COMPETITION AND TIME OF DAMAGE AFFECT THE PATTERN OF SELECTION ACTING ON PLANT DEFENSE AGAINST HERBIVORES

Peter Tiffin[1]

*Department of Zoology—Evolution, Ecology, and Organismal Biology Group, Duke University,
Durham, North Carolina 27708 USA*

Abstract. In this paper I examine the effects that the competitive environment in which plants are grown and the timing of herbivore damage have on the expression and pattern of selection acting on plant tolerance to herbivory and resistance to herbivores. I conducted a field experiment involving 720 plants from 24 full-sib families of the common morning glory, *Ipomoea purpurea*. Plants were grown in one of two competitive environments, and the levels of resistance and tolerance to naturally occurring folivory expressed by the 24 families were estimated three times during the growing season: soon after plants emerged, when plants began to flower, and when seed capsules began to mature. Low- and high-competition environments were established using different densities of interspecific competitors. Herbivory had a negative effect on plant fitness in both competitive environments, and consistent with the compensatory continuum hypothesis, herbivory was more detrimental in the high- than low-competition environments. Phenotypic analyses also revealed that early- and late-season damage were more detrimental than mid-season herbivore damage. Although fitness was more negatively affected by herbivore damage in the high- than low-competition environments, competition did not affect the pattern of selection acting on defense traits. Similarly, the patterns of selection acting on either resistance or tolerance to early-, mid-, and late-season herbivory did not differ significantly. Therefore, there was little evidence that environmental or seasonal differences in the pattern of selection acting on defense traits will constrain their evolution.

Key words: common morning glory; compensatory continuum; competition; costs; genotype by environment; herbivory; herbivory timing; Ipomoea purpurea; *phenotypic plasticity; resistance; tolerance.*

Introduction

Herbivores are generally accepted as having a detrimental effect on plant fitness. Therefore, plant traits that minimize the potentially negative effects of herbivores are expected to offer a fitness benefit and thus may be expected to evolve to maximal levels. Widespread genetic variation for defense traits, including both tolerance (the ability of a plant to incur herbivore damage without a corresponding reduction in fitness) and resistance (the ability of a plant to minimize the amount of herbivore damage incurred; Painter 1958), however, indicates that in many systems defense traits have not evolved to maximal levels. Models developed to explain why defense traits have not evolved to maximal levels have generally assumed that these traits involve costs (Rhoades and Cates 1976, Simms and Rausher 1987, Adler and Karban 1994, Tiffin 2000*a*). Attempts to detect costs of resistance and tolerance have focused primarily on three types of costs: allocation costs, measured as a negative correlation be-

tween defense and some component of fitness in the absence of herbivore damage (Berenbaum et al. 1986, Simms and Rausher 1989, Agren and Schemske 1993, Bergelson 1994, Mauricio 1998, Siemens and Mitchell-Olds 1998, Tiffin and Rausher 1999, Agrawal 2000); ecological costs, defined as a trade-off between different types of defense or between defense to one type of herbivore and susceptibility to a second type of herbivore (Berenbaum et al. 1986, Pilson 1996); and trade-offs between tolerance and resistance (van der Meijden et al. 1988, Fineblum and Rausher 1995, Shen and Bach 1997, Stowe 1998, Mauricio et al. 1997, Tiffin and Rausher 1999). These costs have not, however, been detected in all systems (reviewed in Simms 1992, Bergelson and Purrington 1996) suggesting that other mechanisms may be important in constraining the evolution of plant defense traits. In this study I investigate whether the evolution of either tolerance or resistance may be constrained by environmentally or seasonally dependent changes in the pattern of selection acting on these traits.

Numerous studies have shown that the expression of defense traits may depend on the environment in which plants are grown. There are several mechanistic reasons why environmental conditions may affect the expression of resistance. For example, nutrient availability may directly affect plant nutrient status that may in

turn affect plant attractiveness to herbivores or the production of secondary compounds that confer resistance (Bryant et al. 1983, Coley et al. 1985, Herms and Mattson 1992). There does not, however, appear to be a consistent pattern to the effects of nutrient conditions on plant resistance. In some situations resistance is higher when nutrients are limiting and in others resistance is reduced (reviewed in Gershenzon 1984, Waterman and Mole 1989). In contrast, investigations on tolerance and compensatory ability generally find that herbivore damage has more severe effects on fitness when plants are grown in low-resource or high-competition environments (Maschinski and Whitham 1989, Fay et al. 1996, Juenger and Bergelson 1997, but see Meyer and Root 1993). The mechanistic basis for environmental differences in tolerance is unclear but presumably results from resource limitations preventing plants to replace tissue or resources lost to herbivores.

The expression of tolerance and resistance also may change during a plant's lifetime. Developmental or seasonal changes may cause either increased or decreased levels of resistance and these changes may result for a variety of reasons, including induced resistance (reviewed in Karban and Baldwin 1997), interactions among herbivores (Karban 1987, Pilson 1992), changes in plant chemistry (Feeny 1970, Bowers and Stamp 1993, Jarzomski et al. 2000), or changes in the nutrient status of plant tissues (Feeny 1970, Mattson 1980). Seasonal changes are also expected to affect the expression of tolerance. Previous work in natural systems suggests that an important determinant of tolerance is the time plants have to regrow following damage. The later damage occurs, the less time plants have to induce mechanisms to compensate for that damage. As a result, plants are predicted to be less tolerant of damage that occurs late, rather than early, in the growing season (Maschinski and Whitham 1989, Juenger and Bergelson 1997).

