs of resistance in *I. purpurea* to several different insect herbivores. One possible problem with Simms and Rausher's studies is that the method used to detect costs may not reveal them if resistance is inducible. In particular, Simms and Rausher inferred the resistance genotype of plants from which insects were eliminated (by spraying) from levels of damage incurred by their unsprayed half-sibs. As long as resistance is constitutive, the genetic covariance of fitness of the sprayed plants with damage to the unsprayed plants accurately reflects the genetic relationship between those two traits and hence measures the cost of resistance. However, if resistance is inducible, all genotypes in the sprayed treatment would remain uninduced, allocate no resources to resistance, and hence experience no cost of resistance. The measured genetic covariance would then be zero, indicating an apparent absence of a cost of resistance, even though resistance might be costly when it is induced and might be genetically variable under normal conditions when herbivores are present to induce resistance. In the experiments reported here, failure to detect induction would support Simms and Rausher's contention that allocation to resistance incurs little or no cost in *I. purpurea*. By contrast, a demonstration that induction occurs would call the validity of that contention into question.

**Materials and Methods**

**Organisms and study site**

*Ipomoea purpurea* (Convolvulaceae) is a climbing annual vine that is commonly found in disturbed sites such as agricultural fields and roadsides. It is fed upon by three types of folivores, whose feeding can be distinguished by the distinctive form of the damage each causes (see Rausher and Simms 1989 for details): (1) adult flea beetles, *Chaetocnema confinis* (Chrysomelidae); (2) adult and larval tortoise beetles, *DeloyaLal guttata* and *Metriona bicolor* (both Chrysomelidae); and (3) miscellaneous generalist folivores, primarily orthopterans.

If the genetic variation for resistance in *Ipomoea purpurea* observed by Simms and Rausher (1987, 1989) is due primarily to genetic differences in the amount by which induction increases resistance, the difference in level of resistance between the induced and uninduced states should be greatest for the most resistant genotypes. Consequently, to enhance our chances of detecting induction of resistance, we used as our experimental plants the progeny of genotypes that exhibited high levels of resistance to all three types of herbivores in one of our previous experiments (Simms and Rausher 1989). Specifically, the plants used in all experiments described below were the natural seed progeny of the plants in one full-sib family in our previous experiment. This family ranked 3rd, 13th, and 33rd out of 140 families for resistance to flea beetles, tortoise beetles, and generalist folivores, respectively.

The study site was an old agricultural field in Durham County, North Carolina, that had last been planted in soybeans in 1983. This field was the site of earlier experiments reported by Simms and Rausher (1987, 1989).

**Experiments—general design**

We report the results from six separate experiments. The general design of each experiment was similar: plants were subject to experimentally imposed damage, induction was allowed to occur for a specified period of time, and then changes in the quality of the foliage for insect growth or for susceptibility to damage were assayed. Because the time necessary for induction and subsequent decay of induced response varies greatly among plant species (e.g., Wratten et al. 1984, Haukojoa and Neuvonen 1985, Tallamy 1985, Zangerl 1990), but we had no prior information about these times for any induced changes that might occur in *I. purpurea*, we used different periods between application of experimental damage and assay of induction in different experiments. In particular, in three of the experiments, the period allowed for induction was 10–13 d (long-period experiments), while in three others (short-period experiments), the period allowed for induction was 1–6 d. Induction periods of either length are consistent with the results of Simms and Rausher (1987, 1989). Details specific to individual experiments are described below.

**Long-period experiments**

*Experiment 1A—tortoise beetle preference experiment.*—The purpose of this experiment was to determine whether prior damage reduces subsequent feeding damage by adult tortoise beetles. Ninety-four *I. purpurea* plants were grown from seed in 10 cm diameter pots containing Duke University Department of Botany research mix. The plants were paired randomly and each plant in a pair was assigned randomly to one of two treatments: (1) control plants, on which there was no experimentally produced damage, and hence presumably no induction; and (2) induced plants, on which tortoise beetle larvae were placed to induce resistance. In the damaged treatment, one last-instar *DeloyaLal guttata* larva was placed on each of three leaves, allowed to feed for 48 h, then removed. Plants were then left in a greenhouse for 12 d to permit induction of resistance. After this induction phase, the pairs of plants were placed in 0.6 m tall conical cages made of insect screening and set outside in an experimental garden. Three adult *DeloyaLal guttata* were introduced into each cage and allowed to feed for 48 h.
Feeding damage inflicted during this postinduction phase was quantified by placing a clear plastic grid with 0.59-cm² squares over each leaf; counting the number of squares covering the leaf and the number covering damage provided estimates of total leaf area and damage, respectively. A census of induced plants during the induction phase permitted total damage on these plants to be corrected for the amount of experimentally induced damage. Amount of damage in the two treatments was compared statistically using a Wilcoxon matched-pairs signed-ranks test (Siegel 1956).

