

## TESTING ADAPTIVE PLASTICITY TO UV: COSTS AND BENEFITS OF STEM ELONGATION AND LIGHT-INDUCED PHENOLICS

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**Abstract.**—On exposure to ultraviolet radiation (UV), many plant species both reduce stem elongation and increase production of phenolic compounds that absorb in the UV region of the spectrum. To demonstrate that such developmental plasticity to UV is adaptive, it is necessary to show that the induced phenotype is both beneficial in inductive environments and maladaptive in non-inductive environments. We measured selection on stem elongation and phenolic content of seedlings of *Impatiens capensis* transplanted into ambient-UV and UV-removal treatments. We extended the range of phenotypes expressed, and thus the opportunity for selection in each UV treatment, by pretreating seedlings with either a low ratio of red:far-red wavelengths (R:FR), which induced stem elongation and reduced phenolic concentrations, or high R:FR, which had the opposite effect on these two phenotypic traits. Reduced stem length relative to biomass was advantageous for elongated plants under ambient UV, whereas increased elongation was favored in the UV-removal treatment. Selection favored an increase in the level of phenolics induced by UV in the ambient-UV treatment, but a decrease in phenolics in the absence of UV. These results are consistent with the hypotheses that reduced elongation and increased phenolic concentrations serve a UV-protective function and provide the first explicit demonstration in a wild species that plasticity of these traits to UV is adaptive. The observed cost to phenolics in the absence of UV may explain why many species plastically upregulate phenolic production when exposed to UV, rather than evolve constitutively high levels of these compounds. Finally, pretreatment with low R:FR simulating foliar shade did not exacerbate the fitness impact of UV exposure when plants had several weeks to acclimate to UV. This observation suggests that the evolution of adaptive shade avoidance responses to low R:FR in crowded stands will not be constrained by increased sensitivity to UV in elongated plants when they overtop their neighbors.

**Key words.**—Adaptive plasticity, *Impatiens capensis*, phenolic compounds, phytochrome, production costs, red:far-red cues, shade-avoidance responses, ultraviolet radiation.

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Developmental plasticity is commonly viewed as a mechanism enabling organisms to adaptively match their phenotype to local conditions and overcome environmental stresses. However, to demonstrate that plasticity is adaptive, it is necessary to show that the induced phenotype is explicitly adaptive in the inductive environment and maladaptive in noninductive environments (van Tienderen 1991; Schmitt et al. 1995; Dudley and Schmitt 1996). If the induced phenotype were only selectively advantageous or selectively neutral across all environments, plastic genotypes would have no evolutionary advantage over “ecologically specialized” genotypes with fixed (or canalized) trait expression. Identifying adaptive plasticity therefore requires evidence that selection reverses across environments. Detecting selection against a plastic trait is complicated by the fact that many organisms continually modify their phenotype in response to local conditions, and an induced character state may rapidly disappear in noninductive environments. Phenotypic manipulation, that is, induction of a trait independent of actual environmental conditions, has proven successful in demonstrating adaptive plasticity of several traits (Schmitt et al. 1995; Kingsolver 1995; Dudley and Schmitt 1996; Agrawal et al. 1999). However, the adaptive value of many common plastic developmental responses has never been tested.

For plants, sunlight is both an important cue regarding environmental conditions as well as an important resource, enabling photosynthesis and the acquisition of organic compounds. However, exposure to sunlight also results in ex-