Although the phenotypic expression of resistance and tolerance may depend on the environmental conditions in which plants are grown and may change during a plant's life, studies have not investigated whether the environment in which plants are grown affects the pattern of selection acting on these traits. If the selection favors different optimal levels of these traits when plants are grown in different environments then opposing patterns of selection may constrain evolution and prevent them from evolving to a optimal level (Via and Lande 1985). This, however, assumes that the expression of the trait is correlated positively across environments. If trait expression is negatively correlated then evolution will be constrained only if selection is acting in the same direction in both environments (Via and Lande 1985). If the expression of a trait in one environment is completely independent of the expression of that trait in other environments then there is apparently not a common genetic basis for these traits, they can be considered as distinct traits, and they can

evolve independently in the separate environments (Falconer 1952).

The primary objective of this study was to determine if environmentally dependent changes in the patterns of selection acting on tolerance and resistance affect the evolution of these traits. I examine both tolerance and resistance to early-, mid-, and late-season herbivore damage on plants grown in low- and high-competition environments. I use phenotypic analyses to gain insight into the ecological effects of herbivore damage on plant fitness, and I use genotypic analyses to determine whether the patterns of selection acting on these traits differ in low- and high-competition environments. Although phenotypic analyses may provide insight into the direction and magnitude of selection acting on a trait, phenotypic selection gradients may be biased by environmental factors that affect fitness and the expression of a trait (Lande and Arnold 1983, Mitchell-Olds and Shaw 1987). Analyses of selection acting on genotypic values minimize this potential problem (Rausher 1992b). I address these objectives using data from an experimental population of *Ipomoea purpurea*, the common morning glory, in which plants were grown in one of two competitive environments in a field setting.

MATERIALS AND METHODS

Experimental system

Plant species.—*Ipomoea purpurea* Roth (Convolvulaceae), the common morning glory, is a self-compatible annual vine commonly found in agricultural fields and disturbed areas throughout the southeastern United States. Interspecific competition can have major effects on plant growth and fitness in these environments (*personal observation*; R. E. Miller, *unpublished data*). In North Carolina, plants emerge between May and August and die with the first frost, usually in October or November. Flowers are generally produced within six weeks of emergence. Plants can bear multiple flowers daily and individual flowers last only one day. After plants begin flowering they continue to flower until they begin to senesce or are killed by frost. Dehiscent fruits, each of which contains 5–6 seeds, mature within ~four weeks of fertilization. Mature fruits typically remain on plants for longer than one week before dehiscing. The leaves are eaten by several different herbivores including three specialists on the family Convolvulaceae (two species of tortoise beetles *Deloyla guttata* and *Charidatilla* [=Metriona] *bicolor* [Coleoptera: Chrysomelidae]), and the sweet potato flea beetle *Chaetocnema confinis* (Coleoptera: Altinacae), as well as generalist lepidopteran larvae, grasshoppers and an unidentified weevil (Coleoptera: Curculionidae).

Experimental methods

The experiment involved 720 plants from twenty-four maternal full-sib families grown in a field in Dur-

ham County, North Carolina. Seeds for experimental plants were obtained by mating each of eight pollen parents to three seed parents resulting in 24 full-sib families. Parental plants were tenth generation single-seed-descent inbred lines derived from seeds that were initially collected from an agricultural field in Durham County, North Carolina in the fall of 1989. Inbred lines were used as parent plants in order to generate full-sib families with nearly identical genotypes. Minimizing genetic variation within full-sib families was deemed advantageous since tolerance cannot be measured at the level of an individual (Rausher 1992a). Flowers of seed parents were emasculated the evening prior to flower opening in order to prevent self pollination and were hand pollinated the following morning. All crosses were made from March through May of 1997 using plants growing in a greenhouse.

On 29 June 1997, 30 seeds from each full-sib family (720 total seeds) were planted into a previously plowed and disked field in Durham County, North Carolina. Seeds were scarified the day prior to planting and planted into eight spatial blocks (due to space restrictions, two blocks had only three seeds per family, while the other six blocks each had four seeds from each family). Seeds were planted at 70-cm spacing within rows. Spacing between rows was 100 cm. Each plant was allowed to twine up a 1.5 m tall wooden stake, mimicking growth in agricultural fields and allowing easy identification of individual plants. All plants had emerged within seven days of planting. Plants began flowering on 18 August and seed capsules began maturing on 23 September.

Individuals from each family were grown in two competitive environments: low and high levels of interspecific competition in a randomized block split-plot design in which competition was applied as a split-plot treatment. The low-competition treatment was kept relatively weed free by a combination of hoeing and hand weeding prior to planting, and weekly during the first four weeks after planting. The high-competition treatment was established by seeding crabgrass, *Digitaria sanguinalus*, two weeks prior to planting. The high-competition treatment was not weeded with the exception of a circular area (~10 cm radius) into which experimental plants were seeded. The 10 cm radius area around experimental plants was kept weed free for the first six weeks after planting. In addition, to lessen the severity of competition in the high-competition environment, weeds were removed from an ~30 cm wide strip between each of the rows one month after planting. Nonexperimental morning glories were removed from both treatments throughout the experiment. The density of plant competitors at the end of the season was 233 plants/m^2 and 410 plants/m^2 in the low- and high-competition treatments, respectively.

