INTERACTIONS BETWEEN HERBIVOROUS INSECTS AND PLANT-INSECT COEVOLUTION

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Submitted October 12, 1992; Revised May 17, 1993; Accepted June 7, 1993

Abstract.—Two alternative paradigms exist for coevolutionary processes in plant-herbivore systems: “pairwise” and “diffuse.” There is little empirical evidence indicating which mode is more common, in part, we believe, because of an absence of explicit criteria by which the two modes can be distinguished. We present such a criterion, which states that, for selection imposed by different herbivores to be independent (pairwise coevolution), not only must there be no genetic correlation among plant resistances to the different species of herbivores, but also there must be no ecological interactions whereby one herbivore affects the evolution of resistance to other species. We present a model that demonstrates the validity of this criterion. We tested whether this criterion is satisfied by the annual morning glory Ipomoea purpurea and two of its insect herbivores, Deloyala guttata and Bedellia somnulentella, by conducting both greenhouse and field experiments. We placed various densities of the two insect species on plants in a factorial design and tested for a statistical interaction between densities of the two species on plant fitness. We did not detect an interaction in either experiment. Our results suggest, therefore, that selection exerted by insect herbivores on resistance in I. purpurea is pairwise rather than diffuse.

Although it is widely believed that plants commonly coevolve with their insect herbivores (Ehrlich and Raven 1964; Feeny 1976; Rhoades and Cates 1976; Strong et al. 1984), the nature of the coevolutionary process remains unclear. On the one hand, most theoretical treatments of coevolution assume that there are strong pairwise interactions between two species, but they often assume that these interactions are independent of other species (e.g., Levin and Udovic 1977; Leonard and Czochor 1980; Kiyosawa 1982; Levin 1983; Roughgarden 1983; Kiefer et al. 1984; Taper and Case 1985; Brown and Vincent 1987; but see Levin et al. 1990). As applied to plant-herbivore systems, this view postulates that the selection pressures exerted by any particular herbivore on a plant population is independent of the presence or absence of other herbivore species.

An alternative view is that selection pressures imposed by herbivores are “diffuse” (Janzen 1980; Fox 1981, 1988; Gould 1988). According to this view, the selection pressures imposed by one herbivore species on plant resistance traits are often opposed, constrained, or modified by selection imposed by other herbivore species. Thus, this view postulates that evolution of resistance to one herbivore species is not independent of the presence of other species, often because

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selection pressures imposed by one species are not independent of the selection pressures imposed by a second species.

There is very little empirical evidence indicating which of these two modes of coevolution is more prevalent in nature. We believe that this paucity of evidence is due in part to an absence of explicit consideration of what type of criteria can be used to determine which mode is occurring in any particular plant-insect association (Rausher 1992). The objective of this article is to remedy this deficiency partially by demonstrating a particular criterion that is necessary for coevolution to be pairwise rather than diffuse. We then examine whether this criterion is satisfied by a particular plant-herbivore system, the annual morning glory *Ipomoea purpurea* and two of its insect herbivores.

**THE CRITERION**

It has been argued (Rausher 1992) that one criterion necessary for selection imposed by different herbivores to be independent (pairwise coevolution) is that there must be no genetic correlation between resistance to one herbivore and resistance to others, since, if resistances are genetically correlated, selection imposed by one herbivore will generate indirect selection on resistance to other herbivores. Although this criterion is a necessary condition for selection to be independent, it is not a sufficient condition: even if resistances to different herbivores are genetically uncorrelated, it is still possible, as shown below, for the presence of one herbivore species to influence the evolution of resistance to other species through certain types of ecological interactions. To conclude that selection imposed by different herbivores is independent thus requires a demonstration not only that resistances are not genetically correlated but also that these ecological interactions are absent.

An influence of one herbivore on the type and magnitude of selection imposed by a second herbivore may arise in at least two ways. If there are interactions among herbivores, then the expected density of a particular herbivore species on a plant with a given level of resistance to that herbivore may depend on whether other herbivore species are present. If reduction in fitness is linearly and additively related to damage, then the selective impact of the first herbivore will depend on the presence and density of other herbivores.

In addition, the effects of many combinations of stress on plant fitness are nonadditive (Clantworthy 1960; Harper and McNaughton 1962; Bentley and Whittaker 1979; Lee and Bazzaz 1980; Fowler and Rausher 1985). It is thus reasonable to expect that different types of herbivore damage will often affect plant fitness nonadditively. Under these circumstances the selective impact of a given amount of damage by one herbivore will depend on the presence and density of other herbivore species. In this section we present a simple model that demonstrates the validity of these claims.

The model is a modification of the standard cost-benefit model of the evolution of the level of resistance (Rhoades 1979, 1983; Coley et al. 1985; Fagerström et al. 1987; Simms and Rausher 1987, 1989; Fagerström 1989), expanded to examine the effects of two herbivore species. This model makes three assumptions, which
appear to be true of many, but not all, plant-herbivore systems: first, herbivory reduces plant fitness (see, e.g., Kulman 1971; Morrow and LaMarche 1978; Rausher and Feeny 1980; Louda 1983, 1984; Kinsman and Platt 1984; Marquis 1984; for a review see Crawley 1983; but see Paige and Whitham 1987); second, resistance is costly to plants (see, e.g., Windle and Franz 1979; Dirzo and Harper 1982; Burdon et al. 1984; Berenbaum et al. 1986; Coley 1986; but see Simms and Rausher 1987, 1989); and, third, resistance is a quantitative trait (see, e.g., Berenbaum et al. 1986; Simms and Rausher 1987, 1989; Ågren and Schemske 1993).

