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ENVIRONMENTAL DEPENDENCY IN THE EXPRESSION OF COSTS OF TOLERANCE TO DEER HERBIVORY

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Abstract.—Plant tolerance to natural enemy damage is a defense strategy that minimizes the effects of damage on fitness. Despite the apparent benefits of tolerance, many populations exhibit intermediate levels of tolerance, indicating that constraints on the evolution of tolerance are likely. In a field experiment with the ivyleaf morning glory, costs of tolerance to deer herbivory in the form of negative genetic correlations between deer tolerance and fitness in the absence of damage were detected. However, these costs were detected only in the presence of insect herbivores. Such environmental dependency in the expression of costs of tolerance may facilitate the maintenance of tolerance at intermediate levels.

Key words.—Costs, herbivory, *Ipomoea hederacea,* plant defense, tolerance.

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Tolerance is the ability of plants to sustain damage without a concurrent loss of fitness (Painter 1958). Since herbivore damage usually has a negative impact on plant fitness (e.g., Marquis 1992; but see Paige and Whitham 1987; Lennartsson et al. 1998), more tolerant genotypes should be favored by natural selection because of their ability to mitigate the negative fitness consequences of damage. As such, widespread evidence of intermediate levels of tolerance to herbivore and pathogen damage in natural plant populations (e.g., Simms and Triplett 1994; Fineblum and Rausher 1995; Mauricio et al. 1997; Stowe 1998; Tiffin and Rausher 1999; Stowe et al. 2000) represents a paradox: given the apparent selective advantage of tolerance, what forces prevent maximal levels of tolerance from evolving? One possibility that has been the focus of several investigations is that physiological costs constrain tolerance from evolving to maximal levels.

Physiological costs of tolerance are manifested as negative genetic correlations between tolerance and fitness in the absence of damage—because in the absence of damage, costs but not benefits of tolerance are expressed (Simms and Triplett 1994). Such physiological costs of tolerance can prevent the evolution of maximal levels of tolerance and have been reported twice: for tolerance to fungal pathogen and insect herbivore damage in the common morning glory, *Ipomoea purpurea* (Simms and Triplett 1994; Tiffin and Rausher 1999). However, significant physiological costs of tolerance have not been detected in other systems (Mauricio et al. 1997; Agrawal et al. 1999; Fornoni and Núñez-Farfán 2000).

Although costs are central to our understanding of the evolution of tolerance, little evidence exists on whether the expression of costs is environmentally dependent. Environmentally dependent costs of tolerance may lead to more situations in which costs could prevent the evolution of maximal levels of tolerance. For example, if costs of tolerance are greater than the benefits in nitrogen-poor environments, selection could act to reduce tolerance levels both in low herbivore density and low nitrogen environments. While it has been predicted that physiological costs of resistance traits should be more common in stressful environments (Bergelson 1994), little evidence exists to indicate that this is a widespread pattern (Bergelson and Purrington 1996), or even applies to costs of tolerance. In this brief communication, I present evidence of a significant physiological cost of tolerance to deer damage in the ivyleaf morning glory, *Ipomoea hederacea*, that is realized only in the presence of insect herbivores. The costs of tolerance detected are consistent with the pattern of selection acting against tolerance reported elsewhere (Stinchcombe 2001; Stinchcombe and Rausher 2002).

MATERIALS AND METHODS

Natural History and Experimental Design

Ipomoea hederacea (L.) Jacquin (Convolvulaceae), the ivyleaf morning glory, is a weedy annual vine common to roadsides and agricultural fields in the southeastern United States. Seeds typically germinate in June or July, and plants die with the first fall frost. Plants begin flowering four to six weeks after germination, and seeds mature in papery capsules about four weeks later. In Durham and Orange Counties, North Carolina, *I. hederacea* is attacked by a variety of natural enemies—insect, mammalian, and fungal. Damage imposed by each of these natural enemies is distinctive, and each can be quantified independently (Bright 1998). Damage at my study site was caused only by insect herbivores, fungal pathogens, and deer.