Total leaf area on each plant was measured at three times during the growing season (early season 15–17 July, mid season 9–11 August, and late season 3–5 September) by counting the number of expanded leaves and measuring the length of each leaf. Leaf length was converted to leaf area using the relationship: Area = $0.67 \times$ Length$^{2.088}$ ($R^2 = 0.97$; Tiffin and Rausher 1999). Late- and mid-season correspond to two weeks prior to the time at which the first seed capsules began to mature and one week prior to the time at which plants began to flower, respectively. At the time leaf area was measured, the leaf area missing due to feeding by folivores was also recorded. These measurements were made by overlaying each leaf with a clear plastic 0.10-cm^2 grid and recording the number of squares in which the leaf tissue was missing (Simms and Rausher 1989). Leaf area missing was converted to proportion damage by dividing total leaf area missing by total leaf area.

Seeds were collected from each plant during eight rounds of harvesting lasting from the time the first capsules matured until 17 November when a hard frost killed all plants. Viable seeds were counted and this number was used as an estimate of fitness of each plant. Individuals that did not emerge or died within one week of emergence were excluded from analysis. This resulted in excluding 16 individuals from the analysis. Likelihood ratio chi-square tests revealed no significant effect of full-sib family on the likelihood of individuals to survive ($P > 0.40$) although significantly more individuals survived in the low- than in the high-competition environments (1 compared to 15 deaths, $P < 0.001$).

Data analysis

Phenotypic analyses.—Analysis of variance was used to determine whether the phenotypic effects of herbivore damage on plant fitness differed among competitive environments and at different times during the growing season. To normalize the residuals, relative fitness (fitness divided by mean fitness) values were cube-root transformed prior to conducting this analysis. Following ANOVA, path analyses, a general form of multiple regression (Li 1975, Kingsolver and Shemske 1991, Mitchell 1993), were used to examine the relationships among early-, mid-, and late-season damage, and the putative causal relationships between each of these traits and plant fitness. Because ANOVA revealed that competitive environment had a significant effect on the relationship between damage and fitness path analyses were conducted separately for the low- and high-competition environments. The standardized multiple regression coefficients reported in path diagrams (Fig. 1) were calculated after transforming the independent variables to have a mean equal to zero and a standard deviation equal to one. Transforming fitness could change the magnitude of the relation between fitness and the independent variables and therefore, with the exception of changing fitness values to relative fitness values, fitness was not transformed for these analyses. The TEST option of the REG procedure was used to test for significant differences in the effect ear-
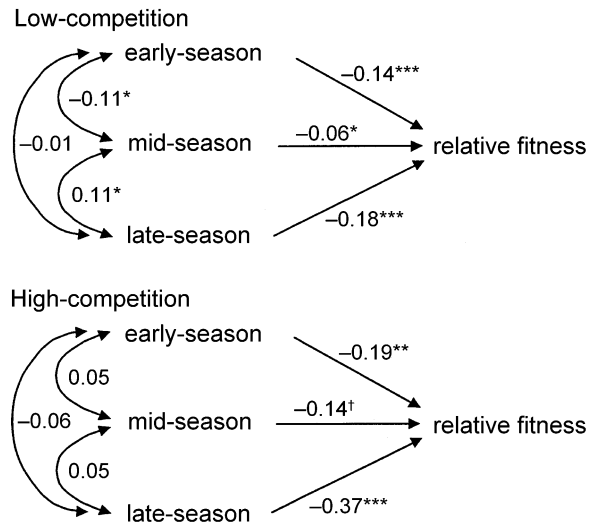
Low-competition

High-competition

FIG. 1. Path diagram showing the relationships between plant fitness and early-, mid-, and late-season herbivore damage in the low-competition and high-competition environments. The double-headed arrows represent phenotypic correlations, and the single-headed arrows represent causal relationships between damage and plant fitness.

$\dagger P < 0.10$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

ly-, mid-, and late-season herbivore damage had on plant fitness.

Pearson correlation coefficients were calculated to evaluate the relationships between the amounts of damage experienced by individual plants at different times during the growing season. Because testing the significance of correlation coefficients using parametric methods assumes a normal distribution of data and because the distribution of early-, mid-, and late-season damage data were skewed, these data were cube-root transformed prior to calculating the correlations. Pearson correlation coefficients calculated from untransformed data were of similar magnitude as those calculated on transformed data and only those calculated on transformed data are reported.

Variation for tolerance and resistance.—To test for the presence of genetic variation for fitness and tolerance, the GLM procedure of the SAS statistical software (version 6.12; SAS Institute 1996) was used to conduct analysis of variance. For these analyses, relative number of seeds produced (an estimate of relative fitness) was the response variable. Relative fitness values were cube-root transformed to achieve a normal distribution of residuals. A significant interaction between full-sib family and leaf-area damage indicates that herbivory did not affect fitness of all families equally and is evidence of genetic variation for tolerance (Simms and Triplett 1994).

To test for the presence of genetic variation for resistance to early-, mid-, and late-season folivory, the MANOVA option of the GLM procedure in SAS was used to conduct multivariate analysis of variance. A significant MANOVA indicates significant treatment effects but does not identify which of the response variables were significantly affected. To identify which response variables differed significantly among families the MANOVA was followed by univariate ANOVAs in which proportion leaf area missing in the early-, mid-, and late-season were the response variables. The proportion of leaf area missing was cube-root transformed prior to analysis in order to minimize deviations from normality.

Estimates of tolerance and resistance.—For individual families, estimates of tolerance to folivory were made by calculating the regression coefficient of relative fitness onto proportion damage separately for each full-sib family (Mauricio et al. 1997, Tiffin and Rausher 1999). Estimates of resistance for each full-sib family were calculated as one minus the mean level of damage experienced all individuals within that family. Estimates of both tolerance and resistance were made on residual variables after the effects of block had been removed and separate analyses were conducted for early-, mid-, and late-season damage and low- and high-competition environments. Preliminary analysis revealed no evidence for nonlinear effects of folivory on fitness within each environment and thus regressions used to estimate tolerance for individual families included only linear terms. Transforming fitness values could affect the shape of the relationship between fitness and damage and therefore fitness values were not transformed prior to conducting these analyses.