For one species of herbivore, the model postulates that the genotypic value for fitness associated with a given additive genotypic value for level of resistance, \( R \), is given by

\[
W(R) = W_0 - C(R) - H(R),
\]

where \( W_0 \) is the expected fitness of a completely susceptible genotype in the absence of herbivores, \( C(R) \) is the cost of resistance, and \( H(R) \) is the reduction in fitness due to herbivory.

It is usually assumed that cost should increase monotonically with \( R \) because materials and energy are being diverted from other functions contributing to fitness (McKey 1979; Rhoades 1979, 1983). In our model, we assume the cost of resistance is a linear function of \( R \).

The shape of the function \( H(R) \) is constrained by three biological considerations. First, the reduction in fitness due to herbivore damage should be maximal for completely susceptible genotypes (i.e., for \( R = 0 \)). Second, increasing levels of resistance by definition imply reduced herbivore densities, reduced damage, and hence a reduction in the detrimental effects of herbivory. We assume that resistance reduces damage by herbivores and is distinct from tolerance to herbivory. The reduction in fitness due to herbivory should thus be a monotonically declining function of \( R \). Finally, as \( R \) increases indefinitely, \( H(R) \) must approach zero, since complete resistance implies absence of damage and hence no detrimental effect of damage. For analysis, we assume that \( H(R) \) is quadratic, although the results do not depend on this assumption.

It has been demonstrated (Simms and Rausher 1987) that, under these assumptions, there is an intermediate level of resistance that represents an evolutionary equilibrium. At this equilibrium, the marginal benefit associated with reducing herbivory as \( R \) increases (i.e., \( dH/dR \)) is equal to the marginal cost (\( dC/dR \); see App. A also).

This model can be expanded to accommodate two herbivore species very simply. In this case, \( R_1 \) and \( R_2 \) represent additive genetic values of investment in resistance to herbivore species 1 and 2, respectively. We assume that costs associated with these two resistances, \( C_1(R_1) \) and \( C_2(R_2) \), are linear and additive. Finally, we let \( H_1(R_1) \) and \( H_2(R_2) \) be the reduction in fitness due to damage by each herbivore in the absence of the other herbivore species.

The total reduction in fitness caused by damage would simply be \( H_1(R_1) + H_2(R_2) \) if the herbivores did not affect each other’s density and if the effects of the two herbivore species’ damage on fitness were additive and independent, that
is, if the impact of one species on plant fitness were not affected by the presence of the other species. To allow for nonindependence, we add a third term, \(a \times H_1(R_1) \times H_2(R_2)\), to the effect of herbivory, which yields the following overall relationship between genotypic values of fitness and resistance:

\[
W(R_1, R_2) = W_0 - C_1(R_1) - C_2(R_2) - H_1(R_1) - H_2(R_2) - a[H_1(R_1)H_2(R_2)] .
\]  

(2)

In this equation, the coefficient \(a\) is positive if one herbivore increases the density of the other herbivore (i.e., reduces the effectiveness of resistance) or if the effects of the total damage by the two herbivores is more than the sum of the effects of their individual damages. By contrast, \(a\) is negative if the herbivores have negative effects on each other’s density or if the effect of the total damage is less than the sum of the effects of the individual species’ damages.

When \(a = 0\), that is, when there is no interaction between the damage effects, this model generates a selection surface of the type shown in figure 1. As with a single species, an optimal combination of levels of resistance to the two species exists at the maximum of the curve, corresponding to \((R_1^*, R_2^*)\). In this case, the optimal level of resistance to each species is equal to the optimal level predicted by the one-herbivore model (see App. A). Consequently, when resistances to two herbivores are genetically uncorrelated and there are no interactions between herbivore densities or between the effects of the two types of damage on plant fitness, the evolution of resistance to one herbivore is independent of the presence or absence of the other. In other words, coevolution is pairwise rather than diffuse.

By contrast, when \(a\) is not equal to zero, the equilibrium levels of resistance are no longer \((R_1^*, R_2^*)\) (see App. A for a general proof). This change in equilibrium
Fig. 2.—Three-dimensional phenotypic fitness surface for plants when herbivores interact, that is, \( a < 0 \). This situation corresponds to competition between the two herbivorous species. Axes \( R_1 \) and \( R_2 \) are concentrations of two defensive chemicals. Points \( R_1^* \) and \( R_2^* \) are optimum values on the curve for the two resistance chemicals. The plus sign represents the optimum values for \( R_1 \) and \( R_2 \) when \( a = 0 \).

is illustrated in figure 2, which portrays the fitness surface using the same functional relationships as in figure 1 but with \( a < 0 \). In this situation, when both types of herbivore occur on a plant, plant fitness is higher than when \( a = 0 \). The equilibrium level of resistance to each herbivore is shifted toward lower levels. When \( a \) is positive, the equilibrium is shifted toward higher levels of resistance to each herbivore. It is thus clear that when \( a \) is not zero, the equilibrium levels of resistance are not the same as in the one-herbivore model. In other words, when herbivore densities interact or when the effects of damage by the two herbivores on plant fitness are not additive, the equilibrium level of resistance to one herbivore will depend on whether the other species is present. Interactions and nonadditivity thus engender diffuse coevolution.