posure to the mutagenic effects and physiological stresses of ultraviolet radiation (UV). Ultraviolet radiation detrimentally affects growth in many plant species (Barnes et al. 1990; Sullivan et al. 1992; Li et al. 1993; Landry et al. 1995), suggesting that plastic responses to UV that reduce susceptibility to damage will confer a fitness advantage. On exposure to ambient levels of UV, plants plastically upregulate the production of UV-B absorbing phenolic compounds (Mazza et al. 2000; Dixon et al. 2001), and many species also show decreased stem elongation when exposed to UV (e.g., Barnes et al. 1990; Sullivan et al. 1992; Ballaré et al. 1995; Dixon et al. 2001). However, to demonstrate that observed developmental plasticity to UV is adaptive, it is necessary to show that the phenotype induced by UV enhances fitness in UV environments but confers a disadvantage in the absence of UV (Bradshaw 1965; Levins 1968; Dudley and Schmitt 1996). A negative relationship between fitness and phenolics in the absence of UV would indicate production costs that select against constitutive expression of high phenolic concentrations in the absence of UV. Again, without such production costs, plastic genotypes would have no advantage over genotypes with fixed phenotypic expression (e.g., Agrawal 1999; Agrawal et al. 1999; Van Tienderen 1991), and canalized versus plastic genotypes would be equally expected to evolve. To date, tests for production costs of phenolics are lacking in wild plants (but see Bieza and Lois 2001), as are tests regarding the adaptive significance of plasticity of elongation to UV.

Opportunity costs, or trade-offs, associated with the expression of a given character state will also affect the evolution of adaptive strategies. As mentioned above, developmental responses that increase exposure to sunlight also

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increase exposure to damaging UV radiation. Plants in natural populations experience variable shading by neighbors and exposure to sunlight. Although morphological responses such as stem elongation enhance leaf exposure (Weinig 2000a) and fitness in crowded settings (Schmitt et al 1995; Dudley and Schmitt 1996; Weinig 2000a), elongated plants will also experience greater UV stress as they overtop neighbors. Increased stem length may also exacerbate the physiological stress of UV exposure by increasing water stress (Maliakal et al. 1999). Plant damage resulting from UV exposure may therefore be an important opportunity cost of competitive phenotypes that increase exposure to sunlight, and this opportunity cost could affect the evolution of plant competitive responses.

When developmental responses to environmental cues include several traits, phenotypic evolution depends not only on the effect of direct selection, but also on indirect selection acting via correlated characters. Plant responses to competition and UV provide a salient example. Low ratios of red:far-red wavelengths (R:FR) provide a reliable indication of neighboring plants and the onset of competition for sunlight, because chlorophyll selectively filters red wavelengths while transmitting far-red (Smith 1982). Light filtered through a vegetative canopy or reflected off the stems of neighboring plants therefore exhibits a predictable reduction in R:FR relative to sunlight (~0.2–0.7 for foliar shade vs. 1.1 for sunlight). The phytochrome photoreceptors perceive low R:FR cues (Smith 2000) and mediate a suite of responses collectively known as “shade-avoidance,” which includes two- to fivefold increases in stem-elongation that increase access to sunlight under crowded conditions. However, aside from having more elongated stems, plants experiencing low R:FR also have dramatically lower concentrations of phenolics relative to those under high R:FR (similar to sunlight) (Beggs and Wellman 1994) and may be more susceptible to UV damage.

Testing the potential effects of shade-avoidance responses to low R:FR on UV-susceptibility is important in light of observed costs to these responses; elongated *I. capensis* plants pretreated with low R:FR have higher fitness in crowded stands but reduced fitness relative to shorter plants pre-treated with high R:FR when grown in low density patches (Dudley and Schmitt 1996). Although UV may impose selection to reduce stem elongation, the reduced fitness of elongated plants at low density observed by Dudley and Schmitt (1996) cannot be explained by direct selection on height and must therefore be due to phytochrome-mediated effects of the pretreatment other than stem elongation. Again, one hypothesis is that elongated plants pretreated with low R:FR were more damaged by UV exposure at low density due to their low phenolic levels. Thus, selection imposed by UV may act directly to reduce elongation or indirectly via phenolic levels and their correlation with elongation.