Because the experimental plants were derived from only eight pollen parents there was little power to detect significant differences among paternal half-sib families in this experiment. For this reason all analyses were conducted using maternal full-sib families. Estimates of genetic variation based on maternal full-sib families are potentially biased by nonadditive genetic and maternal effects (Falconer 1989). Maternal environmental effects in this study are expected to be small because the parents of the experimental plants grew in a common greenhouse environment. I have no estimate, however, of the amount of genetic variation due to dominance. Therefore, the estimates of genetic variation reported in this study should be viewed as an upper limit to possible additive genetic variation that is present in the population from which the experimental plants originated.

Correlations among genotypic values.—Pearson correlation coefficients were calculated to determine if the genotypic expression of resistance and tolerance in the two competitive environments or at different times during the growing season were correlated. In addition, competition by resistance and competition by tolerance interaction terms from ANOVAs were examined to determine if there was statistical support for across-environment correlations being different from one.

Genetic selection analyses.—The partial-regression

TABLE 1. Phenotypic analysis of the effects of competition and herbivore damage on fitness of *Ipomoea purpurea*.

Source	df	Type III SS	F	P
Block	7	30.83	21.6	0.0001
Competition†	1	126.45	17.9	0.0083
Block × Competition	7	7.05	4.9	0.0001
Early-season damage	1	6.37	31.2	0.0001
Mid-season damage	1	1.76	8.6	0.0034
Late-season damage	1	10.89	53.4	0.0001
Early season × Competition	1	1.85	9.1	0.0027
Mid-season × Competition	1	0.28	1.4	0.2431
Late season × Competition	1	4.50	22.1	0.0001

† Significance of competition was tested using the Block × Competition term as the denominator in the F test.

analysis described by Rausher (1992*b*) was used to characterize the type and magnitude of selection acting on tolerance and resistance except that full-sib family means rather than breeding values were analyzed. In this analysis, the directional selection gradient acting on each character was estimated by the partial regression coefficients of a linear regression of the family mean of relative fitness on tolerance. This analysis is similar to that described by Lande and Arnold (1983) except that it is based on genotypic or breeding values rather than the phenotype of individuals. Analyzing genotypic rather than phenotypic values insures that the covariance between fitness and trait values, which is the statistical basis for inferring selection, is determined genetically and not solely by environmental factors (Rausher 1992*b*). Moreover, because tolerance cannot be measured on an individual a genotypic method is more appropriate for estimating selection on this trait, even if environmental factors are not expected to bias estimates of selection. Following standard methods relative fitness was not transformed prior to conducting these analyses (Lande and Arnold 1983, Mitchell-Olds and Shaw 1987). The independent variables were standardized to have a mean equal to zero and standard deviation equal to one. Selection gradient acting in the low- and high-competition environments were estimated separately because competition had a significant effect on the fitness of full-sib families, a marginally significant effect on the expression of tolerance to mid-season damage, and significant effects on the phenoytpic relationship between damage and fitness (see *Results*). Because the genetic selection analysis is only applicable to genetically variable traits, selection gradients were estimated only for traits for which significant variation among families was detected. Preliminary analyses revealed that including all traits had little effect on the estimated selection gradients. Finally, because of limited sample size, nonlinear terms were not included in the selection analyses.

RESULTS

Phenotypic effects of herbivory

Analysis of variance revealed that both competition and herbivore damage had significant effects on plant fitness (Table 1). Moreover, the competitive environment in which plants were grown significantly altered the effects early- and late-season herbivore damage had on plant fitness. There was no evidence that competition had a significant effect on the relationship between mid-season damage and fitness. Despite the significant interactions, path analyses revealed that plant fitness was affected negatively by both early- and late-season herbivore damage in both competitive environments, although damage had more negative effects in the high-competition than low-competition environments (Fig. 1). In both competitive environments early- and late-season herbivore damage had more negative effects on fitness than mid-season damage (Early vs. mid: $F_{1,344}$ = 4.8 and P = 0.03, $F_{1,329}$ = 6.8 and P = 0.01, in the low- and high-competition environments, respectively; mid vs. late: $F_{1,344}$ = 3.9 and P = 0.048, $F_{1,329}$ = 3.2 and P = 0.07, in the low- and high-competition environments, respectively) but the effects of early- and late-season damage did not differ significantly from one another (Early vs. late: $F_{1,344}$ = 0.57 and P = 0.45, $F_{1,329}$ = 0.41 and P = 0.49, in the low- and high-competition environments, respectively). In other words, phenotypic analyses indicate that the plants in this experiment were less tolerant of early-season and late-season damage than mid-season damage and were less tolerant of damage when grown in high-competition than low-competition environments.

Competition and time of season also had significant effects on the amount of damage plants experienced (Tables 2 and 3). Plants grown in the low-competition environment incurred more early-season damage than plants grown in the high-competition environment, but by the end of the growing season, plants grown in the high-competition environment had lost significantly more leaf area to herbivores than those plants growing in the low-competition environment.

Variation among genetic families

Phenotypic analyses indicated that selection may be acting on resistance and tolerance. However, selection acting on phenotypes will result in evolutionary change only if there is genetic variation for these traits, there is genetic variation for fitness, and the covariance be-

TABLE 2. Mean fitness and level of early-, mid-, and late-season damage in the low- and high-competition environments.