My experimental approach was to manipulate whether insect and fungal natural enemies were present or absent and determine whether these manipulations altered the pattern of costs of tolerance to deer damage. I used standard methods to estimate deer tolerance for each inbred line (e.g., Simms and Triplett 1994) and evaluate tradeoffs between tolerance and fitness in the absence of damage (Mauricio et al. 1997). In previous reports on the pattern of selection on resistance and tolerance to deer herbivory estimated from this experiment, I described the experimental methods in greater detail (Stinchcombe 2001; Stinchcombe and Rausher 2001).

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To generate experimental seeds, I let 18 inbred lines self-fertilize in a greenhouse. All experimental seeds used were the products of selfing a single individual per line, so the between-inbred line variation reflects total genetic variation (additive and nonadditive) plus maternal effects. I attempted to equalize maternal effects by growing all parental plants in a common greenhouse environment. Using total genetic variation is appropriate for *I. hederacea* because of its high selfing rate (93%; Ennos 1981). In populations with high selfing rates, natural selection acts primarily on total genetic variation rather than just additive genetic variation (Roughgarden 1979). Furthermore, the crosses necessary to discern additive and nonadditive components of genetic variation would create experimental seeds with artificially high levels of heterozygosity and of questionable relevance to natural populations of this species (Mauricio 1998).

Eighty seeds from each inbred line were planted into an agricultural field in a randomized, spatially blocked design. Rows and columns of plants were separated by 1.25 m; plants were not staked. Individual plants were randomly assigned to receive one of four treatments throughout the experiment: (1) natural levels of insect herbivores and fungal pathogens (sprayed with water); (2) natural levels of insect herbivores but reduced levels of fungal pathogens (sprayed with the fungicide Ridomil Gold® Copper; Syngenta, Greensboro, NC); (3) natural levels of fungal pathogens but reduced levels of insect herbivores (sprayed with a mixture of the insecticides Carbaryl [Ortho, San Ramon, CA] and *Bt* [Certis, Columbia, MD]); and (4) reduced levels of both insect herbivores and fungal pathogens (sprayed with both insecticide and fungicide). Spraying treatments began when plants had four true leaves, and were imposed at approximately 2-week intervals. This pesticide regime is highly effective at reducing insect herbivory and fungal damage, does not alter the amount of deer damage plants suffer, and has no effects on seed set of plants grown in the greenhouse (Stinchcombe and Rausher 2001).

Approximately seven weeks after emergence, I recorded the total number of leaves and the number of leaves removed by deer (determined by leaf scars on stems or the presence of the petiole but no leaf) for the 1225 surviving plants. The proportion of leaves damaged by deer was calculated as the number of leaves removed by deer divided by the total number of leaves. For example, if a plant had 30 leaf scars and 70 extant leaves, proportion deer damage was scored as 0.30 (proportion damaged = 30/30 + 70). This procedure provides an estimate of deer damage that is unbiased by the experimental treatments, as the amount of deer damage plants suffered did not differ between experimental treatments (Stinchcombe and Rausher 2001).

All seeds produced by experimental plants were gathered and counted. I calculated relative fitness for each plant by dividing the number of viable seeds produced by each plant by the mean for all plants. Individuals that survived to the damage census but did not set any viable seed were assigned a fitness of zero. Because of *I. hederacea's* high selfing rate (93%), calculating fitness from seed set included both male and female fitness components. Individuals that did not germinate or survive to the damage census were excluded from analyses. There were no differences between inbred lines in either germination or survivorship to the damage census (Stinchcombe and Rausher 2001), so excluding these individuals is unlikely to alter my results.

Statistical Methods

Genetic variation for tolerance

To determine if the inbred lines differed in their tolerance to deer damage, I evaluated the relationship between damage and fitness with ANCOVA (general linear models procedure; SAS Institute 1990). I started with a full model that included block effects, insecticide and fungicide treatments, inbred line as a random effect, deer damage as a covariate, and all interactions except those involving block. I eliminated nonsignificant higher-order interactions in a stepwise manner, and present results from the reduced model. I interpret a significant inbred line × deer damage term as evidence that there was significant genetic variation for tolerance to deer damage; that is, inbred lines differed in how damage affected fitness (Simms and Triplett 1994). For this analysis I log-transformed fitness to improve the normality of the residuals (e.g., Mitchell-Olds and Shaw 1987; Tiffin and Rausher 1999), although significance was not altered in analyses without the log transformation. Preliminary analyses revealed that inclusion of covariates such as plant size and other types of natural enemy damage, and their interactions, did not alter the significance of the results. Accordingly, I report results only for genetic variation and costs of deer tolerance, and without other covariates.