To evaluate the patterns of selection on phenolics and stem elongation in varying UV environments, we exposed seedlings of 50 inbred lines of the annual species *Impatiens capensis* Meerb. (Balsaminaceae) to either low R:FR or high R:FR conditions for two weeks and then transplanted them into ambient-UV or UV-removal treatments. The lines had been established from two natural populations in a woodland and a nearby clearing that differ genetically in average phe-

nolic production (Dixon et al. 2001) and in stem elongation (Dudley and Schmitt 1996; Donohue and Schmitt 1999; Donohue et al. 2000a, b). The experiment therefore used naturally occurring genetic variation and phenotypic manipulations to expand the distribution of phenolic concentrations and elongation independent of UV levels. These phenotypic manipulations are important in testing for adaptive plasticity because plants continuously modify their phenotype in response to changing environments, thereby minimizing the potential to measure selection. Here, we address the following questions: (1) Does plasticity of stem elongation to UV enhance fitness in variable UV environments? (2) Is plasticity of phenolic production an adaptation to UV-stress, that is, does selection on phenolics differ across UV environments? (3) Is increased susceptibility to UV a cost of expression of phytochrome-mediated shade-avoidance responses?

#### MATERIALS AND METHODS

*Study system.*—*Impatiens capensis* Meerb. (Balsaminaceae) is a native North American annual that inhabits both sunlit clearings and woodlands. Seeds for this experiment were derived from inbred lines maintained for six generations in the greenhouse. The lines originated from two populations located at the Haffenreffer Reserve of Brown University (Bristol, RI); one of the populations was located in a clearing, whereas the second was within the forest understory (Dudley and Schmitt 1995; Donohue and Schmitt 1999; Donohue et al. 2000a, b). Seeds were collected from the greenhouse plants in December 1998 through January 1999 and then wet stratified in microtitre trays filled with distilled water for four months at 4°C to maintain seed viability and promote germination.

*Experimental design.*—To examine whether responses to R:FR affected selection imposed by UV, we exposed seedlings to high and low R:FR pretreatments in the greenhouse prior to transplanting them into ambient-UV and UV-removal field treatments. On May 21, 1999, seeds were planted into 16 128-celled plug trays filled with Metro-mix 360 soil mixture (Grace Sierra Horticultural Products Co., Milpitas, CA). This planting design results in a high density of seedlings (1024 m<sup>-2</sup>) comparable to dense natural stands. Seed trays were placed on benches in the Brown University greenhouse, Providence, RI. A total of 2000 seeds (2 R:FR pretreatments × 2 UV treatments × 50 lines × 10 seeds per line) were planted with two seedlings per line placed randomly within each tray. On June 3, the R:FR pretreatments were applied, at which point all seedlings had germinated. For the high R:FR pretreatment, eight trays of seedlings were placed under plastic panels that selectively filtered FR wavelengths (Mitsui Chemicals, Inc., Tokyo, Japan). R:FR under the panels is approximately 18.1 (Murakami et al. 1996) and thus remains higher than the R:FR of natural sunlight under the seedling canopy, that is, remains in the range where the phytochrome photoequilibrium is insensitive to R:FR (Smith 1982; Dudley and Schmitt 1996; Maliakal et al. 1999). Measurements of photosynthetic photon flux density (PPFD) and light spectral quality with a LI-1800 portable spectroradiometer (LICOR, Inc., Lincoln, NE) confirmed published transmittance of the filters. In the low R:FR treatment, seedling trays were placed

under clear plastic panels covered by layers of cheesecloth to reduce photosynthetically active radiation to 70% of full sunlight, a level equivalent to the high R:FR treatment. Because the cheesecloth acted as a neutral filter and reduced light at all wavelengths equally, ambient R:FR within the canopy dropped as the seedlings grew. Both pretreatments were surrounded by a barrier of tinfoil to prevent exposure to unfiltered sunlight.