Trait	Low competition Mean	SD	High competition Mean	SD
Fitness	817	616	61	99
Early-season damage	0.114	0.169	0.083	0.158
Mid-season damage	0.059	0.045	0.070	0.079
Late-season damage	0.032	0.032	0.082	0.090

Note: As indicated in Table 1, competitive environment had a significant effect on the amount of early-season and late-season damage that plants experienced.

TABLE 3. Multivariate and univariate analyses of variance testing for significant variation among the full-sib families for resistance to early- and late-season folivory.

| | | MANOVA | | | Univariate analyses | | | | | | | | |
| | | | | | Early-season folivory | | | Mid-season folivory | | | Late-season folivory | | |
Source of variation	df†	Wilks' λ	F	P	Type III ss	F	P	Type III ss	F	P	Type III ss	F	P
Block	7	0.84	5.19	0.0001	2.75	6.93	0.0001	0.73	5.50	0.0001	0.30	2.93	0.0051
Competition‡	1	0.86	8.70	0.001	1.11	9.17	0.038	0.09	0.85	0.90	1.10	16.9	0.008
Block × Competition	7	0.88	3.87	0.0001	0.85	2.14	0.038	0.72	5.45	0.0001	0.46	4.44	0.0001
Full sib	23	0.85	1.51	0.005	2.25	1.71	0.019	0.49	1.13	0.31	0.57	1.67	0.026
Full sib × Competition	23	0.89	1.01	0.45	1.61	1.23	0.21	0.36	0.82	0.70	0.37	1.08	0.36
Error	625				36.1			11.8			9.55		

† Degrees of freedom for univariate analyses; df for MANOVA were 21, 1780.86; 3, 620; 21, 1780.86; 69, 1853.09; 69, 1853.09; for Block, Competition, Block × Competition, Full-sib, and Full-sib × Competition, respectively.

‡ Significance of competition was tested over Block × Competition term.

tween fitness and the traits is not due solely to environmental factors (Mitchell-Olds and Shaw 1987, Rauscher 1992b). ANOVA revealed that both fitness and the effect competition had on fitness varied significantly among the families (Table 4). The model used to test for variation in fitness did not include any measure of herbivore damage. If herbivore damage terms had been included the effects of herbivory would have been statistically removed from the analysis. However, herbivore damage may have contributed to variation in fitness among families and therefore it is appropriate to test for variation in fitness using a model that does not contain measures of herbivore damage.

Analysis of variance revealed significant genetic variation for tolerance to early- and mid-season herbivory but no evidence for genetic variation for tolerance to late-season herbivory (Table 5). A multivariate analyses of variance revealed significant genetic variation for resistance to folivory (Wilks' lambda $P < 0.005$) but provided no evidence that genetic variation for resistance was significantly affected by competition (the two-way interaction term family × competition was not significant, Table 3). Univariate ANOVAs revealed that the significant genetic variation detected by the MANOVA was largely due to significant genetic variation for resistance to early- and late-season damage (Table 3). There was no evidence that full-sib families differed in their resistance to mid-season damage.

TABLE 4. ANOVA testing for variation among the full-sib families for fitness.

Source	df	Type III ss	F	P
Block	7	555	22.3	0.0001
Competition†	1	5020	301	0.0001
Block × Competition	7	116.9	4.69	0.0001
Full sib	23	148.8	1.82	0.0115
Competition × Full sib	23	140.0	1.71	0.0210
Error	624	221.4		

† Signficance of competition was tested using the Block × Competition term as the denominator in the F test.

Correlations among environments

There were weak but significant phenotypic correlations between the amount of early- and mid-season damage and between mid- and late-season damage in the low-competition environment. These correlations were, however, opposite in sign. Early- and mid-season damage were negatively correlated whereas the correlation between mid-season and late-season damage was significantly positive. In the high-competition environment, none of the correlations were significantly different from zero.

Whereas the phenotypic correlations were significant, but weak, this experiment revealed no evidence that the genotypic expression of tolerance and resistance was correlated either across environments or across different times in the growing season. The one exception to this was the significant positive correlation between early- and mid-season tolerance in the high-competition environment (Fig. 2). The Pearson correlation coefficients provided no evidence that the expression of resistance, or tolerance at any time during the growth season were correlated across competitive environments.

Although this experiment revealed evidence for only one significant genetic correlation there was little statistical power to detect significant correlations and therefore this finding should be interpreted with caution. As mentioned above, the variances of among-family values are inflated by within-family error variance. These inflated variance terms, in turn, inflate the denominator of the correlation coefficient causing the calculated correlation coefficient to be an underestimate of the true family mean correlation (Arnold 1981, Via 1984). The limited power to detect significant correlations is evident by the fact that although only one of the calculated correlations was significantly different from zero, ANOVA revealed little evidence that the expression of either tolerance or resistance across competitive environments or times during the growing sea-

TABLE 5. Analysis of variance testing for significant genetic variation in tolerance.