INTERACTION EFFECTS OF DAMAGE ON FITNESS IN MORNING GLORIES

The above considerations suggest that determination of whether plant-herbivore systems commonly exhibit such interactions or nonadditivity will aid in determining whether coevolution is commonly diffuse. Interactions between herbivore species are known to affect subsequent densities of the interacting species (see, e.g., McClure and Price 1975; Stiling 1980; Faeth and Bultman 1984; Niemela et al. 1984; Williams and Myers 1984; Faeth 1986; Harrison and Karban 1986; Damman 1989; Tscharntke 1989; Moran and Whitham 1990; Strauss 1991; Pilson 1992), but whether these effects on insect density translate into an effect on plant fitness has seldom been explored.
In addition, there is very little empirical evidence regarding whether damage effects on fitness are additive or nonadditive. A notable exception is the recent study by Strauss (1991), who demonstrated that the effect of deer browse on ramet elongation depends on whether chrysomelid beetles also damage a plant. Because of this paucity of information concerning types of interaction that may determine the nature of coevolution between plants and their herbivores, we attempted to determine whether such interactions characterize the effects of two insect herbivores on the fitness of the morning glory *Ipomoea purpurea*.

In two experiments, one in the field and one in a greenhouse, we experimentally applied various combinations of densities of two herbivores to individual plants and determined the effects of the resulting damage on two primary fitness components, the number of seeds produced and the mean seed mass.

**MATERIAL AND METHODS**

**Experimental Species**

The common or tall morning glory, *Ipomoea purpurea* (L.) Roth. (Convolvulaceae), is an annual hermaphroditic vine that grows throughout southeastern North America in disturbed habitats (Radford et al. 1964). Seeds usually germinate in May or June in central North Carolina (depending on date of disturbance, e.g., spring tillage in agricultural fields), and plants grow until they are killed by the first fall frost, whose average date is October 30 (−2.2°C temperature between 1969 and 1988; National Oceanic and Atmospheric Administration 1969–1988).

We used two common herbivores of *I. purpurea* in both experiments. The mottled tortoise beetle, *Deloyala guttata* (Coleoptera: Chrysomelidae), is a specialist on members of the family Convolvulaceae. It is multivoltine and is present at all times during the growing season of *Ipomoea* (Barrows 1979). The larvae are relatively sessile, usually staying on the same plant for the duration of larval development. Both adults and larvae chew holes in leaves while feeding, causing recognizable damage. The morning glory or sweet potato leaf miner, *Bedellia somnulentella* (Lepidoptera: Lyonetiidae), is also a specialist on members of the family Convolvulaceae. Larvae of this multivoltine moth feed in leaves during five larval instars, creating serpentine mines during the first two instars and blotch mines during the final three instars (Shorey and Anderson 1960; Drâghia 1974; Parrella and Kok 1977). Larvae begin to occur in appreciable densities in the field approximately in late September (D. Hougen-Eitzman, personal observation).

**Insect Colonies**

Larvae used in the experiments were obtained from laboratory colonies maintained on a 14L:10D regime. Temperature and humidity were set to 29°C and 80%, respectively, during the day and to 19°C and 80%, respectively, during the night.

The *Deloyala* colonies used for the two experiments were maintained in an identical manner, except that the colony for the greenhouse experiment was started 3 mo prior to the beginning of the experiment, whereas the colony for the
field experiment was started 1 mo prior to the experiment. The colonies were started with approximately 200 wild adults collected in Durham and Orange counties in North Carolina from various fields and roadides within 5 km of one another. Both adults and larvae were cultured on fresh leaves of *I. purpurea* that were collected in the field or grown in the greenhouse.

As with *Deloyala*, colonies of *Bedellia* used in the two experiments were treated identically, except that the colony for the greenhouse was started 1 mo prior to the experiment and the colony for the field experiment was started 8 mo before the experiment. Colonies were started with approximately 50 miner larvae that were collected from *I. purpurea* leaves in the field. Adults were maintained in a screened flight cage and fed a 5% honey solution. Larvae were raised on *I. purpurea* plants that were grown in the greenhouse.

Transferring Eggs and Larvae

*Deloyala* larvae were applied to plants as third instar larvae (younger larvae suffered high mortality) and fed on the plants for approximately 2 wk. Larvae for each block of the experiment were obtained from a single cohort; that is, they all hatched on the same day. Larvae were censused twice at 2-d intervals after they were applied, and any missing larvae were replaced. This reapplication was intended to control for any mortality due to handling of the larvae.

*Bedellia* were applied to plants in the form of eggs that had been oviposited 2 d earlier on plants in the adult flight cage. We separated the eggs from the leaf surface with a small scalpel blade (Bard-Parker no. 15). We then moistened the leaf of the experimental plant with a saturated gelatin solution (400 mg of dried gelatin in 40 mL of boiling water) and moved eggs to the experimental leaf with the moistened tip of a size 10/0 paintbrush. We placed three eggs on each leaf that required a single *Bedellia* larva since approximately 40% of transferred eggs hatch successfully (D. Hougen-Eitzman, unpublished data). We examined plants 4 d later and removed extra larvae with fine forceps.

