

ENVIRONMENTAL VARIATION MEDIATES THE DELETERIOUS EFFECTS OF *COLEOSPORIUM IPOMOEAE* ON *IPOMOEA PURPUREA*

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Abstract. Variation in the environment is common within and between natural populations and may influence selection on plant resistance by altering the level of damage or the fitness consequences of damage from plant enemies. While much is known about how environmental variation influences the amount of damage a plant experiences, few studies have attempted to determine how variation in the environment may alter the fitness consequences of damage, particularly in plant–pathogen interactions. In this work we manipulated a rust pathogen, *Coleosporium ipomoeae*, in field experiments and showed that this pathogen reduced several components of fitness in its natural host plant, *Ipomoea purpurea*. Furthermore, we showed that the deleterious effects of *C. ipomoeae* were variable. We identified variation in the quality of a plant's microenvironment, the abundance of secondary enemy damage, and the length of a growing season as variable components of the environment that may influence the magnitude of damage and tolerance, causing the interaction between *C. ipomoeae* and *I. purpurea* to vary from parasitism to commensalism. Considering how environmental variation impacts the magnitude and negative fitness effects of pathogen damage is important to understanding spatially variable selection and coevolution in this and other plant–pathogen interactions.

Key words: *Coleosporium ipomoeae*; compensation; environmental variation; *Ipomoea purpurea*; phytometer; plant–pathogen interactions; tolerance.

INTRODUCTION

In theory, environmental variability is likely to have a large effect on the evolution of plant resistance to natural enemies (pathogens and herbivores). For example, if resistance is costly, variation in pathogen abundance can prevent the fixation of resistance alleles (Gillespie 1975, Antonovics and Thrall 1994). Similarly, habitat heterogeneity could potentially maintain resistance polymorphisms if the benefits of resistance are greater than the costs in some habitats, while the reverse is true in others (Levene 1953). On a larger spatial scale, geographic differences in environmental conditions may favor different levels of resistance by tipping the balance between costs and benefits in opposite directions (Thompson's geographic mosaic theory of coevolution; Thompson 1994).

Considerable evidence indicates that variation in abiotic environmental components such as temperature, rainfall, humidity, light intensity, and nutrient gradients can alter the severity of herbivore or pathogen damage, and hence the potential for selection for resistance, by influencing the strength of plant resistance, enemy abundance, and enemy performance (reviewed in Burdon 1987, Smith 1989, Kennedy and Barbour 1992, Jarosz and Davelos 1995). In addition, accumulating

evidence suggests that variation in the biotic environment may have similar effects. For example, damage by one pathogen can reduce the amount of damage imposed by a second pathogen by inducing systemic resistance (Burdon 1987, Karban and Baldwin 1997). Spatial or temporal variation in the abundance of the first pathogen would then presumably cause variation in the magnitude of selection imposed by the second. Similarly, several investigations have demonstrated directly that the presence/absence of one natural enemy influences the magnitude, and even direction, of selection imposed on resistance by a second enemy (Pilson 1996, Juenger and Bergelson 1998, Stinchcombe and Rausher 2001).

A relatively unexplored route through which environmental variation may influence the magnitude of selection on resistance involves environmental effects on tolerance. Tolerance is distinct from resistance, in that resistance reflects the amount of damage imposed by a pathogen or herbivore on a plant under constant conditions, while tolerance reflects the impact on fitness of a given amount of damage (Strauss and Agrawal 1999). Environments that increase the strength of tolerance will reduce the potential benefits of resistance alleles by decreasing the fitness increase associated with being resistant.

A small number of studies show that environmental variation influences the magnitude of tolerance to herbivory in natural populations (Maschinski and Whitham 1989, Juenger and Bergelson 1997, Huhta et al.

Manuscript received 26 August 2005; accepted 8 September 2005. Corresponding Editor: S. H. Faeth.

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2000, Levine and Paige 2004). However, we are aware of only one study showing how the environment may influence tolerance to disease in a natural population. Thrall and Jarosz (1994) reported that the difference in overwintering mortality between *Silene alba* plants infected and not infected with the pathogen *Ustilago violacea* decreases with the severity of winter conditions. Because few investigations have attempted to determine how variation in either the abiotic or biotic environment influences the magnitude of tolerance, it remains unclear how important this type of variation may be in causing variation in the potential for selection on resistance.

In this investigation, we examined the effect of different types of environmental variation on the magnitude of damage and tolerance in the common morning glory, *Ipomoea purpurea*, to a fungal pathogen, *Coleosporium ipomoeae*. We demonstrate that spatial variation in microhabitat suitability appears to modify the magnitudes of both damage and tolerance. We also find that these effects are sometimes large enough to convert the interaction between *C. ipomoeae* and *I. purpurea* from a parasitism to a commensalism. Finally, we demonstrate that variation in damage by other natural enemies, as well as variation in growing season length, may affect *C. ipomoeae* damage, and thereby influence the effect of *C. ipomoeae* on *I. purpurea* fitness.

MATERIALS AND METHODS

Natural history

Ipomoea purpurea is a self-compatible, annual plant that is naturally infected by the fungal rust pathogen, *Coleosporium ipomoeae*. *I. purpurea* ranges from Mexico through the central, southern, and southeastern United States in North America. *C. ipomoeae* is a heteroecious rust fungus that is found throughout the Americas, and in North America is distributed from Mexico east to Florida and north to Pennsylvania and Illinois. Plants in the genus *Pinus* serve as the primary host for *C. ipomoeae* and may include *P. palustris*, *P. rigida*, *P. echinata*, and *P. taeda*; the latter two species are the most likely primary host species in North Carolina (Simms 1993). Secondary hosts include several species in the genus *Ipomoea* (Rhoads et al. 1918). In the location of the present study (Durham County, North Carolina), *C. ipomoeae* is commonly found in natural populations on *I. purpurea*, *I. hederacea*, *I. coccinea*, *I. lacunosa*, and *I. pandurata* (J. Kniskern, *personal observations*).