Experiment IB—tortoise beetle larval growth experiment.—The purpose of this experiment was to determine whether prior damage induces resistance factors that reduce the growth rate or maximum size of tortoise beetle larvae. Plants were germinated, grown in pots as described for Experiment IA, and randomly assigned to three treatments: (1) control plants, which were not subjected to experimentally produced damage, and hence in which there was presumably no induction; (2) mechanically induced plants, which were damaged mechanically with a cork borer; and (3) biologically induced plants, which were damaged experimentally with Deloyala guttata larvae as described for treatment (2) of the preceding experiment. After beetle larvae were removed from the biologically induced plants, all plants were left in a greenhouse for 12 d without damage to allow induction to occur. One newly hatched D. guttata larva was then placed on a damaged leaf on each plant and allowed to grow just prior to pupation. These larvae were obtained from a mass culture and allocated randomly to the three treatments. After larvae had voided their gut contents in preparation for pupation, they were removed from the plants and placed individually in petri dishes until pupation occurred. Larvae were checked twice daily for gut voidance, and the time and date of removal and pupation were recorded. Once larvae pupated they were weighed to the nearest 0.1 mg. Adults were sexed after eclosion.

Pupal mass and time to pupation were analyzed using a standard two-way analysis of variance (Searle 1971). The main effects were treatment and sex. Analyses were performed using the SAS statistical package (SAS 1982). Type III sums of squares are reported.

Experiment IC—field experiment.—The purpose of this experiment was to detect whether induction of resistance occurs in I. purpurea under field conditions similar to those of our earlier experiment (Simms and Rausher 1987, 1989). The experiment consisted of the same three treatments as in Experiment IB: (1) control, (2) mechanically induced, and (3) biologically induced. Three hundred seeds were germinated in a greenhouse in root trainers (i.e., soil plugs that can be inserted in a hole in the field without disturbing the roots of a plant growing in the plug) and transplanted to the field site when they had produced one true leaf. Because the soil plug containing the roots was inserted into the ground intact, little disturbance to roots occurred. The seedlings were planted in a 10 × 30 array, with rows and columns of the array spaced 1 m apart, and randomly assigned to the three treatments. All plants were sprayed with carbaryl (Sevin XLR) every 3 d for 18 d. On day 6, we removed an average of 14.5% of the foliage from each of three leaves on the plants in the mechanically induced treatment (equivalent to removal of an average of 7.5% of total foliage). Also on day 6, we placed one last-instar larva on each of three leaves of each plant in the biologically induced treatment. These leaves were shielded from spraying on day 6 and also had residual insecticide washed off with water to prevent poisoning of the larvae. Plants in the other treatments were given an equal amount of water. The larvae were allowed to feed for 48 h and removed an average of 17.6% of the foliage from each leaf (equivalent to removal of an average of 9.4% of the total foliage). These amounts of damage were greater than or comparable to the total amounts accumulated in Simms and Rausher's (1987, 1989) experiments by plants in the family used in this experiment (mean damages of 15.0, 0.7, and 1.2% of area for flea beetles, tortoise beetles, and generalist folivores, respectively), and thus should have been sufficient to trigger induction in this experiment if it had occurred previously.

On day 19 spraying was discontinued for all treatments and residual insecticide was washed off the plants with water. Plants in the mechanically and biologically induced treatments were thus given a period of 10–13 d for induction of resistance to occur. All plants were then permitted to accumulate damage from native insect herbivores for 11 d.

All plants were censused for damage on day 9 (at the beginning of the induction phase) and on day 29 (at the end of the experiment) as described in Experiment IA. Mechanical damage and the three separate categories of insect damage described above were recorded.