Measurement of tolerance

Because an individual plant cannot simultaneously exist in a damaged and undamaged state, it is impossible to measure tolerance for a single individual (Rausher 1992; Strauss and Agrawal 1999). Instead, tolerance must be measured for a group of genetically related individuals—an inbred line in this case. I calculated deer tolerance values for individual inbred lines as the slope of a regression of relative fitness on deer damage for each inbred line (Simms and Triplett 1994; Mauricio et al. 1997; Tiffin and Rausher 1999). In these analyses I used residuals of relative fitness (untransformed) after the effects of block had been removed to reduce the influence of spatial variation. Only linear terms were included in these regressions because preliminary analyses revealed no evidence of nonlinear effects of damage on fitness (Stinchcombe and Rausher 2002). I measured deer tolerance for each inbred line in each treatment separately because I was explicitly interested in how the presence or absence of other natural enemies affected costs of deer tolerance.

One inbred line in the dual-spray treatment overcompensated for herbivory (i.e., the slope of fitness on damage was significantly positive), and as such its tolerance value was 3.0 standard deviations from the population mean. I performed all analyses with and without data for this inbred line in the dual-spray treatment included in the dataset. Because this inbred line did not have tolerance values that were outliers in other treatments, and there was no obvious source of error for this measurement, I report results that include this inbred line (see below).

TABLE 1. ANCOVA for relative fitness (log-transformed) that demonstrates the existence of genetic variation for tolerance to deer herbivory. Significant effects are shown in bold. Results presented are for the reduced model, after elimination of nonsignificant higher-order interactions from the full model.

Source	df	Type III SS	*F*-value	*P*
Block	**9**	**22.408015**	**12.54**	**$<$0.001**
Inbred line	**17**	**6.916007**	**2.05**	**0.0072**
Insecticide	**1**	**3.431685**	**17.28**	**$<$0.0001**
Fungicide	1	0.061743	0.31	0.5773
Deer damage	**1**	**10.63562**	**53.55**	**$<$0.001**
Deer damage × inbred line	**17**	**5.611011**	**1.66**	**0.0441**
Error	1023	203.173611		

TABLE 2. Correlations and adjusted covariances between deer tolerance and estimated fitness in the absence of herbivory. The adjusted covariances were jackknifed to determine if their 95% confidence limit (CL) overlapped zero. Significant costs of tolerance are shown in bold.

Treatment	Correlation coefficient	Adjusted covariance	95% CL	95% CL overlaps zero?
Control	**−0.95**	**−0.48725**	**0.44025**	**No**
Fungicide	**−0.98**	**−1.37378**	**0.91881**	**No**
Insecticide	−0.81	−0.09275	0.28346	Yes
Dual-spray	−0.87	−0.44083	0.82822	Yes

Tradeoffs between tolerance and fitness in the absence of damage

To measure physiological costs of tolerance, it is necessary to have estimates of tolerance and fitness in the absence of damage for each inbred line. I estimated fitness in the absence of damage for each inbred line as the intercept of the regression of fitness on damage that had been used to estimate tolerance for each inbred line in each treatment. I then calculated the correlation between tolerance values and fitness in the absence of damage for each treatment. These correlations are inflated due to the artifactual covariance between slope (tolerance) and intercept (fitness in the absence of damage) of a regression. I calculated this artifactual covariance and subtracted it from the calculated covariance according to the methods described by Mauricio et al. (1997) and Tiffin and Rausher (1999). I then used standard jackknifing procedures and a one-tailed *t*-statistic to determine if the 95% confidence limit of the corrected covariance overlapped zero (Sokal and Rohlf 1995). I used a one-tailed *t*-statistic because I was testing an a priori, directional hypothesis that these corrected genetic covariances were significantly *less than zero* (e.g., Tiffin and Rausher 1999). Although the use of a one-tailed *t*-statistic precludes testing the hypothesis that a positive genetic correlation exists between tolerance and fitness in the absence of damage, such a positive genetic correlation would not function as a cost. In that scenario, inbred lines that were more tolerant of herbivore damage would have higher fitness in the absence of damage—exactly the opposite of what would be expected if tolerance is costly.