The data on environmental effects on damage and tolerance reported here are derived from three similar field experiments performed in successive years. Populations of *I. purpurea* are typically polymorphic at a single locus that segregates for resistance and susceptibility alleles to *C. ipomoeae* (Kniskern 2004), and because one of the goals of this project was to examine the fitness effects of variation at a resistance locus in

I. purpurea, we employed a somewhat complicated crossing design to generate seeds of all three genotypes at the resistance locus (see Appendix A). However, the data examined in the current report represent only the plants lacking the resistance allele, since tolerance in this interaction is best estimated in susceptible plants.

2000 Field experiment

A total of 864 seeds (288 each of the three genotypes at the resistance locus) were planted in a randomized block design in an experimental field in Durham, North Carolina, on 17 July 2000. Seeds were planted at 0.75 × 1 m intervals and allowed to twine up 2-m wooden stakes. Three different treatments were imposed to manipulate the abundance of *C. ipomoeae*: (1) A pathogen-exclusion treatment was established by spraying plants with the systemic fungicide Ridomil Gold (Syngenta Crop Protection, Greensboro, North Carolina, USA). A separate field experiment in 2000 showed Ridomil Gold had no effect on *I. purpurea* size or seed production in the absence of *C. ipomoeae* (see Appendix B). (2) A pathogen-addition treatment was imposed by spraying the underside of each leaf with an aqueous solution of urediospores. This solution was made by rinsing the urediospores from leaves collected from many plants from local populations of *I. purpurea* and *I. hederacea*. Although we do not know the number of different pathogen genotypes included in this solution, all experimental plants received the same mixture of genotypes at the same relative concentrations and density. (3) Finally, a control treatment was administered by spraying plants with water to allow unmanipulated infection by *C. ipomoeae*. Treatments were first imposed when local populations of *I. purpurea* provided enough urediospores for the pathogen-addition treatment and were administered every two weeks thereafter.

Damage by *C. ipomoeae* was estimated near the end of the growing season as the total percent of leaf area covered by uredia (fruiting bodies that produce urediospores) or telia (fruiting bodies that produce teliospores that do not reinfect *Ipomoea*). Total leaf area on a plant was estimated from a regression of leaf length on leaf area. Damage was estimated using a clear plastic grid to measure leaf area occupied by *C. ipomoeae*. Proportion damage was then estimated by dividing leaf area damaged by total leaf area. Plant size was estimated by counting the total number of leaves per plant. The female component of plant fitness was estimated by collecting all seeds and counting the total number of seeds per plant. While no microbial pathogens other than *C. ipomoeae* were visible in 2000, we estimated the percent leaf area removed by insect herbivores as described previously. Individual species of insect herbivores were not explicitly identified, but species found at this site on *I. purpurea* in a previous year included tortoise beetles (*Deloya guttata* and *Charidotilla bicolor*), the sweet potato flea beetle (*Chaetocnema con-*



PLATE 1. The microhabitat quality of each experimental plant (on right) was estimated by measuring the size of the corresponding phytometer (on left). Photo credit: J. Kniskern.

finis), and generalist lepidopteran larvae (Tiffin and Rausher 1999).

2001 Field experiment

The design of this experiment was similar to that of the 2000 field experiment with the following differences, which were implemented to increase statistical power: A total of 1056 seeds (528 homozygous resistant and 528 susceptible seeds) were planted on 1 August 2001, at a spacing of 0.75×0.8 m. There were only two treatments in this experiment: a pathogen-exclusion and pathogen-addition treatment. In addition to the experimental plants, a “phytometer” seed (sensu Clements and Goldsmith 1924) was planted within 20 cm of each experimental plant to estimate local microhabitat quality (see Plate 1). These phytometer seeds were all from an *I. purpurea* line that had been inbred for 13 generations by single seed descent. Early-season size (leaf number) was measured on each phytometer approximately two weeks following the average date of germination, which was prior to the first treatment application. Because the phytometers were genetically highly homogeneous, any variation in their performance likely reflected variation in the quality of the environment (Turkington et al. 1979, Antonovics et al. 1988). In order to minimize the effects of phytometer presence on disease transmission among experimental plants, phytometer plants were sprayed with fungicide during treatment applications and guided to twine up smaller 0.75-m bamboo stakes for the remainder of the experiment. Herbivore damage was not measured this year, and there was no visible damage by other pathogens.

In the 2001 experiment an unseasonably early frost killed plants in the midst of their reproductive period and damaged many of the developing fruits. Consequently, in this year female fitness for an individual plant was estimated by multiplying the total number of fruits produced by that plant at the time of frost by the average number of seeds per undamaged fruit produced by all plants for each treatment-by-genotype group. In this experiment, we also counted the total number of flowers produced by each plant and used this measure as a surrogate for male fitness. A previous marker-assisted paternity analysis showed that lifetime flower production was highly correlated with the total number of seeds sired (J. Lau, R. Miller, and M. Rausher, *unpublished data*), a result that has been observed in other annual plants as well (Delvin and Ellstrand 1990, Conner et al. 1996).