After 14 days in the R:FR pretreatment, 256 plants were destructively harvested for extraction and measurement of phenolic compounds. The number of plants used for extraction was 256 rather than the expected 300 (three individuals per line  $\times$  50 lines  $\times$  two pretreatments), due to low germination in some lines. Because seedlings were small, whole plants were ground in liquid nitrogen and placed in a  $-80^{\circ}\text{C}$  freezer. Samples were then removed successively from the freezer for processing and quantification of phenolic concentrations. One gram of thawed, ground plant material was placed in test tubes containing 3 ml of extraction buffer (81% distilled water, 18% methanol, and 1% glacial HCL by volume), after which samples were boiled for three minutes in a hot water bath and incubated in the dark at room temperature for 24 hours. Following the 24-hour incubation, samples were centrifuged at 6000 g and  $4^{\circ}\text{C}$  for 40 min. The supernatant was diluted to 0.005 g/ml, because absorbance values of the undiluted samples were too high to provide accurate estimates of concentrations. Samples were pipetted into spectrophotometer cuvettes transparent to light at wavelengths longer than 285 nm (Fisher Scientific, Springfield, NJ) and scanned for absorbances between 285–700 nm in a Varian Cary 50scan Spectrophotometer (Varian Scientific Instruments, Victoria, Australia). Peaks in absorbance falling between 280 and 380 nm were attributed to the presence of flavonoids or related phenolic compounds, because these substances absorb in the UV region and because the procedure used here selectively extracts these compounds (Li et al. 1993; Robberecht and Caldwell 1983). We observed two absorbance peaks (at 285 and 327 nm) following both the R:FR and UV treatments, suggesting that R:FR and UV induce similar phenolic compounds in *I. capensis*.

For the UV treatments, seedlings were transplanted into raised beds outside the Brown University greenhouse on June 14. Seedlings were positioned 10-cm apart in alternating rows of individuals from the low and high R:FR pretreatments. Lines were fully randomized within the rows. At the time of transplanting, seedlings were censused for height and length of the longest leaf. Three beds were designated for the ambient-UV treatments and three for the UV-removal. In the ambient-UV plots, Acrylite OP-4 panels (Cryo Industries, Woodcliff Lake, NJ) were positioned on top and along the sides of wooden frames (Bothwell et al. 1994). The OP-4 filter transmits 80% of incident light at 300 nm and 89% of light at 380 nm; plants in the ambient-UV treatment therefore experience a slight reduction UV relative to natural settings. The UV-removal plots were established using a similar arrangement of UV-opaque, Acrylite OP-3 panels (Cryo Industries) on wooden frames. These panels transmit less than 1% of light at wavelengths shorter than 380 nm. This treatment therefore removes both UV-A and -B. This broad-spectrum removal is appropriate to our questions of the evolution

of plasticity, because plants from differing canopy environments likely experience simultaneous decreases in all UV wavelengths. Because the experimental treatments excluded rainfall, plants were watered as needed throughout the experiment, that is, as soon as plants showed a loss in leaf turgidity. After three weeks in the UV treatments, 463 plants (two to three individuals  $\times$  50 lines  $\times$  two R:FR pretreatments  $\times$  two UV treatments) were harvested and censused for phenolic levels and dry biomass accumulation. At this harvest, plants were large enough to permit phenolic extractions using leaf tissue exclusively, rather than a combination of stems and leaves. We chose to extract from leaves alone, because phenolic compounds in leaves might reasonably be expected to affect fitness more strongly (e.g., by reducing the damaging effects of UV on photosynthesis) than phenolics in stems. These plants, in addition to the 479 remaining in the experimental plots, were also censused for leaf length and height. After an additional three weeks in the UV treatments, the remaining 479 plants were harvested and again censused individually for leaf length, height, and biomass accumulation. The sample sizes were reduced from the expected 600 plants for each of these two harvests as a result of low germination in some lines and mortality of a few plants ( $n = 12$ ) prior to the completion of the experiment. Plants that died prior to the conclusion of the experiment were scored as having zero fitness.

*Data analysis.*—We used PROC MIXED (SAS 1999) to perform mixed model analysis of variance and test the effects of genetic line, population, and R:FR pretreatment on phenolic production. Line nested within population was included as a random effect and estimated using restricted maximum likelihood. Population and R:FR were treated as fixed effects. We also tested for differences in elongation, which, following Schmitt et al (1995), is defined as greater increases in height relative to overall plant size. To test differences in elongation responses, we performed an ANOVA for height similar to the one described for phenolics, but which included leaf size as a covariate. Leaf size closely approximates differences in total plant size in *I. capensis* ( $r^2 = 0.62$ ,  $P < 0.0001$  from the current data). Including leaf size therefore controls for genetic or treatment effects on height that result from changes in overall plant size rather than changes in elongation and plant allometry per se (see Schmitt et al. 1995). In contrast to height, elongation is not confounded with plant size or vigor. However, means for plant height are presented in the Results for comparison with elongation.