Greenhouse Experiment Methodology

Plants used in this experiment were grown from seeds of 10 field-pollinated plants that were randomly selected in a field near Durham, North Carolina. In October 1988, we planted seeds in 6-inch pots in the Duke University greenhouse, with seeds from each field-collected plant placed in a separate spatial block on the greenhouse bench. The plants were trained onto 1-m bamboo stakes and situated such that neighboring plants did not touch one another. All plants were sprayed periodically with Safer insecticidal soap to control mites and aphids, and the spraying was done when the experimental insects were not present.

The experimental design consisted of nine treatments in a randomized block design, with one replicate of the nine treatments in each block. Three densities of larvae per plant for each species—zero, one, and two—were used in a completely crossed design. Larvae were placed on one or two neighboring leaves in the center of each vine, with one larva per leaf.

We applied *Bedellia* eggs to the plants when plants were 1 mo old. One week later, we applied *Deloyala* larvae to the same two leaves on which *Bedellia* eggs
were placed. Therefore, *Deloyala* and *Bedellia* were present on plants simultaneously. Three weeks after *Bedellia* eggs were applied, when both species were starting to pupate, we censused the total leaf area of each plant and the leaf area damaged by each insect species. We used a transparent piece of acetate on which was copied a grid scaled in 5-mm increments to estimate the area of each leaf and damage by each species on that leaf in increments of 25 mm². We estimated damage by serpentine mines as the number of grid sections containing serpentine mines.

All seeds were collected and the experiment was stopped when most plants had senesced, on February 18, 1989. Seed set and seed weight were used as estimators of plant fitness.

*Field Experiment Methodology*

In early July 1989, we planted seeds from eight fifth-generation inbred lines into Roottrainers in the greenhouse, with each line represented by 48 offspring from each of two dams. The inbred lines were chosen randomly from a set of 50 lines that were maintained in the greenhouse by allowing plants to self-pollinate. Two weeks later, seedlings were transplanted in a recently ploughed field near Durham, North Carolina, with plants situated 1 m from the nearest neighbor in three spatial blocks. As plants grew, they were trained onto 1.3-m bamboo stakes, to simulate growth in corn fields.

The experimental design consisted of four densities of the two insect species in a $4 \times 4$ randomized block design. This design allowed us to test for the effect of each species on plant fitness alone and in combination. We used larval densities that spanned the range of natural densities we have observed in the field for these species—zero, one, two, and four beetle larvae per plant and zero, two, four, and eight leaf miner larvae per plant. Larvae and eggs were placed on one or two leaves in the center of each vine. The entire factorial design, which was completely balanced with respect to treatments, inbred lines, and blocks, used 768 plants. The dam effect was nested in the inbred line effect.

Two weeks after plants were transplanted, when plants had three or four true leaves, beetle larvae were placed on plants. Approximately 3 wk later, when plants had five to eight leaves and after all beetle larvae had pupated, leaf miner eggs were applied. This sequence of beetles followed by leaf miners was similar to the natural phenology of the two insects.

We conducted three plant censuses, in which we measured leaf area and damage by all insects. As in the greenhouse experiment, leaf area and damage were measured with a transparent grid. The first census was conducted before any larvae were applied, in order to have an initial measure of plant size. The second census was conducted after the beetles started to pupate, and the third after the leaf miners started to pupate. The two late censuses were used to measure damage caused by the two herbivore species. All plants were checked weekly to remove any naturally occurring herbivores, which were relatively few (D. Hougen-Eitzman, personal observation).

All seeds were collected until a frost occurred, on November 20, 1989. Seeds
from each plant were counted and weighed, and the seed set and the seed weight were used as estimators of plant fitness.

STATISTICAL METHODS

We used two types of analyses to examine the data. We first used ANOVA or ANCOVA to investigate possible interactions between effects of each species of insect on plant fitness. We then used path analysis to investigate possible ecological interactions between the two species of insects.

Greenhouse Experiment with ANCOVA

Data from the greenhouse experiment were analyzed with ANCOVA using the SAS generalized linear model (GLM) procedure (SAS Institute 1988), with leaf area as the covariate. Though leaf area was measured after insects were applied, it was not correlated with any of the treatments. All effects were considered to be fixed effects. Although spatial block is often assumed to be a random effect, we consider the block effect here to be a fixed effect; our spatial blocks in the greenhouse were located in different parts of the greenhouse and perhaps experienced different microclimates (light, humidity, and temperature)—however, we do not consider the blocks to represent a meaningful random sample of environments across which we would desire to make inferences. Note that variation due to plant family was included in the block effect since the two sources of variation were confounded. In all analyses, seed set and seed weight were log transformed to normalize the data and equalize treatment variances.

Field Experiment with ANOVA

Data from the field experiment were analyzed with mixed-model ANOVA using SAS GLM statistical software (SAS Institute 1988). Seed set and seed weight were used as response variables. Appropriate denominators for source effects were calculated according to the mixed model described by Hocking (1985), which is implemented by SAS. These denominators were based on the expected mean squares for each effect, with the assumption that inbred line and dam were random effects and that the two herbivore density effects and block were fixed effects. We considered the block effect to be fixed since the three spatial blocks were relatively close to one another within a small field and consequently did not represent a meaningful random sample of environments. The dam effect was treated as a nested effect in inbred line. When compound denominators were necessary, nonsignificant mean square components were dropped to simplify the approximations (see App. B). In addition, interactions that were not significant and not of direct interest in the analysis were pooled with error. Both seed set and seed weight were log transformed to normalize the data and equalize treatment variances.