Source	df	Type III ss	F	P
Block	7	391.4	20.1	0.0001
Competition (Comp)†	1	1055	380	0.0001
Block × Competition	7	85	4.37	0.0001
Full sib	23	109	1.71	0.022
Early-season damage (Propd 1)	1	0.3	0.12	0.72
Mid-season damage (Propd 2)	1	2.9	1.04	0.31
Late-season damage (Propd 3)	1	57.8	20.8	0.0001
Competition × Full sib	23	69.0	1.08	0.36
Early season × Competition	1	4.7	1.71	0.19
Mid-season × Competition	1	0.00	0.02	0.89
Late season × Competition	1	26.8	9.67	0.002
Propd1 × Full sib	23	102	1.60	0.039
Propd2 × Full sib	23	157	2.45	0.0002
Propd3 × Full sib	23	71.8	1.13	0.31
Propd1 × Comp × Full sib	23	72.7	1.14	0.29
Propd2 × Comp × Full sib	23	95.7	1.50	0.06
Propd3 × Comp × Full sib	23	77.2	1.21	0.23

Note: Significant family-by-damage interaction terms are equivalent to significant genetic variation for tolerance.

† Significance of competition was tested using the Block × Competition term.

son were significantly different from one (the trait by competition interactions were not significant).

Selection acting on tolerance and resistance

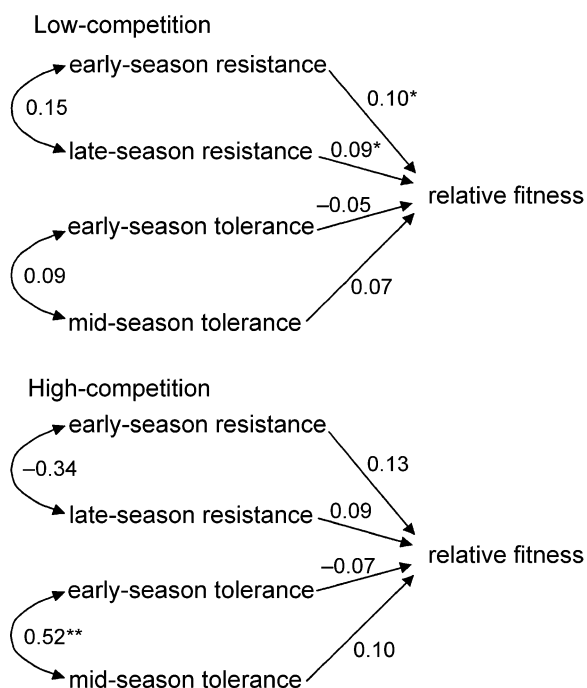The genotypic selection gradients acting on defense traits were similar in the two environments. Higher

FIG. 2. Path diagram showing genotypic selection gradients acting on early- and late-season resistance and on early- and mid-season tolerance in the low-competition and high-competition environments. The double-headed arrows represent genotypic correlations, and the single-headed arrows represent selection gradients. Only genetically variable traits are included in the diagram.

*P < 0.05; **P < 0.01.

fitness was associated with higher levels of early- and late-season resistance, with lower levels of early-season tolerance, and with higher levels of mid-season tolerance (Fig. 2). Only selection gradients acting on resistance in the low-competition environment were, however, significantly different from zero. Moreover, there was no evidence for significant differences in the strength of selection acting on early- vs. late-season resistance or on early- vs. mid-season tolerance. In both competitive environments the estimates of selection acting on early- vs. mid-season tolerance were opposite in sign but the estimates were not significantly different from one another ($P = 0.49$ and $P = 0.12$ in the low- and high-competition environments, respectively).

DISCUSSION

This experiment revealed that the effect herbivore damage had on plant fitness differed significantly between the low-competition and high-competition environments and at different times during the growing season. Despite these significant effects, phenotypic analyses revealed that herbivore damage negatively affected plant fitness at all times during the growing season and in both competitive environments. Therefore in the absence of allocation or ecological costs, selection is expected to favor increased levels of resistance, which would reduce levels of herbivore attack, and increased levels of tolerance, which would reduce the negative impacts of herbivory on plant fitness. The estimates of the genotypic selection gradients, although generally not significantly different from zero, were generally consistent with expectations from the phenotypic analysis. In both competitive environments selection favored increased levels of early- and late-season resistance as well as mid-season tolerance. In contrast, the estimated selection gradients acting on early-season tolerance were negative, indicating that

selection favored lower levels of early-season tolerance. However, the genotypic selection gradients acting on both resistance and tolerance did not differ significantly either across environments or at different times in the growing season. For this reason, the results from this experiment do not provide evidence that environmentally or seasonally dependent changes in selection gradients will constrain defense traits from evolving to optimal levels.

Even if the selection gradients acting in different environments had opposed one another the results from this experiment would not provide strong evidence that the evolution of these traits is constrained either by the environment in which plants are grown or by changes in the expression of defense during the growing season. In order for environmentally dependent changes in selection to constrain trait evolution it is necessary that the expression of the trait be strongly correlated across those environments (Via and Lande 1985). This experiment revealed little evidence that the genotypic expression of either tolerance or resistance is correlated either across environments or at different times during the growing season. The only significant correlation detected was between early- and mid-season tolerance expressed in the high-competition environment.

Because of limited statistical power the absence of significant correlations needs to be viewed with caution. Nevertheless, there are at least two reasons to think that the expression of resistance may not always be highly correlated across environments. First, correlations between expression of resistance across different environments have not been detected in all systems in which they have been investigated (Pilson 1992, Bowers and Stamp 1993, Stinchcombe and Rausher 2001, but see Siemens and Mitchell-Olds 1998), and second, the phenotypic correlations in this experiment, although significantly different from zero, were weak. Previous studies have not examined if genotypes that express high tolerance in one environment also express high tolerance in other environments. Therefore there are no other data to determine whether weak across-environment correlations are common. However, given that mechanisms of tolerance may be affected by the environment in which plants are grown (Trumble et al. 1993) it may be reasonable to expect that genotypes that have high tolerance in one environment or to damage at one time during the growing season may not be highly tolerant to damage in all environments or at all times during the growing season.