We examined damage both to old leaves (leaves on the plant at the time of experimentally imposed damage) and new leaves (leaves produced subsequent to experimentally imposed damage). To calculate proportion damage, we first determined total damage (by insect category) and total leaf area per leaf-age category by summing damage and leaf area from the day 29 census over all leaves of that category. We then divided total damage by total leaf area to obtain proportion damage. Because some beetle damage to old leaves in the biologically induced treatment on day 29 was caused by beetle larvae placed on the plants to induce resistance, the amount of this pre-induction damage (as measured on day 9) was subtracted from the total beetle damage on day 29 to yield an accurate estimate of damage sustained during the damage accumulation phase of the experiment. Before subtraction, the experimentally produced damage was multiplied by 1.424 to compensate for expansion of the area of damage as a leaf grew during the experiment. This correction factor was obtained by measuring the increase in the size
of mechanically produced holes from the day 9 census to the day 29 census. Since no additional mechanical damage was added after the beginning of the induction phase of the experiment, the average ratio of mechanical damage on day 29 to mechanical damage on day 9 represents the factor by which a hole expanded between the two censuses. We assume that holes produced experimentally by beetle larvae expand by the same factor.

Proportion damage for the three treatments was compared by a Kruskal-Wallis one-way analysis of variance by ranks (Siegel 1956). The analysis was performed using the Statgraphics statistical software (Statistical Graphics Corporation 1985).

Short-period experiments

Experiment IIA—tortoise beetle preference experiment. —This experiment was similar to Experiment IA, except that the two different short induction periods were used: 3 and 6 d. For each of these periods, 48 pairs of control and induced plants were used. Damage was quantified by photographing leaves, projecting the negatives onto a digitizing pad, and digitizing leaf and damage perimeters.

Experiment IIB—tortoise beetle larval growth experiment. —This experiment was similar to Experiment IB, except that the induction period was 2 d. In addition, this experiment was blocked over time: each of 17 temporal blocks (begun over a period of 20 d) consisted of 4–7 control plants and an equal number of plants with damage experimentally imposed on the lowest leaf by tortoise beetle larvae. Induced responses were assayed by placing two nearly hatched larvae on the lowest leaf of plants in each treatment. Larvae were allowed to grow for 7 d, at which time they were removed and weighed. In some blocks, a sufficient number of larvae were available to assay larval growth on the neighboring (upper) leaf. This allowed us to determine whether any induced changes that occurred were confined to the experimentally damaged leaf or affected neighboring leaves. For analysis, larval masses were averaged for larvae on the same leaf.

Experiment IIC—armyworm growth experiment. —A short-period field experiment similar to Experiment IC was not feasible. Since the purpose of such an experiment would be to assay the effects of induction that take place over 1 or 2 d, the period of exposure of induced and control plants to natural damage could not be longer than this; otherwise, control plants would presumably also become induced. Yet the amount of damage that would accumulate over 2 d would be too small to allow any meaningful comparison between treatments. Consequently, instead of a field experiment, we attempted to determine whether prior damage induces changes in foliage quality that affect a generalist herbivore, Spodoptera eridania, the southern armyworm, which has been used as a representative generalist bioassay agent by previous workers (Rehr et al. 1973, Berenbaum 1978, Blau et al. 1978).

The design of this experiment was similar to that of Experiments IB and IIB. Fifty plants in each of four temporal blocks were allocated in equal numbers to control and induced treatments in a greenhouse. In the induced treatment, initial damage was imposed by allowing a third- or fourth-instar armyworm larva to feed on the lowest leaf for 24 h. After a 2-d period to allow for induction to occur, a third- or fourth-instar larva was placed on the lowest leaf and on the neighboring leaf of plants in both treatments. These bioassay larvae were starved for 3 h to void their guts, weighed prior to placement, then confined on a leaf in a gauze bag for 24 h. After this period, they were removed, starved for 3 h, and reweighed.

Three indices of growth performance were calculated for each larva (see Waldhauser 1968, Rausher 1982): (1) relative growth rate (RGR); (mass increase)/(initial larval mass); (2) relative consumption rate (RCR); (leaf area eaten)/(initial larval mass); and (3) gross growth efficiency (EG); (mass increase)/(leaf area eaten).

Leaf area eaten was estimated by subtracting final leaf area from original leaf area, correcting the latter for preliminary damage in the induced treatment. For statistical analysis, an individual plant was treated as the basic unit of analysis. For each plant, the analysis of a particular growth index involved a vector of two response variables corresponding to the value of the index for the larvae on the upper and lower leaves. Multivariate analysis of variance (MANOVA) was used to determine whether overall this vector differed among treatments and blocks. If a significant multivariate effect was observed, upper and lower leaves were analyzed separately to determine which contributed to the overall significant variation. Initial masses of larvae were included as covariates in the analyses.