2002 Field experiment

The 2002 experimental design was similar to that of 2001 with the following differences: A total of 1080 seeds (360 of each resistance genotype) were planted on 7 June 2002 at 0.75×0.9 m intervals. There were again only two experimental treatments, but in this year these were pathogen exclusion and control because sufficient urediospores could not be collected this year in time to establish a pathogen-addition treatment. A single phytometer seed was once again sown next to each experimental plant as in 2001. The phytometers used in this experiment were derived from a 13th generation inbred line possessing the resistance allele to avoid the need for fungicide applications. Plant size was measured three times during the season. All seeds were

counted for each plant to estimate the female component of fitness. In this year, the duration of *I. purpurea*'s reproductive period was estimated as the difference between the date of first and last flowering, and total life span was estimated as the difference between the dates of germination and last flowering.

Damage from the fungus *Rhizoctonia solani* (identification by North Carolina State University Plant Disease and Insect Clinic) was measured in the same manner as *C. ipomoeae* damage on 3 July 2002, approximately three weeks before the first application of fungicide. *R. solani* is a common soil-borne pathogen that infects many different plant families (Ogoshi 1987) and has been recorded previously on *I. purpurea* by P. Tiffin and R. Zufall in separate field experiments (*personal communications*). *R. solani* appeared after a heavy thunderstorm approximately two weeks after germination when plants were very small (mean of 4.85 leaves) and was most likely transmitted by rain splash. Damage by *R. solani* remained visible until infected leaves senesced, which was prior to the visible emergence of *C. ipomoeae*.

Statistical analyses

The analyses presented here evaluate the magnitude of damage and negative fitness effects of *C. ipomoeae* on *I. purpurea*, and identify environmental components that affected levels of tolerance. Multivariate analyses of variance (MANOVA) were run in the GLM procedure of SAS (SAS Institute 1990) to determine the average effects of *C. ipomoeae* on plant size and seed number (2000–2002), flower number (2001 and 2002), life span (2002) and reproductive period (2002). Univariate analyses of variance (ANOVA) were performed on individual response variables when the MANOVA was significant. These ANOVAs were adjusted for spatial correlation between individuals using the MIXED procedure of SAS (Littell et al. 1996), using data from all plants (resistant and susceptible). Preplanned hypotheses involving susceptible plants were tested using orthogonal contrasts. All main effects including block (in the MANOVA), genotype (included in spatially adjusted models only), treatment, and sire/dam lines (see Appendix A for description of sire and dam line terms) were considered fixed. In general, fitness and life-history response variables were log-transformed to improve the fit of residuals to a normal distribution, but plant size and seed number in 2000 were square-root transformed.

We used early-season phytometer size as a proxy for the quality of the microenvironment in analyses of covariance (ANCOVA) to determine how the quality of the microenvironment influenced the magnitude of *C. ipomoeae* damage and the negative effects of this damage on plant fitness. Specifically, we interpreted the presence of a significant interaction between treatment and the covariate early-season phytometer size for estimates of fitness coupled with the absence of such an

interaction for damage as an indication that micro-environmental quality influences the magnitude of tolerance. To quantify in more detail how tolerance to *C. ipomoeae* changed across the microenvironmental gradient, we tested for the presence of a treatment effect within susceptible plants in low, medium, and high quality microenvironments. These categories were operationally defined as the minimum, median (which was equivalent to the mode in these experiments), and maximum values of early-season phytometer size that had at least 30 observations. In 2001, these values were 1, 3, and 6 leaves, while in 2002 they were 2, 4, and 6 leaves. For statistical reasons, treatment effects were evaluated with orthogonal contrast tests by running three separate ANCOVAs where the minimum, median/mode, or maximum covariate value (early-season phytometer size) was subtracted from the covariate value for each experimental plant. Consequently, plants with the covariate value of interest have an adjusted covariate value of zero such that response variable values lie along the y-axis, which allows the use of simple contrast tests to evaluate the presence of a treatment effect (Milliken and Johnson 2002). A Bonferroni correction was used to adjust alpha values to 0.017 for these tests.

We also used ANCOVA to determine whether the amount of damage by *C. ipomoeae* may have been influenced by herbivores (2000) and by *Rhizoctonia solani* (2002). Herbivore damage was treated as a covariate, while damage by *R. solani* was categorized as either present or absent. We also used ANOVA with time included as a fixed effect to determine how the deleterious effects of *C. ipomoeae* progressed in 2002. In these repeated-measures analyses, errors from a single plant over time are not independent. Consequently, we tested different error covariance structures available within the MIXED procedure in SAS (Littell et al. 1996) and determined that the autoregressive option provided the best covariance structure for these data.

RESULTS

Average effects of Coleosporium ipomoeae on Ipomoea purpurea performance

In all three years, the experimental treatments were effective in generating distinctly different levels of *C. ipomoeae* damage (Fig. 1). Moreover, treatment affected the performance of susceptible *I. purpurea* plants in all three years, as revealed by MANOVA (Appendix C). Consequently, separate ANOVAs were used to assess the average effects of *C. ipomoeae* on each measure of plant performance trait separately.