Compensatory continuum hypothesis

This experiment provides little evidence that differences in the time of herbivore damage or the competitive environment in which plants are grown will constrain the evolution of tolerance and resistance. However, the data from this experiment do indicate that the effects of herbivory on plant fitness differ among environments. The phenotypic analyses of the data revealed that damage is more detrimental in high-competition (low-resource) than in low-competition (high-resource) environments and that mid-season herbivore damage was less detrimental to plant fitness than either early- or late-season damage; whereas early- and late-season damage was equally detrimental.

The effect that competitive environment had on the relationship between herbivory and plant fitness is consistent with the prediction of the compensatory continuum hypothesis (Maschinski and Whitham 1989) that plants grown in resource-rich environments will be more tolerant of herbivore damage than plants grown in resource-limited environments. In contrast, the results are less consistent with predictions of the compensatory continuum hypothesis that plants are more tolerant of damage that occurs early compared to late in the growing season. The data from this experiment are more consistent with data from agricultural systems that suggest that the expression of tolerance to herbivore damage is (1) low immediately following germination, (2) increases until the onset of flowering, (3) is reduced during flowering, and (4) again increases as reproductive structures mature (Trumble et al. 1993).

Much of the data that support the prediction that early-season damage should be less deterimental than late-season damage has come from experiments in which plants have been subjected to removal of vegetative and/or floral meristems or single episodes of severe defoliation (Maschinski and Whitham 1989, Juenger and Bergelson 1997, Lennartsson et al. 1998). These types of episodic damage may be typical of the damage that plants experience in grazing systems. However, damage by insect herbivores, the type of damage that was studied in this experiment, is likely to occur throughout a growing season and in many cases leaf area lost to insects is likely to be much less than leaf area lost to grazers.

It is reasonable to expect that the mechanisms and thus the expression of tolerance will depend on the timing and pattern of damage that plants experience (Whitham et al. 1991, Trumble et al. 1993, Tiffin 2000b). For example, in order for plants to reproduce following severe defoliation or meristem removal it may be necessary for them to replace tissue that was lost to herbivores. It is certainly true that nonclonal plants subject to loss of floral meristems need to regrow before they are able to reproduce. If a plant needs to replace parts that are consumed by herbivores there may be a direct relationship between the time available to regrow and tolerance to that damage. In contrast, tolerance to the loss of a small percentage of leaf area to insect herbivores may not induce mechanisms that replace lost tissue. Rather, tolerance to minor defoliation may result from changes in resource allocation or the degree to which plant fitness is limited by photosynthate relative to water or other nutrients (Welter 1989, Trumble et al. 1993). The effectiveness of these mechanisms may be more dependent on a plant's phys-

iological state at the time of damage and thus less dependent on the time between when damage occurs and the end of the growing season. Regardless of mechanisms, the results presented here suggest that there may not be a simple relationship between the time at which damage occurs and the ability of plants to tolerate that damage. Moreover, these results suggest that it may be necessary to consider the type and magnitude of damage in order to gain a fuller understanding of the ecological dynamics of tolerance to herbivore damage.

Literature Cited

Adler, F. R., and R. Karban. 1994. Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. American Naturalist **144**: 813–832.

Agrawal, A. A. 2000. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). Ecology **81**:1804–1813.

Agren, J., and D. W. Schemske. 1993. The cost of defense against herbivores: an experimental study of trichome production in *Brassica rapa*. American Naturalist **141**:338–350.

Arnold, S. J. 1981. Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. Evolution **35**:489–509.

Berenbaum, M. R., A. R. Zangerl, and J. K. Nitao. 1986. Constraints on chemical coevolution: wild parsnips and the parsnip webworm. Evolution **40**:1215–1228.

Bergelson, J. 1994. The effect of genotype and environment on costs of resistance in lettuce. American Naturalist **143**: 349–359.

Bergelson, J., and C. B. Purrington. 1996. Surveying patterns in the cost of resistance in plants. American Naturalist **148**: 536–558.

Bowers, M. D., and N. E. Stamp. 1993. Effects of plant age, genotype, and herbivory on *Plantago* performance and chemistry. Ecology **74**:1778–1791.

Bryant, J. P., F. S. Chapin, III, and D. R. Klieg. 1983. Carbon/ nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos **40**:357–368.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science **230**: 895–899.

Falconer, D. S. 1952. The problem of environment and selection. American Naturalist **86**:293–298.

Falconer, D. S. 1989. Introduction to quantitative genetics. Third edition. Longman Scientific and Technical, Essex, UK.

Fay, P. A., D. C. Hartnett, and A. K. Knapp. 1996. Plant tolerance of gall-insect attack and gall-insect performance. Ecology **72**:521–534.

Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology **51**:565–581.

Fineblum, W. L., and M. D. Rausher. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature **377**:517–520.

Gershenzon, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. Recent Advances in Phytochemistry **10**:273–320.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. Quarterly Review of Biology **67**:284–335.

Jarzomski, C. M., N. E. Stamp, and M. D. Bowers. 2000. Effects of plant phenology, nutrients and herbivory on growth and defensive chemistry of plantain, *Plantago lanceolata*. Oikos **88**:371–379.

Juenger, T., and J. Bergelson. 1997. Pollen and resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. Ecology **78**:1684–1695.

Karban, R. 1987. Herbivory dependent on plant age: a hypothesis based on acquired resistance. Oikos **48**:336–337.

Karban, R., and I. T. Baldwin. 1997. Induced responses to herbivory. Chicago University Press, Chicago, Illinois, USA.