RESULTS

Long-period experiments

Experiment IA—tortoise beetle preference experiment. —Plants subjected to prior larval feeding damage experienced slightly less subsequent damage by adult beetles (proportion foliage removed = 0.0248 ± 0.0117 [mean ± 1 se]) than undamaged controls (proportion foliage removed = 0.0305 ± 0.0152). This result is not statistically different from that expected under the null hypothesis of no difference in susceptibility between treatments (Wilcoxon test statistic = −0.267, P < .8, ns). The results of this experiment thus provide no evidence that prior damage induces resistance to feeding by tortoise beetle adults.

Experiment IB—tortoise beetle larval growth experiment. —This experiment was designed to determine whether prior damage leads to changes in plant quality that affect the growth rate and final size of developing tortoise beetles. No such effects were detected. For both
Table 1. Growth of tortoise beetle (Deloyala guttata) larvae on plants that had been previously damaged (Experiment IB). (A) Means ± 1 se by treatment and sex. (B) Analysis of variance.

<table>
<thead>
<tr>
<th>A) Larval growth</th>
<th>Days to pupation</th>
<th>Pupal mass (mg)</th>
<th>Growth rate (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Control</td>
<td>14.80</td>
<td>14.33</td>
<td>10.03</td>
</tr>
<tr>
<td>±0.41</td>
<td>±0.59</td>
<td>±0.45</td>
<td>±0.20</td>
</tr>
<tr>
<td>Biologically induced</td>
<td>14.54</td>
<td>14.64</td>
<td>10.41</td>
</tr>
<tr>
<td>±0.50</td>
<td>±0.39</td>
<td>±0.39</td>
<td>±0.29</td>
</tr>
<tr>
<td>Mechanically induced</td>
<td>14.57</td>
<td>14.86</td>
<td>10.53</td>
</tr>
<tr>
<td>±0.59</td>
<td>±0.35</td>
<td>±0.25</td>
<td>±0.17</td>
</tr>
</tbody>
</table>

B) Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Days to pupation</th>
<th>Pupal mass</th>
<th>Growth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>df</td>
<td>ss</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.401</td>
<td>0.06</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.015</td>
<td>0.00</td>
</tr>
<tr>
<td>Treatment × sex</td>
<td>2</td>
<td>2.386</td>
<td>0.36</td>
</tr>
<tr>
<td>Error</td>
<td>88</td>
<td>294.3</td>
<td>113.3</td>
</tr>
</tbody>
</table>

sexes there was a trend toward lower pupal mass in the control treatment than in the induced treatments (Table 1). Under the assumption that damage is proportional to pupal mass, this trend suggests that prior damage may actually increase subsequent damage slightly. However, this trend was not statistically different from the null hypothesis of no effect of prior damage on pupal mass. Days to pupation also did not differ statistically among treatments (Table 1). Finally, growth rate, estimated by the ratio (pupal mass)/(days to pupation) exhibited a slight trend suggesting slower growth in the control treatment (Table 1), the opposite of what is expected if prior damage induces a growth inhibitor. Like the other trends, however, this one was not statistically significant. None of the interactions between treatment and sex for these variables were significant, though females exhibited significantly greater pupal mass and more rapid growth rates than males (Table 1).

Experiment IC—field experiment. The purpose of this experiment was to determine whether prior damage reduces subsequent susceptibility of Ipomoea purpurea under field conditions. For none of the three types of herbivores examined did we obtain evidence of such an effect. When total foliage (old and new leaves) is considered, plants subject to prior biological and mechanical damage (induced treatments) actually exhibited a tendency toward greater susceptibility to tortoise beetle and flea beetle damage compared to controls that experienced no prior damage (Table 2). Damage by generalist insects was slightly lower in the biologically induced treatment, and slightly higher in

Table 2. Percent leaf area damaged by native herbivores in the field when plants had previously been damaged (Experiment IC). Data are means ± 1 se. Test statistic is value of Kruskal-Wallis test statistic and P is its probability under the null hypothesis of no difference among treatments.