Except for the 2001 experiment (see next paragraph), exposure to *C. ipomoeae* reduced all measures of plant performance (Table 1). Moreover, the strength and statistical significance of this effect tended to increase with the magnitude of damage. For example, in 2000, both plant size and female fitness were depressed more

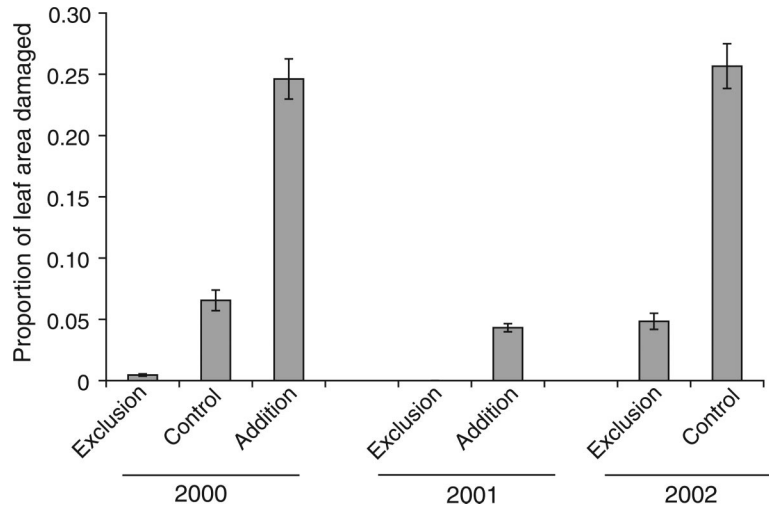


FIG. 1. Proportion of leaf area (mean \pm SE) damaged by *Coleosporium ipomoeae* in control, pathogen exclusion, and pathogen addition trials, 2000–2002. Damage was consistently lower in pathogen exclusion treatments despite extensive variation in absolute damage between years.

in the pathogen addition treatment, in which 25% of the leaf area was damaged, than in the control treatment, in which only 6.5% of the leaf area exhibited damage. When damage was on the order of 25% (pathogen addition treatment in 2000, control treatment in 2002), plant size, male fitness, the length of the reproductive period, and life span all showed highly significant reductions. Although female fitness exhibited a nonsignificant reduction in 2002, the reduction in 2000 was highly significant. In addition, a combined probability test (Sokal and Rohlf 2001) indicates that over both years, female fitness was significantly reduced by pathogen damage ($X^2_4 = 11.33$, $P < 0.025$).

Only in the 2001 experiment were any of the nominal estimates of the effects of damage positive. Both female and male fitness components exhibited an apparent increase in the pathogen addition treatment, compared to the pathogen exclusion treatment (Table 1). However, neither of these increases was statistically significant, indicating that there was no definitive effect, either positive or negative, of *C. ipomoeae* on these fitness components. We suspect that our failure to observe negative effects on these fitness components in this experiment is more likely due to the low amount of damage (4.3%, see Fig. 1) experienced by plants in the pathogen addition treatment than to a true absence of such an effect.

Effects of microenvironment on Coleosporium ipomoeae damage and tolerance

In the 2000 experiment, it was evident from observation that both plant size and severity of damage from *C. ipomoeae* were spatially clumped within blocks, suggesting that microenvironmental variation may influence both of these characters. Consequently, in order to assess the impact of such variation on both damage

and tolerance, in 2001 and 2002 we used phytometers to measure microhabitat quality.

In 2001, there was no detectable correlation between phytometer size and damage by *C. ipomoeae*; nor was there an interaction between phytometer size and treatment, as assessed by ANCOVA ($df = 1, 439$: $F = 0.11$, $P = 0.7419$ and $F = 0.07$, $P = 0.7929$). This outcome was not surprising, because damage in 2001 was induced by imposing a pathogen-addition treatment equally across all microenvironments. By contrast, in 2002, pathogens were permitted to colonize plants naturally in the control treatment, and in this experiment, damage by *C. ipomoeae* was positively correlated with phytometer size ($F = 9.09$, $df = 1, 254$, $P = 0.0028$). Although the fungicide was very effective at reducing damage overall (see Fig. 1), there was no statistical interaction between phytometer size and treatment ($F = 0.09$, $df = 1, 254$, $P = 0.7601$), indicating that the difference in damage between the two treatments was similar for environments of different quality. The contrasting results from these two years suggest that the quality of the microenvironment may influence the number of urediospores a plant is exposed to, but not the performance of urediospores on plants.

As would be expected, phytometer size was positively correlated with all three measures of plant performance that were quantified in 2001, as revealed by ANCOVA (plant size, $F = 51.3$, $df = 1, 886$, $P < 0.0001$; female fitness, $F = 48.09$, $df = 1, 884$, $P < 0.0001$; and male fitness, $F = 62.31$, $df = 1, 880$, $P < 0.0001$). More importantly, there was a significant interaction between phytometer size and treatment for each of these measures (plant size, $F = 6.63$, $df = 1, 886$, $P = 0.0102$; female fitness, $F = 4.65$, $df = 1, 884$, $P = 0.0314$; and male fitness, $F = 4.86$, $df = 1, 880$, $P = 0.0278$). The pattern of these interactions

TABLE 1. *Coleosporium ipomoeae* reduced several *Ipomoea purpurea* fitness components.

Trait	2000 control-exclusion			2000 addition-exclusion			2001 addition-exclusion			2002 control-exclusion		
	Diff. (%)	F_{\dagger}	P	Diff. (%)	F_{\dagger}	P	Diff. (%)	F_{\ddagger}	P	Diff. (%)	F_{\S}	P
Plant size	-18.3	11.97	0.0006	-22.6	18.23	<0.0001	-4.3	6.05	0.014	-47.6	57.76	<0.0001
Female fitness	-11.1	3.17	0.0758	-14.9	6.2	0.0128	+17.1	0.12	0.7274	-5.5	1.21	0.2703
Male fitness	NA			NA			+11.7	0.14	0.7042	-12.8	7.67	0.0058
Reproductive period	NA			NA			NA			-9.4	24.9	<0.0001
Life span	NA			NA			NA			-5.1	22.28	<0.0001

Notes: All trait values are expressed as the mean difference between susceptible plants from a control (natural infection) or pathogen addition treatment relative to susceptible plants in the pathogen exclusion treatment from that year. NA indicates these data are not available because they were not collected.