RESULTS

Genetic Variation for Tolerance

The inbred lines differed in their tolerance to deer damage, as indicated by a significant deer damage × inbred line interaction in an ANCOVA for fitness (Table 1). Thus, there was significant genetic variation for tolerance to deer damage in the experimental population, as has been reported elsewhere (Stinchcombe 2001; Stinchcombe and Rausher 2002).

Tradeoffs between tolerance and fitness in the absence of damage

In each of the four treatments I detected strong negative genetic correlations between tolerance to deer damage and fitness in the absence of damage, as would be expected if tolerance was costly (Table 2). In two cases (insecticide and dual-spray treatments) this relationship appears to be due to the artifactual relationship between slope and intercept. Once this covariance is removed, the 95% confidence limits of these corrected covariances include zero. Exclusion of the inbred line that overcompensated in the dual-spray treatment altered neither the pattern nor significance of these results. It is possible that significant costs of tolerance exist in the insecticide and dual-spray treatments, but that my design (with 18 inbred lines) did not have sufficient power to detect them. However, I detected significant physiological costs of tolerance in the control and fungicide treatments (i.e., the 95% confidence limit of the corrected covariances does not include zero; Fig. 1). Consequently it is unlikely that the experimental design had sufficient power in these treatments but not in the others. Therefore, a significant cost of tolerance to deer herbivory was expressed only in the presence of insect herbivores.

Assessing costs in this manner depends on the assumption that the relationship between tolerance and fitness in the absence of damage is linear. I evaluated this assumption by testing the significance of a multiple regression of estimated fitness in the absence of damage on tolerance, and evaluating the significance of the quadratic term (tolerance^2). For the control, insecticide, and fungicide treatments I detected no curvature in the cost function ($P = 0.27$, $P = 0.53$, and $P = 0.87$, respectively). In the dual-spray treatment, however, I detected nearly significant curvature in the cost function ($F = 4.22$, df = 1, $P = 0.06$). This curvature is entirely due to the inbred line which overcompensated for deer damage in this treatment. Since regression analysis is particularly sensitive to extreme values, I performed the analysis again without this inbred line. With the extreme inbred line excluded, there was no evidence for curvature in the cost function for this treatment ($F = 0.20$, df = 1, $P = 0.66$). Over the majority of the range of tolerance values I detected, therefore, costs of tolerance appear to be linear.

DISCUSSION

The results reported here represent the third demonstration of physiological costs of tolerance, as manifested as a negative correlation between tolerance and fitness in the absence of damage (Simms and Triplett 1994; Tiffin and Rausher 1999), and the first in a species other than *I. purpurea,* albeit in a congener. In contrast to previous findings, however, in this experiment tradeoffs between deer tolerance and fitness