Mixed model ANOVA were used to test the phenotypic and fitness effects of plot, line, population, R:FR pretreatment, and UV exposure. Plot(UV) and line(population) were treated as random effects, whereas population, R:FR treatment, UV treatment were included as fixed effects. The data were analyzed as a hierarchical ANOVA using a conservative hypothesis test, in which UV was tested over plot(UV). Crowding and competition for sunlight increased over the course of the experiment as the plants grew, such that plants in edge rows were larger in terms of biomass than those located in the center of the blocks. We therefore calculated the row residuals for all traits measured when plants were in the UV treatments (i.e., phenolics after three weeks, height after three and six weeks, and biomass after three and six

weeks), and used these residuals in both the ANOVA for genetic and treatment effects as well as in the selection analyses (described below). We included leaf size measured after the R:FR pretreatments as a covariate in the ANOVA for phenolics and for biomass measured after three and six weeks exposure to UV to control for initial differences in plant size and vigor when plants were transplanted into the UV treatments. In ANOVA models for measurements of height taken at three and six weeks in the UV treatment, biomass rather than leaf size was included as a covariate in the ANOVA to estimate line, population, and treatment effects on elongation (increases in height relative to size).

All data, other than height at three weeks in UV, met the assumptions of ANOVA. The transformation suggested by SAS ASSIST (SAS 1999), squaring the values of height, resulted in equal variances across treatments but had no effect on significance tests in the ANOVA. We therefore present results from the original data for consistency among traits and between the quantitative genetic and selection analyses.

We performed genotypic selection analysis (Rausher 1992) to determine the fitness effects of stem elongation and phenolic induction under both ambient UV and in the absence of UV. Selection analysis uses multivariate regression to quantify relative selection on multiple phenotypic traits. In these analyses, an estimate of fitness is regressed on the traits of interest, and the resulting partial regression coefficients are the selection gradients for the focal traits. Among plants similar in size to those used here, prior experiments with *I. capensis* have shown that biomass is strongly correlated with seed production ( $r = 0.95$  for outcross seed,  $r = 0.67$  for self seed production, Waller 1979). Biomass accumulation was therefore used as the fitness estimate, and, again, plants that failed to survive to the completion of the experiment received a score of zero for biomass. Following convention, biomass was relativized to the mean within each UV treatment, and genotypic (i.e., line) means of leaf length, height, and phenolic concentrations were standardized to mean of zero and a variance of one (Lande and Arnold 1983). Two selection models were used to test the relative fitness effects of elongation and phenolics. In the first analysis, relative biomass accumulation at six weeks was regressed on standardized leaf length, height, and phenolic concentrations present immediately following the R:FR pretreatments. Phenolic levels therefore reflect those induced by the high and low R:FR pretreatments. In the second model, relative biomass at six weeks was regressed on leaf length and height after the R:FR pretreatment and phenolic concentrations induced by three weeks exposure to UV. Tolerances were uniformly high ( $>0.95$ ), demonstrating that multicollinearity does not bias the regression results. For both selection models, the residuals were normally distributed.

As mentioned above, we detected absorbance peaks at 285 and 327 nm. Preliminary analyses showed that phenolics absorbing at 285 and 327 nm experienced similar selection (data not shown); we used absorbance at 285 nm for both selection models. Using the same absorbance peak for both models increases the likelihood that selection on the same trait is being measured, despite the fact that the underlying phenolics were induced by different environmental factors. However, it is possible that a somewhat different trait is being measured

if there is functional redundancy such that multiple phenolics absorb at a given wavelength and if the redundant phenolics are induced to differing degrees by R:FR versus UV.