\dagger For plant size and female fitness, $df = 1, 761$.

\ddagger For plant size, $df = 1, 991$; for female fitness and male fitness, $df = 1, 992$.

\S For plant size, $df = 1, 899$; for female fitness and male fitness, $df = 1, 918$; for reproductive period, $df = 1, 902$; for life span, $df = 1, 911$.

(Fig. 2a) is similar and striking for all three performance measures: the effect of *C. ipomoeae* tends to diminish as microenvironmental quality increases. In the highest quality microenvironments, *C. ipomoeae* even appears to be beneficial, though this effect is not significant following a Bonferroni correction ($\alpha = 0.017$; plant size, $F = 1.3$, $df = 1, 886$, $P = 0.2562$; female fitness, $F = 2.99$, $df = 1, 884$, $P = 0.0836$; male fitness, $F = 3.92$, $df = 1, 880$, $P = 0.0479$). Because microenvironmental quality did not affect the magnitude of the difference in *C. ipomoeae* damage treatments, these performance differences are ascribable to microenvironmental effects on tolerance. In particular, tolerance appears to be low in low-quality environments, and complete in high-quality environments.

In 2002, early-season phytometer size once again explained a significant portion of the variation in seed number ($F = 5.15$, $df = 1, 878$, $P = 0.0235$) and flower number ($F = 6.93$, $df = 1, 891$, $P = 0.0086$). In addition, there was a significant interaction between early-season phytometer size and treatment for both plant size ($F = 4.25$, $df = 1, 870$, $P = 0.0396$) and flower number ($F = 4.71$, $df = 1, 891$, $P = 0.0302$) but not seed number ($P > 0.1$). As in 2001, the reduction in flower production caused by *C. ipomoeae* was greater in poor microenvironments (Fig. 2b). *C. ipomoeae* significantly reduced flower production only in the low-quality microenvironment ($F = 8.82$, $df = 1, 891$, $P = 0.0031$), but not in the medium- or high-quality microenvironment ($\alpha = 0.017$; $df = 1, 891$; $F = 5.29$, $P = 0.0214$ and $F \sim 0$, $P = 0.9976$, respectively). The reduced adverse effect of *C. ipomoeae* on flower production in good microenvironments cannot be ascribed to reduced damage because, again, the difference in damage between pathogen exclusion and control treatments was unaffected by microenvironmental quality. Consequently, it appears that, with respect to flower production, plants growing in good microenvironments are more tolerant to *C. ipomoeae* damage, as was found in the 2001 experiment.

In summary, increased microenvironmental quality, reflected in phytometer growth, was associated with reduced fitness differences between plants in the pathogen exclusion relative to the pathogen addition and control treatments of 2001 and 2002, respectively, and yet increasing microenvironmental quality had no effect on the differences in *C. ipomoeae* damage between plants in these treatments, implying level of tolerance increases with environmental quality. This association was more general in 2001, in which plant size, seed production, and flower production all exhibited complete tolerance in high-quality environments, while in 2002 this association was limited to flower production.

Effect of natural enemies on *Coleosporium ipomoeae* damage

Damage by naturally occurring insect herbivores was quantified in 2000 to determine if herbivory may have influenced damage by *C. ipomoeae*. There was a significant, negative correlation between the proportion of leaf area consumed by insect herbivores and the proportion of leaf area occupied by *C. ipomoeae* on susceptible plants in the control treatment of 2000 ($F = 5.11$, $df = 1, 89$, $P = 0.0265$). In contrast, susceptible plants infected by *R. solani* in 2002 had significantly more damage from *C. ipomoeae* than susceptible plants uninfected by *R. solani* ($t = 2.36$, $df = 1, 143$, $P = 0.0196$). Microenvironmental variation may have promoted the positive correlation between *C. ipomoeae* and *R. solani* damage in 2002, because a logistic regression of the proportion of individuals damaged by *R. solani* on phytometer size was significantly positive ($X^2 = 47.73$, $df = 1, 988$, $P < 0.0001$) and *C. ipomoeae* damage exhibited a similar pattern in this year (see Results: Effects of microenvironment). However, microenvironmental variation may not fully account for this correlation, because an ANCOVA of *C. ipomoeae* damage in which early-season phytometer size was included as the covariate to control for the influence of microenvironmental variation, and in which the presence/absence of *R. solani* was included as a main effect,