Comparison of Treatment Means

Treatment means were compared with linear contrasts conducted in the ANCOVA and ANOVA analyses.
Path Analysis

We used path analysis, a graphical representation of linear regression (Wright 1920, 1921; Li 1975), to search for the presence of ecological interactions between the two insect species. In each experiment, we examined relationships between the following variables: initial densities of both herbivores, densities of herbivores measured 2 wk after application, and damage caused by the herbivores. Path analysis allowed us to determine to what degree these variables affected each other and provided information about ecological interactions not shown in the ANOVAs above. For example, if densities of the two species exhibited nonadditive effects on plant fitness, path analysis might allow us to determine the nature of ecological interactions that might have been responsible for such nonadditivity. If, however, no statistical interaction was present between herbivore densities and plant fitness, the two insect species could nevertheless have affected one another. Knowledge of these ecological interactions might lead one to suspect that the effects of densities of the two species on plant fitness might have interacted under other conditions.

The path diagrams for the greenhouse and field experiments were qualitatively different. In the field experiment, the paths between the two insect species always went from Deloyala to Bedellia. This was because all Deloyala larvae had pupated before Bedellia eggs were applied. Therefore, Deloyala could affect Bedellia in the experiment, but Bedellia could not affect Deloyala.

In the greenhouse experiment, however, both species of insects occurred on plants simultaneously. This situation presented a problem: both species could theoretically affect each other simultaneously; however, in path analysis the direction of each path must be determined a priori. A solution to this problem is to calculate interactions between a measured variable for one species and a later variable for the other species. For example, the effect of Deloyala on the density of Bedellia can be measured by the path between initial Deloyala density and Bedellia density at 2 wk. This type of comparison is valid because, even though interactions could be instantaneous, the effect of the interaction on a species should still be present at a later time. Such paths are necessarily one-way since both species were placed on plants at approximately the same time and have similar developmental rates.

RESULTS

Greenhouse Experiment with ANCOVA

In the greenhouse experiment, there was no detectable interaction between effects of the two species of herbivores on plant fitness, although Bedellia damage clearly affected fitness (table 1, pt. A). Increases in density of Bedellia, averaged across all Deloyala densities, caused an increase in seed set (fig. 3). Plants with a density of two Bedellia larvae per plant had a significantly higher seed set than did plants with no Bedellia larvae (linear contrast in ANCOVA: \( F = 11.10, \text{df} = 1,35, P = .002 \)). A separate analysis with seed weight as the response variable
### TABLE 1

ANOVA for Seed Set (A) and Seed Weight (B) in the Greenhouse Experiment

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Response variable = ( \log_{10} ) (seed set):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf area</td>
<td>1</td>
<td>.2496</td>
<td>31.85***</td>
</tr>
<tr>
<td>Block</td>
<td>9</td>
<td>.05614</td>
<td>7.16***</td>
</tr>
<tr>
<td>Deloyala</td>
<td>2</td>
<td>.008784</td>
<td>1.12</td>
</tr>
<tr>
<td>Bedellia</td>
<td>2</td>
<td>.04371</td>
<td>5.58**</td>
</tr>
<tr>
<td>Block × Deloyala</td>
<td>18</td>
<td>.009662</td>
<td>1.23</td>
</tr>
<tr>
<td>Block × Bedellia</td>
<td>18</td>
<td>.007293</td>
<td>.93</td>
</tr>
<tr>
<td>Deloyala × Bedellia</td>
<td>4</td>
<td>.007681</td>
<td>.98</td>
</tr>
<tr>
<td>Error</td>
<td>35</td>
<td>.007837</td>
<td></td>
</tr>
<tr>
<td>B. Response variable = ( \log_{10} ) (seed weight):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf area</td>
<td>1</td>
<td>.0141</td>
<td>5.45*</td>
</tr>
<tr>
<td>Block</td>
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<td>6.41***</td>
</tr>
<tr>
<td>Deloyala</td>
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<td>.82</td>
</tr>
<tr>
<td>Bedellia</td>
<td>2</td>
<td>.00044</td>
<td>.08</td>
</tr>
<tr>
<td>Block × Deloyala</td>
<td>18</td>
<td>.0010</td>
<td>.39</td>
</tr>
<tr>
<td>Block × Bedellia</td>
<td>18</td>
<td>.0022</td>
<td>.85</td>
</tr>
<tr>
<td>Deloyala × Bedellia</td>
<td>4</td>
<td>.0017</td>
<td>.66</td>
</tr>
<tr>
<td>Error</td>
<td>35</td>
<td>.0026</td>
<td></td>
</tr>
</tbody>
</table>

**Note.**—Mean squares were calculated using Type III sums of squares from SAS GLM (SAS Institute 1988). All source variables were considered to be fixed effects. There were 90 total observations.

* .01 < \( P \) < .05.
** .001 < \( P \) < .01.
*** \( P \) < .001.