<table>
<thead>
<tr>
<th>Damage type</th>
<th>Foliage</th>
<th>Control</th>
<th>Biologically induced</th>
<th>Mechanically induced</th>
<th>Test statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tortoise beetle</td>
<td>Old</td>
<td>0.173 ± 0.140</td>
<td>0.000 ± 0.275</td>
<td>0.201 ± 0.170</td>
<td>0.345</td>
<td>&gt;.84</td>
</tr>
<tr>
<td></td>
<td>New</td>
<td>1.01 ± 0.16</td>
<td>0.760 ± 0.101</td>
<td>0.848 ± 0.163</td>
<td>0.016</td>
<td>&gt;.99</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>0.682 ± 0.100</td>
<td>0.689 ± 0.116</td>
<td>0.814 ± 0.155</td>
<td>0.629</td>
<td>&gt;.73</td>
</tr>
<tr>
<td>Flea beetle</td>
<td>Old</td>
<td>0.307 ± 0.146</td>
<td>0.003 ± 0.085</td>
<td>0.007 ± 0.123</td>
<td>18.29</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>New</td>
<td>0.208 ± 0.079</td>
<td>0.361 ± 0.105</td>
<td>0.306 ± 0.113</td>
<td>9.49</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>0.229 ± 0.074</td>
<td>0.340 ± 0.107</td>
<td>0.298 ± 0.110</td>
<td>3.02</td>
<td>&gt;.22</td>
</tr>
<tr>
<td>Generalist</td>
<td>Old</td>
<td>2.20 ± 0.41</td>
<td>1.25 ± 0.37</td>
<td>1.29 ± 0.54</td>
<td>14.94</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>New</td>
<td>0.477 ± 0.155</td>
<td>0.800 ± 0.166</td>
<td>1.53 ± 0.54</td>
<td>34.29</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>0.992 ± 0.151</td>
<td>0.909 ± 0.167</td>
<td>1.47 ± 0.47</td>
<td>1.41</td>
<td>&gt;.50</td>
</tr>
</tbody>
</table>

* The number of plants in the three treatments were: Control—94 for new and for all leaves (new and old pooled), 87 for old leaves; Biological Damage—94 for new and all leaves, 67 for old leaves; and Mechanical Damage—95 for new and all leaves, 39 for old leaves.
the mechanically induced treatment, than in controls (Table 2). None of these differences, however, were statistically significant.

Separate analyses of old and new leaves reveal a very different result. For old leaves, plants in the induced treatments exhibited significantly less flea-beetle damage and generalist-insect damage than plants in the control treatment (Table 2). By contrast, for new leaves, plants in the induced treatments exhibited significantly more flea-beetle and generalist damage (Table 2). This result suggests that both old and new leaves undergo physiological changes that affect susceptibility to attack by these insects, but that the effect in old leaves is opposite to that in young leaves, leading to no overall effect when all leaves are examined together. Whatever these physiological changes are, however, there is no evidence that they affect susceptibility to tortoise beetles, which did not differ significantly for induced vs. control treatments for either old or new leaves (Table 2).

Short-period experiments

Experiment II A—tortoise beetle preference experiment. — The results of this experiment were similar to those of Experiment IA: plants subject to prior damage experienced slightly less subsequent damage (proportion foliage removed = 0.00146 ± 0.00029 [mean ± 1 se]) than control plants (0.00169 ± 0.00038) when the induction period was 3 d and slightly more damage (0.00150 ± 0.00027 for induced plants vs. 0.00119 ± 0.00025 for control plants) when the induction period was 6 d. Neither of these trends approached statistical significance, however (Wilcoxon test statistic = −0.151 and −0.652, \( P < .9 \) and \( P < .5 \), respectively). This experiment thus provides no evidence that prior damage induces resistance to feeding by adult tortoise beetles.

Experiment II B—tortoise beetle larval growth experiment. — The proportion of larvae that survived to the end of the experiment was 0.828 and 0.802 for induced and control treatments on lowest leaves, and was 0.820 and 0.886 for induced and control treatments on upper leaves. None of these differences is statistically significant. Although there was a marked effect of block on larval mass on both lower and upper leaves, there was no apparent effect of treatment (induced vs. control), nor was there any evidence of a treatment × block interaction (Table 3). The results of this experiment are thus similar to those of Experiment IB in providing no evidence that prior damage affects the growth performance of tortoise beetle larvae.

Experiment II C—armyworm growth experiment. — The relative growth rate of larvae on induced plants was significantly lower than on control plants (Table 4A; by MANOVA, Wilks' \( \lambda = 0.934 \), df = 2, 127, \( P < .013 \)). Separate analyses of upper and lower leaves indicate that this effect is due primarily to effects of prior damage on growth on lower leaves, e.g., leaves upon which damage was imposed experimentally (Table 4B). There was also a highly significant block effect on growth rate for both upper and lower leaves (Table 4A; Wilks' \( \lambda = 0.421 \), df = 6, 254, \( P < .0001 \)), but no treatment × block effect (Wilks' \( \lambda = 0.9702 \), df = 6, 254, \( P < .695 \)).