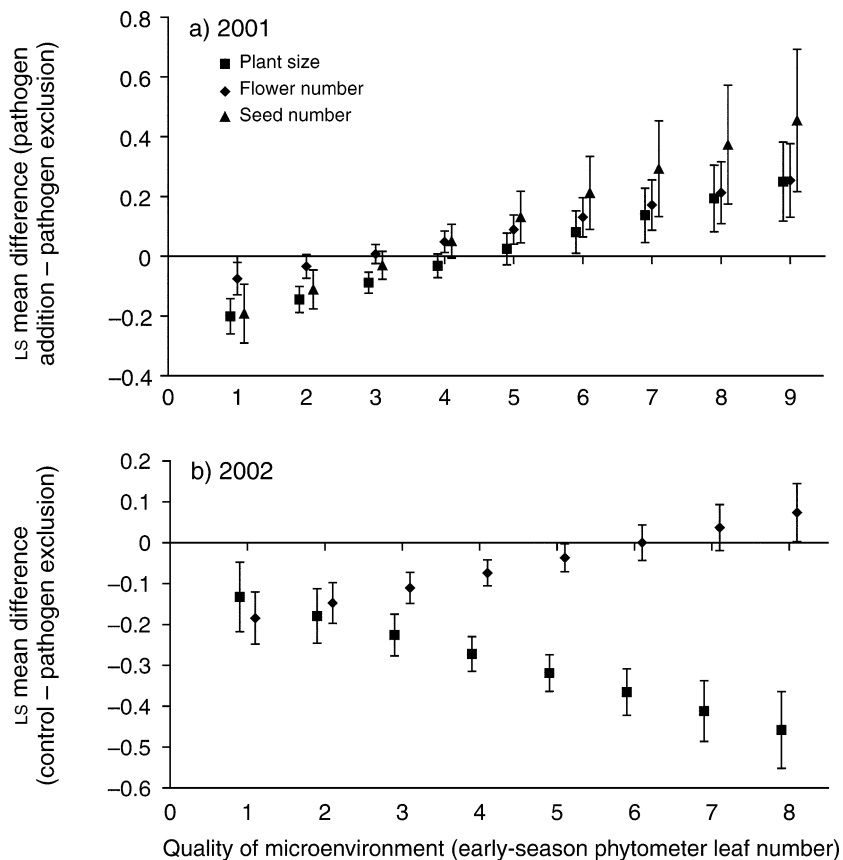


FIG. 2. Negative effects of *C. ipomoeae* on plant traits across the microenvironmental gradient. (a) The negative effects on plant size, flower number, and seed number decreased in 2001. (b) The negative effects on plant size increased while negative effects on flower number decreased in 2002. Data points indicate the mean difference (\pm SE) between susceptible plants in (a) the pathogen addition treatment of 2001 or (b) the control treatment of 2002 relative to the pathogen exclusion treatment from the same year as determined from least-square means. Early-season phytometer leaf number was used as a proxy for microenvironmental quality.

shows that *R. solani* had a moderately significant positive effect on *C. ipomoeae* damage ($F = 3.66$, $df = 1, 136$, $P = 0.0578$).

Season length and the parasitic effects of Coleosporium ipomoeae on Ipomoea purpurea

One of the most apparent forms of variation in the deleterious effects of *C. ipomoeae* was year to year variation, particularly the absence of negative effects on seed number and flower number in 2001. While there are a number of factors that could have differed in 2001 relative to 2000 and 2002, one striking difference was the relatively short experimental growing season of 2001, defined as the number of days between planting and the first hard frost. The experiment of 2001 was planted approximately two weeks later than in 2000 and about seven weeks later than in 2002. Additionally, 2001 had an unseasonably early hard frost in late October while the 2000 and 2002 experiments continued into late November and mid-November, respectively. The absence of negative effects of *C. ipomoeae* in 2001 was probably a result of low *C. ipomoeae*

damage (see Fig. 1), and we believe the low damage from *C. ipomoeae* may have resulted from the relatively short experimental season length of 2001, although other explanations are possible.

This hypothesis assumes that the negative effects of damage by *C. ipomoeae* increase in intensity over the growing season, either because the amount of damage increases, or the level of tolerance to damage decreases, or both. We evaluated this assumption by asking whether the negative effects of *C. ipomoeae*, as measured in plant size and fitness, increase in magnitude over the course of the growing season. To do this, longitudinal analyses were conducted on the only plant traits that had repeated measures and were significantly affected by *C. ipomoeae*: plant size and flower production in 2002. There was a significant treatment by time interaction for both plant size ($F = 30.72$, $df = 1, 466$, $P < 0.0001$) and flower production ($F = 12.32$, $df = 1, 14\ 655$, $P = 0.0004$) indicating that the deleterious effects of *C. ipomoeae* did in fact increase in intensity over the course of the growing season (Fig. 3). To emphasize how a short growing season could reduce

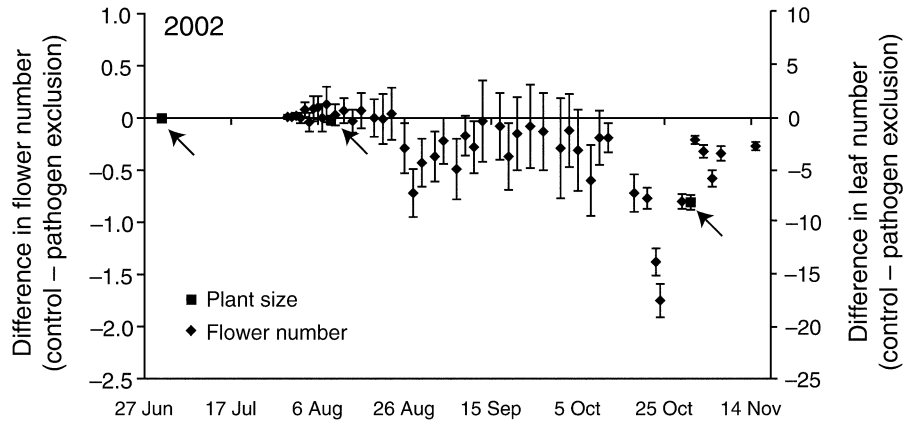


FIG. 3. The negative effects of *C. ipomoeae* on plant size (see arrows for location of data points) and flower number increased over the course of the growing season in 2002. The left and right axes represent differences (mean \pm SE) in flower number and plant size (leaf number), respectively, between the control and pathogen exclusion treatments. *C. ipomoeae* was first observed on 9 August in this experiment, most likely reflecting the initial stages of the epidemic of 2002.

the negative effects of *C. ipomoeae*, consider that if the total reproductive period of 2002 (108 days) had been similar to the reproductive period of 2001 (35 days), *C. ipomoeae* would not have significantly reduced flower number (ANOVA of the first 35 days of flower production in 2002; treatment effect, $F = 0.16$, $df = 1, 946$, $P = 0.6861$). These results support the hypothesis that the absence of negative effects of *C. ipomoeae* on seed number and flower number in 2001 might have been a consequence of the short growing season. Consequently, variation in the length of a growing season could contribute to variation in the overall impact of *C. ipomoeae* on its host.