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**Fig. 3.**—Plot of seed set for all density combinations of *Deloyala* and *Bedellia* in the greenhouse experiment. Each data point represents the average value for 10 plants, and error bars represent SEs. Seed set has been log transformed and presented as a residual after the effects of block and leaf area have been removed.
showed neither an effect of either herbivore species alone nor an interaction between effects of the two species of herbivores (table 1, pt B).

Path Analysis

Path analysis demonstrated a lack of ecological interactions between the two herbivorous species (fig. 4). Note that, although all path coefficients had nonzero values, many coefficients were not significantly different from zero in the multiple regression. The diagram shows that, for each species, initial larval density had a strong effect on density measured 2 wk later, and that this later density had a significant effect on damage. The diagram further shows that the density of Bedellia measured 2 wk after application was not affected by initial density of Deloyala, and damage by Bedellia was not affected by density of Deloyala after 2 wk. Similarly, density of Deloyala measured 2 wk after application was not affected by initial density of Bedellia, and damage by Deloyala was not affected by density of Bedellia after 2 wk. These results therefore indicate that the presence of Deloyala had negligible effects on the mortality and feeding of Bedellia.

Field Experiment with ANOVA

We conducted an ANOVA with the seed set as the response variable using a full model, with all possible interactions included; subsequently, all effects that were not of interest and that were nonsignificant were pooled with the model error, as described in Statistical Methods. This reduction in the size of the statistical model did not appreciably affect the magnitude of the F values of the remaining treatment effects. Analysis of this reduced model showed that there was no significant interaction between the effects of the two herbivorous species,
### TABLE 2

**Mixed-Model ANOVAs for Seed Set (A) and Seed Weight (B) in the Field Experiment**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Effect Type</th>
<th>Mean Square</th>
<th>Denominator</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Response variable = log&lt;sub&gt;10&lt;/sub&gt; (seed set + 1):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block [A]</td>
<td>2</td>
<td>Fixed</td>
<td>18.333</td>
<td>MS&lt;sub&gt;AB&lt;/sub&gt;</td>
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<td>(\ldots)</td>
<td>3.48**</td>
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<td>MS&lt;sub&gt;E&lt;/sub&gt;</td>
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</tr>
<tr>
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<td>MS&lt;sub&gt;E&lt;/sub&gt;</td>
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<tr>
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<td>MS&lt;sub&gt;BF&lt;/sub&gt;</td>
<td>0.06</td>
</tr>
<tr>
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<td>Random</td>
<td>0.341</td>
<td>MS&lt;sub&gt;E&lt;/sub&gt;</td>
<td>2.18**</td>
</tr>
<tr>
<td>Line \times Delloyala [BD]</td>
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<td>MS&lt;sub&gt;E&lt;/sub&gt;</td>
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<tr>
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<td>MS&lt;sub&gt;E&lt;/sub&gt;</td>
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</tr>
<tr>
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<td>MS&lt;sub&gt;E&lt;/sub&gt;</td>
<td>1.27</td>
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<tr>
<td>Error [E]</td>
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<td>.156</td>
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</table>

B. Response variable = log<sub>10</sub> (seed weight):

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<th>Source</th>
<th>df</th>
<th>Effect Type</th>
<th>Mean Square</th>
<th>Denominator</th>
<th>F</th>
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<td>MS&lt;sub&gt;AB&lt;/sub&gt;</td>
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<td>.156</td>
<td></td>
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</table>

**Note.**—Type III sums of squares from SAS GLM were used for the analysis for seed weight because some plants did not set any seed, and the design was consequently unbalanced. Denominator mean square terms for F-tests were determined by the method described in App. B.

* Denominator mean square = MS<sub>AB</sub> + MS<sub>BF</sub> - MS<sub>E</sub>, df \(\approx\) 38.

† \(P = .070\).

** \(P < .01\).

*** \(P < .0001\).

---

**Fig. 5.**—Plot of seed set for all density combinations of Delloyala and Bedellia in the field experiment. Each data point represents the average value for 48 plants, and error bars represent SEs. Seed set has been log transformed and presented as a residual after the effects of block and line have been removed.
which is indicated by the nonsignificant interaction effect between *Deloyala* and *Bedellia* (table 2, pt. A). The ANOVA further showed that damage by *Deloyala* significantly affected seed number, whereas damage by *Bedellia* did not (see fig. 5). Plants with the intermediate density of two *Deloyala* larvae, averaged across all densities of *Deloyala*, produced significantly more seeds than did plants with zero, one, or four *Deloyala* larvae per plant (linear contrasts in ANOVA for densities of *Deloyala*: 2 vs. 0: $F = 4.26$, $df = 1, 679$, $P = .0395$; 2 vs. 1: $F = 5.31$, $df = 1, 679$, $P = .0215$; 2 vs. 4: $F = 14.01$, $df = 1, 679$, $P = .0002$).

A separate ANOVA detected neither an interaction between effects of the two species of insects on seed weight nor any main effect of either species (table 2, pt. B). We used a reduced model, as with the above analysis on seed set. The data were somewhat unbalanced, since 59 plants (7.6% of total plants) produced no seeds. However, a three-way contingency analysis (Fienberg 1970, 1980; Sokal and Rohlf 1981, pp. 747–765), with mortality crossed with the densities of *Deloyala* and *Bedellia*, showed that the density of neither insect species affected the probability that plants would produce zero seeds, nor was there a significant interaction between the two species (*Deloyala*: $\chi^2 = 1.57$; *Bedellia*: $\chi^2 = 0.06$; interaction: $\chi^2 = 8.31$).