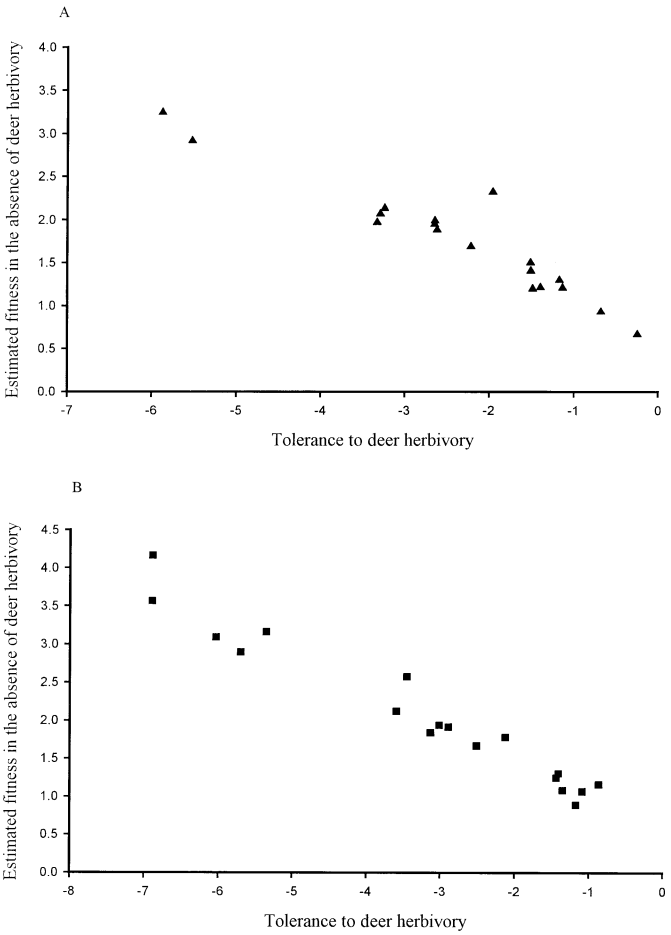

FIG. 1. Physiological costs of deer tolerance, as indicated by negative correlations between tolerance values and fitness in the absence of herbivory. These correlations, as depicted, are not corrected for the artifactual covariance between slope and intercept. Panels A and B represent the control and fungicide treatments, where a significant cost of deer tolerance was found after correcting for the artifactual covariance between slope and intercept (see text for more details).

in the absence of damage were expressed in an environmentally dependent manner—that is, only in the presence of insect herbivores.

Previous theoretical work (Abrahamson and Weis 1997; Tiffin and Rausher 1999) has established that when costs of tolerance are linear, directional selection is expected to act on tolerance. In particular, if herbivore loads are high, selection should be positive and act to increase levels of tolerance. If herbivore loads are low, directional selection should be negative and act to decrease tolerance. These models, however, incorporate only the abundance of a focal natural enemy. The results of the present study clearly indicate that physiological costs of tolerance can vary natural enemy community composition. Such variation in physiological costs of traits, as determined by community composition, suggests that patterns of selection on tolerance will often be diffuse; that is, dependent on the presence or absence of other, auxiliary species (Iwao and Rausher 1997; Stinchcombe and Rausher 2001). If diffuse selection on tolerance is common, predictions from single-species models about the form and direction of selection on tolerance may often be misleading.

The interpretation that environmentally dependent physiological costs of tolerance is likely to produce diffuse selection is consistent with the pattern of natural selection acting on deer tolerance in this experimental population (described in Stinchcombe 2001; Stinchcombe and Rausher 2002). In that study, directional selection was found to be acting against deer tolerance throughout the experiment, regardless of whether insects were present or not. Nevertheless, selection acted more severely against deer tolerance in the presence of insects than in their absence. This difference can be explained by the differences in the physiological costs in these environments, as described here, though other factors may also contribute.

Without knowing the physiological details of tolerance to deer herbivory in *I. hederacea*, it remains difficult to determine the basis for environmental dependency in physiological costs of tolerance. One possibility is that defenses or repair mechanisms that are induced by insect herbivory lead to greater physiological stress, and that this physiological stress increases the costs of tolerance. Although this scenario is consistent with the data presented here, I have no empirical evidence for or against this hypothesis.

These findings suggest that during years of high deer herbivory, when the benefits of tolerance are likely to outweigh its costs, and during years of low insect abundance, when the magnitude of costs is reduced, selection might favor increased tolerance to deer herbivory. It is possible, therefore, that the effects of focal herbivore density and environmental dependency in the expression of costs could interact to change the direction and magnitude of selection on tolerance to herbivore damage from year to year or site to site. These combined effects might be sufficient to maintain tolerance at intermediate levels.

Plant populations commonly have substantial genetic variation for tolerance to natural enemy damage (e.g., Simms and Triplett 1994; Fineblum and Rausher 1995; Mauricio et al. 1997; Stowe 1998; Tiffin and Rausher 1999; Fornoni and Núñez-Farfán 2000). Determining the forces that maintain tolerance at intermediate levels has been a recent challenge in plant evolutionary ecology. The results presented here indicate that the costs of tolerance will depend on the abundance of multiple species. This finding, together with recent evidence that the expression and patterns of selection acting on tolerance may also be environmentally dependent (Stinchcombe and Rausher 2002; Tiffin 2002) suggests understanding the evolutionary dynamics of tolerance will require empirical studies that examine multiple, complex environments.

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