DISCUSSION

Coleosporium ipomoeae is potentially a potent parasite of *Ipomoea purpurea*. In this study, *C. ipomoeae* significantly reduced plant size, an estimate of the male component of plant fitness (flower number), an estimate of the female component of plant fitness (seed number), reproductive period, and life span in at least one of the three years of experiments. These effects suggest that *C. ipomoeae* may also be a potent agent of evolutionary change in *I. purpurea*, a hypothesis that will be evaluated in a subsequent publication. However, it was also clear that the negative effects of *C. ipomoeae*, and thus the potential for this pathogen to exert selection on levels of resistance and tolerance in *I. purpurea*, were quite variable. Our results implicate several environmental variables contributing to this variation, including variation in the quality of the microenvironment, variable damage from consumers, and differing growing season length. Moreover, this overall effect of environmental variation appears to be mediated through both effects on the level of damage by *C. ipomoeae* and effects on the level of tolerance to damage.

Effects of environmental variation on tolerance

While the influence of environmental factors on damage or infestation level by pathogens and other natural enemies has been extensively documented (Burdon 1987, Smith 1989, Kennedy and Barbour 1992, Jarosz and Davelos 1995), similar effects on tolerance to damage or infestation has received far less attention. A small number of studies have shown that variation in microenvironmental conditions including levels of soil nutrients, water, competition, and timing of damage altered tolerance to herbivory (Maschinski and Whitham 1989, Juenger and Bergelson 1997, Huhta et al. 2000, Levine and Paige 2004). These results were interpreted to mean that the physiological state of a plant influences its degree of tolerance. Results from our study support this suggestion. In particular, we found that in each of two years (despite variation between years in length of the growing season and levels of *C. ipomoeae* infection) microenvironmental variation, as reflected by phytometer growth, greatly influenced the degree of tolerance in *I. purpurea* to infection by *C. ipomoeae*. In general, increased environmental quality was associated with increased tolerance, as was also reported by Maschinski and Whitham (1989), Huhta et al. (2000), and Levine and Paige (2004). Moreover, the variation in tolerance observed here is likely to be both ecologically and evolutionarily meaningful, in that it ranged from under-compensation to complete compensation.

Our detection of microenvironmental effects on tolerance depended on the accurate assessment of microenvironmental quality using phytometers. In order to minimize variation in the measurement of environmental quality, we employed a single, genetically homogeneous inbred line for our phytometers. Although the possibility exists that genotypes could differ in the way they assess quality, and therefore that use of a

single genotype might bias our results, we believe this effect is minimal in our experiment. If such bias were extensive, we would not expect to see the strong relationships that we observed between phytometer size and performance measures of the plants in our experiments. These strong correlations indicate that our phytometers captured a large portion of the meaningful variation in environmental quality.

Another possible caveat to our interpretation is that we measured tolerance indirectly. Tolerance is normally measured directly as a regression of fitness on amount of damage (Strauss and Agrawal 1999). Because in our experiment there were many different microenvironments that could not be characterized before the experiment, it was impractical to measure tolerance directly in each one. Instead, we inferred that tolerance was greater in good microenvironments because the fitness difference between plants exposed to *C. ipomoeae* and those protected with a fungicide was lower in these microenvironments, but damage was not lower.

Diffuse interactions and the effects of damage

A current issue of interest regarding the evolution of plant–enemy interactions is whether detrimental effects of one natural enemy on a host plant are altered by the abundance of other natural enemies (Hougen-Eitzman and Rausher 1994, Rausher 1996, Inouye and Stinchcombe 2001, Strauss et al. 2005). In particular, independence of the effects of one enemy on plant fitness from the effects of another enemy is a necessary condition for pairwise selection and pairwise coevolution (Iwao and Rausher 1997).

One way in which one natural enemy may alter the fitness effects of a second is to modify the amount of damage. In our experiments, we found that both herbivores and *Rhizoctonia solani* may have altered the amount of damage caused by *C. ipomoeae*. In particular, herbivore damage was correlated with reduced damage by *C. ipomoeae*. The simplest explanation for this result is that herbivory reduced the susceptible leaf area available for epidemic growth of *C. ipomoeae*, although other explanations are possible. By contrast, damage by *C. ipomoeae* and damage by *R. solani* were positively correlated. Given that *C. ipomoeae* and *R. solani* did not overlap temporally on *I. purpurea*, we believe there are three plausible mechanisms that could explain this association.