Relative growth rate is the product of relative consumption rate and gross growth efficiency. Consequently, we examined these two growth indices to determine which contributed to the difference in growth rate between treatments. Relative consumption rate showed no overall treatment effect (Wilks' \( \lambda = 0.9443 \), df = 2, 80, \( P < .10 \)) and no treatment × block effect (Wilks' \( \lambda = 0.9884 \), df = 4, 160, \( P < .919 \)), although there was a strong block effect (Wilks' \( \lambda = 0.7015 \), df = 4, 160, \( P < .0001 \)). By contrast, larvae growing on induced plants exhibited a reduced gross growth efficiency (Table 4A; Wilks' \( \lambda = 0.8010 \), df = 2, 80, \( P < .0001 \)). This effect is due primarily to reduced efficiency on lower leaves, although there is also a marginally significant reduction of efficiency on upper leaves (Table 4B). Block also influences growth efficiency on both upper and lower leaves (Table 4B), but there are no detectable treatment × block interactions (Table 4B; Wilks' \( \lambda = 0.9459 \), df = 4, 160, \( P < .345 \)). These results indicate that on damaged (lower) leaves, armyworm larvae grow more slowly because of reduced growth efficiency compared to control leaves. There is also some indication that damage affects growth on neighboring undamaged leaves.

**Discussion**

The experiments reported here indicate that induced changes affecting insect preference and/or performance
Table 4. Relative growth rate (RGR), relative consumption rate (RCR), and gross growth efficiency (ECI) of armyworm (Spodoptera eridania) larvae on plants that had been damaged 2 d prior to the onset of herbivory (Experiment IIC). (A) Means and standard error by treatment. (B) Analysis of variance.

A) Larval growth parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample size†</th>
<th>Lower leaves</th>
<th>Upper leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RGR</td>
<td>RCR</td>
<td>ECI</td>
</tr>
<tr>
<td>Damaged</td>
<td>0.301 ± 0.030</td>
<td>0.789 ± 0.052</td>
<td>0.540 ± 0.042</td>
</tr>
<tr>
<td>Control</td>
<td>0.638 ± 0.035</td>
<td>0.648 ± 0.032</td>
<td>0.827 ± 0.043</td>
</tr>
</tbody>
</table>

B) Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df§</th>
<th>Lower leaves</th>
<th>Upper leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RGR</td>
<td>RCR</td>
</tr>
<tr>
<td>Initial mass (lower)</td>
<td>1</td>
<td>5.01*</td>
<td>0.02</td>
</tr>
<tr>
<td>Initial mass (upper)</td>
<td>1</td>
<td>0.02</td>
<td>3.60</td>
</tr>
<tr>
<td>Block</td>
<td>3 (2)</td>
<td>34.49****</td>
<td>12.48****</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>8.97****</td>
<td>4.09*</td>
</tr>
<tr>
<td>Block × treatment</td>
<td>3 (2)</td>
<td>0.79</td>
<td>0.09</td>
</tr>
<tr>
<td>Error</td>
<td>128 (81)</td>
<td>0.0411</td>
<td>0.0688</td>
</tr>
</tbody>
</table>

* P < .05; ** P < .001; *** P < .0001.
† Numbers in parentheses are sample sizes for RCR and ECI.
‡ Numbers for error are mean squares, from which mean squares of other sources can be calculated.
§ Numbers in parentheses are df for RCR and ECI.

occur in foliage of Ipomoea purpurea exposed to prior damage. Two types of evidence warrant this conclusion. First, the reduced growth efficiency of armyworms indicates that prior damage causes some physiological change in foliage quality. Second, in the field experiment, foliage on plants subjected to experimentally imposed prior damage exhibited a different pattern of flea beetle and generalist insect damage than similar foliage on plants not subjected to such damage.

Advantages of induced response

It is commonly assumed that induced changes in foliage quality are advantageous to plants because they reduce the amount of herbivore damage experienced by a plant while minimizing the costs associated with defense (see Introduction). We found no evidence indicating that total damage to induced plants was less than to control plants. This result could be taken to indicate that induced changes in Ipomoea purpurea are not adaptive as defenses against herbivores (though of course they could be adaptive as defenses against pathogens introduced by herbivores). This conclusion would be premature, however, for two reasons. First, our finding of no net difference among treatments in flea-beetle or generalist damage results from the cancelling of opposite effects of induction on damage to new and old leaves. This cancelling may have been an accident of the age and size of the plants at the time the measurements were made. In particular, had we waited until later in the season, when the plants had relatively more old foliage and less new foliage, it is possible that the increased damage to new foliage on “induced” plants would not compensate for the decreased damage to old foliage. There could then be a net advantage to induction. This consideration suggests that experiments designed to examine the effects of induced changes on overall susceptibility of plants to insect damage need to integrate effects measured at a series of times over the course of a season.