**Path Analysis**

As with the greenhouse experiment, path analysis demonstrated a lack of ecological interactions between the two herbivorous species (fig. 6). There were strong relationships for both species between initial density and density measured at 2 wk and between this later density and damage. The density of *Bedellia*
measured 2 wk after application was unaffected by the initial density of *Deloyala*, *Deloyala* density after 2 wk, and damage by *Deloyala*. Similarly, the damage by *Bedellia* was not affected by *Deloyala* density at 2 wk or damage by *Deloyala*. These results therefore indicate that the presence of *Deloyala* had negligible effects on the mortality and feeding of *Bedellia*.

**DISCUSSION**

Although the distinction between pairwise and diffuse coevolution was first made over 10 yr ago, little consideration has been given to concrete criteria that can be used to distinguish between these two modes of coevolution. On the basis of the model presented here and arguments given in an earlier article (Rausher 1992), we suggest that there are two conditions necessary for selection imposed by different herbivores to be independent rather than diffuse: (1) resistances to the different herbivores must be genetically uncorrelated; and (2) the presence versus absence of one herbivore species must not affect the impact of other species on plant fitness. We note that condition 2 may fail either because interactions among herbivores influence the densities of, and hence the amount of damage caused by, those herbivores or because the impacts of damage by different herbivores on plant fitness are not additive.

When combined with previous results (Simms and Rausher 1989), the present analysis provides little reason to believe that selection exerted by insect herbivores on resistance in *Ipomoea purpurea* is diffuse. Genetic variation for resistance to each of four different types of insect herbivores exists in natural populations of *I. purpurea* (Simms and Rausher 1987, 1989). Nevertheless, genetic correlations are low and not significant for all six pairwise combinations of these resistances (Rausher and Simms 1989). Consequently, there is no indication that condition 1 is violated.

The current study provides no evidence that condition 2 is violated, at least not for the two herbivores examined. In particular, neither the presence or absence nor the relative abundance of either herbivore species influenced the abundance of the other species, the amount of damage caused by that species, or the impact of that species' damage on plant fitness. At least under the conditions of this study, then, the selective impact of each of these herbivores was independent of that of the other herbivore.

The failure to reject either condition 1 or 2 suggests that any selection insect herbivores exert on resistance in *I. purpurea* is most likely to be pairwise rather than diffuse. However, this conclusion cannot yet be considered definitive for several reasons. First, we have not yet attempted to determine whether other pairs of species exhibit either interactions or nonadditivity of impacts on fitness. It is possible that some pairs of herbivores exert pairwise selection while others exert diffuse selection. The observation (Simms and Rausher 1993) that the adaptive landscape for resistance to tortoise beetles and to the anthracnose fungus *Colletotrichum dematium* has two peaks, indicating that selection exerted by each of these species depends on the presence of the other species, suggests that similar dependence may occur among other pairs of herbivores on *I. purpurea*. 
Second, even for tortoise beetles and leaf miners, a potentially important aspect of interaction was prevented from occurring because of the design of the experiment. Because these insects were placed on plants at predetermined combinations of densities, effects of one species on the ovipositional tendencies of the other were not measured. Many insect species avoid plants (Shapiro 1981; Giga and Smith 1984; Faeth 1985, 1986; Klimstra 1985; Jones and Finch 1987; Schoonhoven et al. 1990) or are attracted to plants (Strauss 1991) that have already been fed or oviposited on by other insects, and we cannot rule out this possible interaction between Deloyala and Bedellia.

Third, it is well established that the impact of herbivory on plant fitness may depend on environmental conditions such as soil nutrient levels and degree of competition the plants experience (Maschinski and Whitham 1989; Whitham et al. 1990). This dependence may carry over onto the effects of interactions among herbivores on plant fitness. If so, it is possible that we would observe tortoise beetles and leaf miners exerting diffuse selection on I. purpurea in other habitats or in other years.

Finally, it might be argued that no effect of interactions among herbivores is expected unless both herbivores exhibit an impact on host fitness, and that, since in each of our experiments only one herbivore exhibited such an impact, our experiments did not provide an adequate test of whether interaction effects occurred. However, the absence of a detectable main effect of one herbivore in a factorial ANOVA of the type presented here does not necessarily preclude detection of an interaction effect between the two herbivores. Moreover, it does not imply that the herbivore has no effect on fitness. Rather, it implies that, averaged over all treatments involving the second herbivore, there is no detectable effect. For example, if one herbivore species by itself reduces plant fitness but does not do so in the presence of a second herbivore species, the main effect of the first herbivore, which is the average of the effects with and without the second herbivore, may be too small to detect even if the interaction is significant. This possibility seems very real, given numerous examples of one-herbivore species' influencing the abundance of a second (see, e.g., McClure and Price 1975; Stiling 1980; Faeth and Bultman 1984; Niemala et al. 1984; Williams and Myers 1984; Faeth 1986; Harrison and Karban 1986; Damman 1989; Gange and Brown 1989; Tscharnkte 1989; Moran and Whitham 1990; Strauss 1991; Pilson 1992). Moreover, if when alone one species actually increases plant fitness, as the trends in figures 3 and 5 at least suggest may be occurring, there may be no expected main effect of herbivory even though an interaction is present. It is of course possible that both main effects of the two herbivores and their interactions would have become detectable if higher insect densities had been used in our experiment. However, such densities would have been far above those normally seen in natural populations, and the relevance of such results to understanding selection imposed by these herbivores in nature would be questionable.