Kingsolver, J. G., and D. W. Schemske. 1991. Path analyses of selection. Trends in Ecology and Evolution **6**:276–281.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210–1226.

Lennartsson, T., P. Nilsson, and J. Tuomi. 1998. Induction of overcompensation in the field gentian, *Gentianella campestris*. Ecology **79**:1061–1072.

Li, C. C. 1975. Path analysis: a primer. Boxwood, Pacific Grove, California, USA.

Maschinski, J., and T. G. Whitham. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. American Naturalist **134**:1–19.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics **11**: 119–161.

Mauricio, R. 1998. Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. American Naturalist **151**:20–28.

Mauricio, R., M. D. Rausher, and D. S. Burdick. 1997. Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? Ecology **78**:1301–1311.

Meyer, G. A., and R. B. Root. 1993. Effects of herbivorous insects and soil fertility on reproduction of goldenrod. Ecology **74**:1117–1128.

Mitchell, R. J. 1993. Path analysis: pollination. Pages 211–231 *in* S. M. Scheiner and J. Gurevich, editors. Design and analysis of ecological experiments. Chapman and Hall, New York, New York, USA.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution **41**:1149–1161.

Painter, R. H. 1958. Resistance of plants to insects. Annual Review of Entomology **3**:367–390.

Pilson, D. 1992. Relative resistance of goldenrod to aphid attack: changes through the growing season. Evolution **46**: 1230–1236.

Pilson, D. 1996. Two herbivores and constraints on selection for resistance in *Brassica rapa*. Evolution **50**:1492–1500.

Rausher, M. D. 1992*a*. Natural selection and the evolution of plant-insect interactions. Pages 20–88 *in* B. D. Roitberg and M. B. Isman, editors. Insect chemical ecology: an evolutionary approach. Routledge Chapman and Hall, New York, New York, USA.

Rausher, M. D. 1992*b*. The measurement of selection of quantitative traits: biases due to environmental covariances between traits and fitness. Evolution **46**:616–626.

Rhoades, D. F., and R. G. Cates. 1976. Towards a general theory of plant antiherbivore chemistry. Recent Advances in Phytochemistry **10**:168–213.

SAS Institute. 1996. SAS/STAT users' guide. Version 6. Fourth edition. SAS Institute, Cary, North Carolina, USA.

Shen, C. S., and C. E. Bach. 1997. Genetic variation in resistance and tolerance to insect herbivory in *Salix cordata*. Ecological Entomology **22**:335–342.

Siemens, D. H., and T. Mitchell-Olds. 1998. Evolution of pest-induced defenses in *Brassica* plants: tests of theory. Evolution **79**:632–646.

Simms, E. L. 1992. Costs of plant resistance to herbivory. Pages 392–425 *in* R. S. Fritz and E. L. Simms, editors. Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. University of Chicago Press, Chicago, Illinois, USA.

Simms, E. L., and M. D. Rausher. 1987. Costs and benefits of plant resistance to herbivory. American Naturalist **130**:570–581.

Simms, E. L., and M. D. Rausher. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. Evolution **43**:573–585.

Simms, E. L., and J. Triplett. 1994. Costs and benefits of plant response to disease: resistance and tolerance. Evolution **48**:1973–1985.

Stinchcombe, J. R., and M. D. Rausher. 2001. Diffuse selection on resistance to deer herbivory in the Ivyleaf morning glory, *Ipomoea hederacea*. American Naturalist **158**:376–388.

Stowe, K. A. 1998. Experimental evolution of resistance in *Brassica rapa*: correlated response of tolerance in lines selected for glucosinolate content. Evolution **52**:703–712.

Tiffin, P. 2000*a*. Are tolerance, avoidance, and antibiosis evolutionarily and ecologically equivalent responses of plants to herbivores? American Naturalist **155**:128–138.

Tiffin, P. 2000*b*. Mechanisms of tolerance to herbivore damage: what do we know? Evolutionary Ecology **14**:523–536.

Tiffin, P., and M. D. Rausher. 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory, *Ipomoea purpurea*. American Naturalist **154**:700–716.

Trumble, J. T., D. M. Kolodny-Hirsch, and I. P. Ting. 1993. Plant compensation for arthropod herbivory. Annual Review of Entomology **38**:93–119.

van der Meijden, E., M. Wijn, and H. J. Verkaar. 1988. Defense and regrowth, alternative plant strategies in the struggle against herbivores. Oikos **51**:355–363.

Via, S. 1984. The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlations in larval performance within and across host plants. Evolution **38**:896–905.

Via, S., and R. Lande. 1985. Genotype–environment interaction and the evolution of phenotypic plasticity. Evolution **39**:505–522.

Waterman, P. G., and S. Mole. 1989. Extrinsic factors influencing production of secondary metabolites in plants. Pages 107–134 *in* E. A. Bernays, editor. Insect–plant interactions. CRC Press, Boca Raton, Florida, USA.

Welter, S. C. 1989. Arthropod impact on plant gas exchange. Pages 135–150 *in* E. A. Bernays, editor. Insect–plant interactions. Volume 1. CRC Press, Boca Raton, Florida, USA.

Whitham, T. G., J. Mashinski, K. C. Larson, and K. N. Paige. 1991. Plant responses to herbivory: the continuum from negative to positive and underlying physiological mechanisms. Pages 227–256 *in* P. W. Price, T. M. Lewinshogn, G. W. Fernandes, and W. W. Benson. Plant–animal interactions: evolutionary ecology in tropical and temperate regions. John Wiley and Sons, New York, New York, USA.