## RESULTS

Consistent with prior studies examining photomorphogenic responses, plants in the low R:FR treatment were more elongated (i.e., taller relative to overall size) than those in the high R:FR pretreatment (Table 1A, Means  $\pm$  SE: Low R:FR = 30.09 cm  $\pm$  0.27 vs. High R:FR = 16.26 cm  $\pm$  0.26). Genetic lines also differed significantly in both average elongation and plasticity of elongation to R:FR, as shown by the significant line and line  $\times$  R:FR interactions respectively. The effect of early exposure to low R:FR on elongation remained three weeks after the plants were removed from the pretreatment and transplanted into the UV treatments (Table 2A, Means  $\pm$  SE: Low R:FR = 48.64 cm  $\pm$  1.31 vs. High R:FR = 45.35 cm  $\pm$  1.34). There was no effect of UV at this stage, either directly or in combination with R:FR. Differences among genetic lines in average elongation remained significant in this census (Table 2A). Six weeks after removal from the pretreatment, the effect of R:FR on elongation was no longer significant (Table 2B). However, when biomass was removed from the ANCOVA model, plants from the low R:FR pretreatment were significantly shorter than those exposed to high R:FR ( $P < 0.01$ —Means  $\pm$  SE: Low R:FR = 122.84 cm  $\pm$  2.39 vs. High R:FR = 126.84 cm  $\pm$  2.69); this indicates that plants from the low R:FR pretreatment were smaller overall, potentially due to the allocation costs associated with elongating at an early developmental stage. Populations differed in the sensitivity of elongation to UV after six weeks in the ambient-UV and UV-removal treatments (Table 2B); although the woodland population was more elongated than the clearing population in the UV-removal treatment, this elongation advantage disappeared when plants were exposed to UV (Fig. 1). Ultraviolet exposure also affected absolute values of height in a similar manner, such that woodland population was roughly 5% (6.07 cm) taller on average in the UV-removal relative to ambient-UV treatment. It is worth noting that the statistical analyses use a conservative hypothesis test, in which UV is tested over plot (UV). This hypothesis test severely limits the denominator degrees of freedom, and the negative effect of UV on elongation is in fact significant at both three ( $P < 0.0001$ , Means  $\pm$  SE: UV-removal = 49.22 cm  $\pm$  0.48 vs. ambient-UV = 44.51 cm  $\pm$  0.48) and six weeks ( $P = 0.046$ , Means  $\pm$  SE: UV-removal = 126.46 cm  $\pm$  1.28 vs. ambient-UV = 123.48 cm  $\pm$  1.29) when the marginal plot (UV) term is pooled with the error.

Plants in the high R:FR pretreatment had higher phenolic concentrations than those pretreated with low R:FR, as expected (Table 1B, Fig. 2). Genetic lines and populations differed in average concentrations of phenolics, with the woodland population having higher average levels than the clearing population at this stage (Fig. 2). There was no genetic variation for plasticity of phenolic production to R:FR, as evidenced by the nonsignificant line (population)  $\times$  R:FR interaction term. Nor was there evidence of population differentiation for plasticity of phenolic production to R:FR

TABLE 1. ANOVA for effects of R:FR pretreatment, genetic line, and population on (A) elongation and (B) phenolic concentrations.

A. Elongation				
Random effects				
	Variance component estimate	Error	Z-value	P-value
Line(pop)	1.038	0.556	1.87	0.031
R:FR × line(pop)	1.363	0.522	2.61	0.005
Residual	12.697	0.530	23.95	<0.001
Fixed effects				
	Numerator df	Denominator df	F-value	P-value
Leaf	1	1173.0	204.58	<0.001
R:FR	1	43.0	1945.49	<0.001
Population	1	42.1	0.19	0.664
R:FR × population	1	42.1	2.17	0.148
B. Phenolic concentrations				
Random effects				
	Variance component estimate	Error	Z-value	P-value
Line(pop)	0.007	0.002	3.10	<0.001
R:FR × line(pop)	<0.001	Inestimable		
Residual	0.019	0.002	10.12	<0.001
Fixed effects				
	Numerator df	Denominator df	F-value	P-value
R:FR	1	210.0	164.83	<0.001
Population	1	46.0	7.48	0.009
R:FR × population	1	210.0	0.90	0.344

(nonsignificant R:FR × population interaction, Table 1B, Fig. 2). After three weeks in the UV treatments, the inductive effect of seedling exposure to high R:FR on the production of leaf phenolics remained significant (Table 3, Fig. 3A). Consistent with prior results in *Impatiens* (Dixon et al. 2001), plants exposed to UV showed a 16% increase in phenolic concentrations relative to those sheltered from UV (Fig. 3B). The effect of UV on phenolics was nonsignificant using the conservative hypothesis test where UV is tested over plot(UV), but highly significant ( $P < 0.0001$ ) when the marginal plot(UV) term was pooled with the error. Genetic lines again differed significantly in average phenolic levels, as did populations. However, the clearing population produced higher levels of phenolics than did the woodland population at this stage (Table 3, Fig. 3A).