Bearing these caveats in mind, we tentatively conclude that selection exerted by herbivores on I. purpurea is primarily pairwise rather than diffuse. It is not clear how generalizable this conclusion is to other systems. Several studies have detected primarily low genetic correlations between resistances to different in-
sects (Fritz and Price 1988; Simms and Rausher 1989; Fritz 1992), suggesting that condition 1 is often satisfied by plant-herbivore systems (but see Pilson 1992). On the other hand, the only other study that has explicitly examined the influence of interactions among herbivores and of nonadditivity of damage on plant fitness (Strauss 1991) provides evidence of nonindependence of selection exerted by different herbivores. Along with demonstrations that interactions among herbivore species affect the abundances of those species, this result suggests that condition 2 may often fail to be satisfied by multispecies plant-herbivore interactions. Diffuse selection on plant resistance may therefore be common. Our results, however, indicate that it is not universal. Determining the conditions under which selection exerted by herbivore communities tends to be diffuse rather than pairwise remains a largely unexplored question.

ACKNOWLEDGMENTS

We would like to thank J. Antonovics, F. Gould, R. Karban, F. Nijhout, S. Nowicki, J. Van Buskirk, and H. Wilbur for reviewing previous versions of the manuscript and D. Burdick for assisting with statistical analyses. A. McCollum, B. Subramanian, and J. Van Buskirk assisted in collecting field data. This study was supported financially by a grant from the Sigma Xi Foundation, the Duke University Department of Zoology graduate student research fund, and National Science Foundation grant BSR-8817899 to M.D.R.

APPENDIX A

DEMONSTRATION THAT EQUILIBRIA FOR ONE- AND TWO-SPECIES MODELS ARE EQUIVALENT WHEN NO INTERACTION OCCURS BETWEEN SPECIES

In this appendix we demonstrate that, when the parameter $a$ is equal to zero in the model presented in the text, the equilibrium level of resistance under the two-herbivore model is the same as it is under the one-herbivore model. First, consider the one-herbivore model. The equilibrium level of resistance is obtained by solving $dW/dR_1 = 0$. Differentiating equation (1) yields $dW/dR_1 = -dC_1/dR_1 - dH_1/dR_1$. Setting this equal to zero and rearranging gives

$$-dC_1/dR_1 = dH_1/dR_1.$$

(A1)

For the two-herbivore model, the equilibrium is obtained by solving the pair of equations $dW/dR_1 = 0$ and $dW/dR_2 = 0$. Differentiating equation (2), when $a = 0$, yields $dW/dR_1 = -dC_1/dR_1 - dH_1/dR_1$ and $dW/dR_2 = -dC_2/dR_2 - dH_2/dR_2$. The first of these, on rearrangement, yields equation (A1). Consequently, when $a = 0$, the equilibrium level of resistance to herbivore 1 in the two-herbivore model must satisfy the same condition as it does in the one-herbivore model, and thus the equilibria are the same for the two models.

When $a$ is not equal to zero, then the system of equations that must be satisfied at equilibrium is $dW/dR_1 = -dC_1/dR_1 - dH_1[1 - aH_2(R_2)]/dR_1 = 0$ and $dW/dR_2 = -dC_2/dR_2 - dH_2[1 - aH_1(R_1)]/dR_2 = 0$. It is clear that the solution of this set of equations yields an equilibrium level of resistance to herbivore 2 that differs from that specified by equation (A1). Thus, when $a$ is not equal to zero, the two-herbivore model predicts a different equilibrium level of resistance from that of the one-herbivore model.
APPENDIX B

DETERMINATION OF DENOMINATORS IN F-TESTS

For many source effects in mixed-model ANOVA, the appropriate denominator (error) for an F-test for that effect is not the model error mean square. These denominators were determined according to the model of Hocking (1985), by examining the expected mean squares generated with SAS GLM using a RANDOM statement (SAS Institute 1988). In many cases, the effect should be tested with an interaction mean square as the denominator. In other cases, however, the appropriate denominator must be approximated with a combination of mean square terms, which we will call a "compound denominator" (Satterthwaite approximate F-test; see Neter et al. 1985, pp. 851–853). The degrees of freedom associated with each compound denominator must also be estimated.

We used two criteria by which mean squares associated with nonsignificant variance components could be dropped from the F-test denominator both to simplify the calculation of the F-test and to increase the denominator degrees of freedom. In the case of the compound denominator, we tested whether the variance component associated with each mean square of the compound denominator was significantly different from zero; if so, that component was dropped from the denominator. In the case of the interaction as the F-test denominator, we tested the significance of the variance component associated with the interaction mean square. If it was not significant, we used the model error mean square as the denominator, with its higher degrees of freedom. In both cases, no component was dropped if the F statistic calculated for it was greater than 1.5. This second criterion controlled for Type I error in the subsequent F-test, thereby reducing bias that might have been introduced by incorrectly reducing the denominator mean square.

LITERATURE CITED


*Guest Editor: Douglas J. Futuyma*