Even if our results are indicative of the total amounts of damage incurred at other times of the season, however, the difference between induced and noninduced plants in the dispersion of damage could influence plant fitness, as first suggested by Edwards and Wratten (1983; see also Fowler and Lawton 1985). In particular, if a given amount of damage to new leaves causes less of a fitness reduction than the same amount of damage to old leaves in Ipomoea purpurea, induced plants would exhibit a higher fitness, and there would thus be an advantage associated with induction.

Diffuse vs. pairwise evolution

The pattern of effects of induced responses of Ipomoea purpurea on different herbivores is consistent with both the “diffuse” and “pairwise” views of the evolution of induced responses (see Introduction). On the one hand, of three types of herbivores examined, the only type unaffected by induced changes was the specialist tortoise beetle; the generalist herbivores, including Spodoptera, were clearly affected. This pattern is expected if induced responses are, in general, broad-spectrum, generalized defenses to which specialists occasionally evolve counteradaptation.

On the other hand, this pattern is also consistent with the “pairwise” view, since the induced resistances to flea beetles and to generalists observed in the field experiment could reflect two distinct mechanisms of induced resistance. Previously, Rausher and Simms (1989) observed no genetic correlation and a small but significantly negative phenotypic correlation between
resistances to flea beetles and generalist insects in *Ipomoea purpurea* plants naturally exposed to herbivores. To the extent that *Ipomoea purpurea* plants in the field are continually induced, the absence of a genetic correlation implies that induced responses to these different insects are genetically independent, and thus presumably represent different resistance mechanisms. Additionally, if induced resistance to these two types of insects is underlain by a common mechanism, variation in degree of induction should contribute to a positive phenotypic correlation among the resistances. The negative correlation observed suggests that this contribution is minimal, and thus that distinct mechanisms may underlie induced resistances to these two herbivores. Definitive evidence on the "targeting" of induced responses in *Ipomoea purpurea* will, however, require further experiments designed to identify the mechanisms underlying those responses.

**Induction and costs of resistance**

The occurrence of induced changes affecting susceptibility to generalist insects and flea beetles suggests that our prior conclusion that resistances to these insects have little or no cost associated with them may be premature. However, this result by itself does not necessarily invalidate that conclusion. It is possible that all genotypes exhibit the same degree of induction of resistance, with variation in levels of resistance resulting primarily from differences among genotypes in constitutive levels of resistance. In this situation, costs associated with induction would be the same for all genotypes and our previous estimates of net costs of resistance would be valid. Only if genotypes vary in the amount by which induction changes resistance levels (e.g., Zangerl and Berenbaum 1990) would our previous estimates of costs of resistance to generalist insects and flea beetles be invalid. Our current results indicate that we cannot dismiss the latter possibility and that more complex experiments designed to distinguish between these two possibilities are warranted.

Failure to detect effects of prior damage on susceptibility to tortoise beetles or on tortoise beetle performance gives us no reason to reject our earlier conclusion (Simms and Rausher 1989) that resistance to these insects incurs little cost. It also implies that genetic variation in tortoise beetle resistance observed by Simms and Rausher (1989) is likely to be due largely to genetic variation in constitutive levels of resistance. However, several caveats must be added to this conclusion. First, it is possible that a mechanism producing induced resistance to tortoise beetles may have been suppressed in our greenhouse experiments, perhaps because plants were root bound (e.g., Baldwin 1988b) or experienced altered light quality or intensity compared with the field. We believe this possibility to be unlikely, however, because we observed no induced resistance to tortoise beetles in the field experiment, and this experiment should have been sensitive enough to detect induced resistance of the magnitude needed to account for the variation in resistance found by Simms and Rausher (1989) (see Appendix).

A more important caveat is that induced tortoise-beetle resistance that develops and decays over very short periods (i.e., a few hours) may occur in *Ipomoea purpurea*. Although such short-term responses have been identified in other plant species (see Karban and Myers 1989), we have not yet perfected sufficiently sensitive bioassays to permit their detection in morning glories. Until such assays become available, our conclusion that resistance to tortoise beetles costs *Ipomoea purpurea* plants very little, if anything, remains somewhat tentative.