Although the low R:FR pretreatment resulted in low initial levels of phenolics, the pretreatments did not affect the relative induction of phenolics by UV as shown by the nonsignificant R:FR × UV interaction term (Table 3). Plasticity of phenolic induction to R:FR (calculated as the difference between the R:FR pretreatments) was also uncorrelated with plasticity of the same line to UV (calculated as the difference between the UV treatments after three weeks in UV) ( $r = -0.061$ ,  $P = 0.419$ ).

Biomass accumulation was greater in suppressed (non-elongated) plants pretreated with high R:FR than in elongated plants treated with low R:FR three weeks after transplanting to the UV treatments (Table 4A: Means ± SE High R:FR = 1.45 gm ± 0.04 vs. Low R:FR = 1.19 gm ± 0.04), suggesting a cost of elongation in the initially uncrowded experimental conditions. Plants exposed to ambient-UV for three weeks also accumulated less biomass than did those sheltered from UV (Means ± SE UV-removal = 1.39 gm ± 0.04 vs. am-

bient-UV = 1.30 gm ± 0.04), indicating that exposure to UV was stressful. Genetic lines differed in average biomass accumulation, but there was no genetic variation for the effect of R:FR on biomass accumulation or for susceptibility to UV damage. The absence of a population effect or population × UV interaction indicates that no local adaptation has occurred in response to the populations' history of UV exposure. The size advantage of plants pretreated with high R:FR remained at six weeks after transplanting into UV (Table 4B, Fig. 4), and was more pronounced in the clearing than woodland population (significant population × pretreatment interaction, Table 4B, Fig. 4). Plants sheltered from UV accumulated nonsignificantly more biomass than those exposed to UV at this later census, perhaps due to UV acclimation of exposed plants during the six-week treatment.

Under ambient-UV, selection favored decreased elongation among the tall plants (pretreated with low R:FR) and increased elongation among the suppressed plants (pretreated with high R:FR) (Table 5A). In the UV removal treatment, selection acted only to increase height of the suppressed plants (Table 5A). Table 5A shows the differences in selection in plant height that exist between the two R:FR pretreatment groups within each of the UV treatments; pooling data across R:FR pretreatments results in significant stabilizing selection (data not shown), consistent with directional selection observed within each pretreatment. Under both ambient-UV and in the UV-removal treatment, selection differed significantly between the pretreatment groups (heterogeneity of slopes' tests:  $F_{1,91} = 4.34$ ,  $P = 0.044$  under ambient UV and  $F_{1,90} = 5.37$ ,  $P = 0.014$  in the UV-removal treatment). Selection to increase elongation among suppressed plants presumably results from crowding and ensuing competition for light as the plants grew. The presence of selection to

TABLE 2. ANOVA for effects of R:FR pretreatment, ultraviolet level, genetic line, and population on height after three weeks and six weeks in UV.