One possibility is that resistance to *C. ipomoeae* and *R. solani* is positively genetically correlated. We believe this explanation to be unlikely because we could detect no genetic variation for resistance to either pathogen among sire lines or dam lines in this study (data not shown), presumably because of the small number of lines employed. A second possible explanation is that both pathogens were similarly influenced by the external environment. This possibility is supported by positive correlations between microenvironmental quality and both *C. ipomoeae* damage and frequency

of *R. solani* infection. Finally, a third possible explanation is that *R. solani* facilitated damage by *C. ipomoeae*, perhaps by suppressing inducible systemic resistance to *C. ipomoeae*. This hypothesis was also supported by an analysis showing that *R. solani* still had a marginally significant effect on *C. ipomoeae* damage when the effect of the microenvironment on both fungi was statistically controlled. In summary, it is possible that damage by *R. solani* and *C. ipomoeae* was correlated because both pathogens responded similarly to the quality of the microenvironment and because early-season damage by *R. solani* may have in some way promoted greater late-season damage by *C. ipomoeae*.

Our finding that both herbivores and other pathogens may influence damage by *C. ipomoeae* is in line with previous investigations in other systems. Negative associations between insect herbivory and pathogen damage, like that found in this study, have been reported previously (Karban et al. 1987, Hatcher et al. 1994), although positive associations are also known to occur (Gange 1996). Likewise, both positive and negative associations in microbial damage have been observed between pathogens (Burdon 1987). Our results thus support the emerging generalization that interactions among natural enemies, either direct or indirect, are common in plant–enemy associations, and that therefore, coevolution in such systems is likely to be diffuse rather than pairwise.

Year to year variation in the deleterious effects of Coleosporium ipomoeae

The magnitude of the negative impact of *C. ipomoeae* on *I. purpurea* varied substantially across the three years of the experiments. Most strikingly, negative effects on seed number and flower number were not detectable, and negative effects on plant size were minimal, in 2001. By contrast, these effects were strong in 2000 and 2002. Although the cause of this difference cannot be definitively proven, we suggest that the dramatically shorter experimental growing season of 2001 likely explains this pattern. In particular, we suspect that the 2001 experimental season was too short to permit sufficient growth and reinfection by *C. ipomoeae* to cause a measurable impact on seed and flower production. This hypothesis is supported by the prediction that longer growing seasons will tend to increase plant pathogen damage (Harvell et al. 2002) and by our longitudinal analysis of the effects of *C. ipomoeae* in the 2002 season, in which detrimental effects of *C. ipomoeae* on plant growth and flower production were evident only after the length of the 2001 growing season had been exceeded.

Epidemic growth curves of phytopathogen populations are typically sigmoidal in shape, with a plateau beginning when ~50% of susceptible host tissue is infected (Burdon 1987). Damage from *C. ipomoeae* never exceeded 25% of total susceptible leaf area in any year of our experiments, suggesting that even in a

long growing season, *C. ipomoeae* abundance is continuing to increase approximately exponentially when growth is interrupted by adverse fall conditions. These considerations suggest that the maximal abundance of *C. ipomoeae*, and hence the magnitude of its negative impact on *I. purpurea*, is likely determined in part by the length of the growing season.

Implications of variation in the deleterious effects of Coleosporium ipomoeae

These experiments suggest the quality of a plant's microenvironment, the presence of a second plant consumer, and growing season length as components of the environment that may modify the negative effects of *C. ipomoeae* and may thereby change the potential for selection that *C. ipomoeae* may impose upon *I. purpurea*. In addition to potentially influencing the coevolution of *C. ipomoeae* and *I. purpurea*, these variable components of the environment serve as three general mechanisms that could influence coevolutionary dynamics in a variety of plant–pathogen systems. First, the range of variation in the microenvironment identified by our phytometers probably represents a fraction of the environmental variation present across a species distribution. If plant tolerance and pathogen performance are commonly linked to environmental quality, then geographic differences in environmental quality would likely result in population differences in the intensity of reciprocal selection. Second, plants interact with a large community of consumers in natural populations that may alter damage or the fitness consequences of damage from a focal pathogen, causing selection to be diffuse. Given that suites of species are unlikely to completely overlap in their distributions (Thompson 1994), it is possible that diffuse selection will cause spatially variable selection (Stinchcombe and Rausher 2001). Finally, many phytopathogens show epidemic growth curves that could generate a correlation between season length and the strength of selection inferred in this work (Burdon 1987). The length of an annual plant growing season covaries with latitude and elevation, and the hypothesis that season length alters pathogen-mediated selection would predict the presence of latitudinal or elevational clines in pathogen-mediated selection intensity in plant–pathogen systems that span these gradients. All three of these hypotheses support the existence of selection mosaics that can influence the dynamics of coevolution (Thompson 1994). Further studies detailing the environmental components that mediate selection in plant–pathogen interactions are needed to complement work on the coevolutionary consequences of variable selection to develop a better understanding of plant–pathogen coevolution.

ACKNOWLEDGMENTS

We thank R. Miller, J. Stinchcombe, and P. Tiffin for statistical help and *Ipomoea* advice. B. Calhoun, R. Smith, and M. Vallejo-Marin provided help in the field and greenhouse,

and Clint King generously plowed the field site. X. Dong, S. Faeth, W. Morris, B. Traw, R. Vilgalys, and W. Wilson all provided helpful comments on previous drafts. Financial support of this work was provided by an NSF Dissertation Improvement Grant on behalf of J. M. Kniskern to M. D. Rausher (DEB-0107172) and the Biology Department of Duke University.

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APPENDIX A

A diagram of the crossing design used to generate experimental plants (*Ecological Archives* E087-038-A1).

APPENDIX B

A description of an experiment that tests the fitness effects of the fungicide, Ridomil Gold, on plant fitness in the absence of *Coleosporium ipomoeae* (*Ecological Archives* E087-038-A2).

APPENDIX C

A summary of MANOVA for several fitness components (*Ecological Archives* E087-038-A3).