### Possible mechanisms of induced resistance

The pattern of changes induced in *Ipomoea purpurea* is similar to patterns of induced resistance to pathogens in cultivated cucumbers and tobacco (Dean and Kuc 1985) and of alkaloid-based resistance induced in wild tobacco by insect herbivores (Baldwin 1989). In each case, resistance is systemic. Induction occurs only while damage is continually imposed by the pathogen or herbivore. Leaves produced before or during damage are protected, but when the infected leaf is removed or herbivory ceases, subsequently produced leaves exhibit less and less resistance (Dean and Kuc 1985, 1986). One apparent difference in our results was the increased damage, relative to control plants, experienced by leaves produced after damage had ceased. We offer two hypotheses to explain this result. First, induced resistance may involve short-term allocation trade-offs. A plant that allocates resources to induced resistance may have fewer resources available for resistance in subsequently produced leaves. Alternatively, increased damage in newer leaves may simply reflect a behavioral response by the herbivores. Upon encountering unpalatable leaves with systemically induced resistance, the insects may move up the plant and begin feeding when they find a palatable leaf.

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APPENDIX

This appendix examines the power of Experiment IC to 
detect induced changes affecting susceptibility to tortoise beetle 
damage. If variation in degree of induction were responsible 
for the variation in resistance found by Simms and Rausher 
(1989), then the expected difference in resistance between the 
induced and uninduced states for resistant genotypes should 
correspond roughly to the difference in resistance between resistant and susceptible genotypes found by Simms and Rausher in their control treatment, in which all plants were presumably induced because they were exposed to herbivores. To see this, let Ψ, and Ψ, be the mean proportion damage 
(inverse of resistance) in a given environment experienced, 
respectively, by resistant and susceptible genotypes in the 
uninduced state. In addition, let Ψ, and Ψ, be the correspond- 
ing incremental reductions in proportion damage caused by induction, so that the amount of damage, D, in the induced state is given by

\[ D = Ψ - Ψ, \]

where the subscripts and indicate resistant and susceptible 
genotypes, respectively.

Postulating that the variation in level of damage (resistance) observed by Simms and Rausher (1989) in their control (un-
sprayed) treatment was due primarily to differences in level of induction implies two relationships involving these variables:

1) \( \Psi_e \approx \Psi_w \), i.e., damage to uninduced plants is roughly equal for resistant and susceptible genotypes, and

2) \( \Psi_e > \Phi_r \), i.e., the induction response is greater for resistant than for susceptible genotypes.

These relationships in turn imply

\[
\frac{D_e}{D_r} = \frac{\Psi_e - \Phi_r}{\Psi_w - \Phi_r} \approx \frac{\Psi_e - \Phi_r}{\Psi_w - \Phi_r} \leq \frac{\Psi_e}{\Phi_r}.
\]

(1)

This relationship says that the ratio of damage in the uninduced state to damage in the induced state for resistant genotypes, \( [\Psi_e/(\Psi_w - \Phi)] \), is greater than or equal to the ratio of damages, \( D_e/D_r \), of susceptible and resistant genotypes in the induced state. Eq. 1 can be rearranged to yield

\[
\Phi_r \geq \left[ 1 - \frac{D_e}{D_r} \right] \Psi_w.
\]

(2)

which allows one to calculate the expected difference in damage between induced and uninduced states for the resistant genotype if \( D_e/D_r \), and \( \Psi_e \) are known.

An estimate of \( D_e/D_r \), can be obtained from Simms and Rausher's (1989) data by dividing the mean proportion damage for plants in the least resistant family into the mean proportion damage for plants in the family used in Experiment IC (a very resistant family). This ratio is 0.072 for tortoise beetle damage. Estimates of \( \Psi_e \) are provided in Table 2 (control treatment), i.e., \( \Psi_e = 0.682 \). Substituting these values into Eq. 2 yields a critical value of \( \Phi_r \leq 0.632 \). In other words, the reduction in percent damage observed in Experiment IC must approach this value if variation in degree of induction accounts for observed genetic variation in resistance to tortoise beetles.

The 99% confidence interval for the observed reduction in percent damage in the biological damage treatment is \( -0.007 \pm 0.459 \), which is much lower than the critical value above. Consequently, for tortoise beetles our estimates of percent damage are almost certainly precise enough to have detected induced changes in resistance of the expected magnitude if such changes had occurred. We can thus be fairly confident that induction of a magnitude sufficient to explain genetic variation in resistance to tortoise beetles did not occur.