A. Height after three weeks in UV				
	Random effects			
	Variance component estimate	Error	Z-value	P-value
Plot(UV)	8.930	6.450	1.38	0.083
Line(pop)	7.003	2.022	3.46	<0.001
R:FR × line(pop)	1.356	1.065	1.27	0.102
UV × Line(pop)	0.000	Inestimable		
R:FR × UV × line(pop)	0.598	1.038	0.58	0.283
Residual	13.958	1.175	11.88	<0.001
Fixed effects				
	Numerator df	Denominator df	F-value	P-value
Biomass	1	428.0	497.90	<0.001
R:FR	1	46.6	28.30	<0.001
UV	1	4.0	2.61	0.181
Population	1	46.6	0.36	0.552
R:FR × UV	1	85.4	1.67	0.199
R:FR × pop	1	44.7	0.91	0.346
UV × pop	1	84.3	0.27	0.606
R:FR × UV × pop	1	84.2	0.11	0.745
B. Height after six weeks in UV				
	Random effects			
	Variance component estimate	Error	Z-value	P-value
Plot(UV)	27.395	21.087	1.30	0.097
Line(pop)	30.479	12.353	2.47	0.007
R:FR × line(pop)	9.162	12.030	0.76	0.223
UV × Line(pop)	0.000	Inestimable		
R:FR × UV × line(pop)	3.607	13.357	0.27	0.394
Residual	183.600	15.324	11.98	<0.001
Fixed effects				
	Numerator df	Denominator df	F-value	P-value
Biomass	1	462.0	216.86	<0.001
R:FR	1	91.4	0.26	0.614
UV	1	4.2	0.42	0.551
Population	1	50.3	2.06	0.157
R:FR × UV	1	87.8	1.71	0.195
R:FR × pop	1	89.8	0.30	0.584
UV × pop	1	48.6	4.42	0.041
R:FR × UV × pop	1	87.5	0.52	0.473

decrease elongation of tall plants under ambient UV, but not in the absence of UV supports the hypothesis that reduced elongation is an adaptation to UV and that plasticity of elongation to UV is adaptive.

Selection on phenolic compounds induced by the R:FR pretreatments was undetectable under ambient UV (Table 5A). There was, in contrast, significant selection under ambient UV to increase the concentration of phenolic compounds produced in response to three weeks of UV exposure (Table 5B). Differences in the extraction process at the end of the R:FR and UV treatments may account for the observed differences in selection. Because of the low mass of the seedlings following the R:FR pretreatment, it was necessary to extract phenolics from both leaves and stems. For the harvest after exposure to UV, phenolics were only extracted from leaves. The observed differences in selection on phenolics induced by R:FR versus UV may therefore result from organ-specific benefits to the compounds, that is, only phenolics induced in leaves affect fitness in UV environments.

In the UV-removal treatment, there was significant selec-

tion to decrease the levels of phenolic compounds that were present in plants after the R:FR pretreatment (Table 5A); selection to decrease phenolic concentrations in the absence of UV suggests a cost to the production of these compounds. Selection on phenolic levels found in plants after three weeks in the UV-removal treatment was nonsignificant (Table 5B). The absence of selection on phenolics produced in the UV-removal treatment is consistent with the fact that plants in the UV-removal treatment were not induced by that light environment to produce phenolics. Thus, little cost of phenolic production was expected.

## DISCUSSION

When selection on a given trait reverses across environments, plastic genotypes will experience a fitness advantage over genotypes with a fixed trait expression. We observed that selection on elongation and phenolics differed across UV environments. Specifically, selection acted to decrease elongation in the ambient-UV but not in the UV-removal

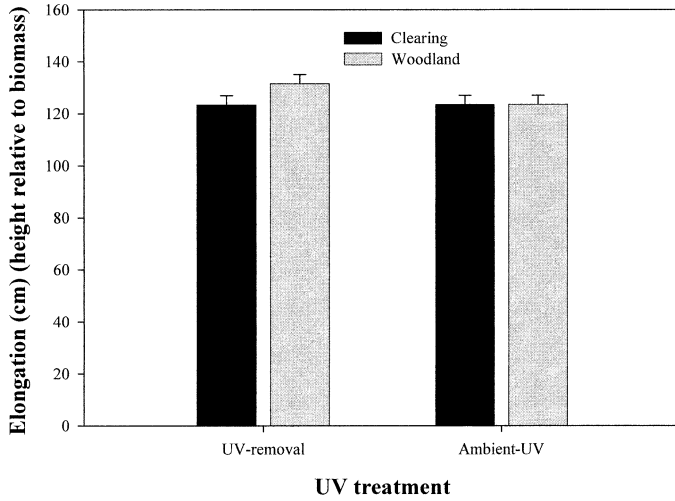


FIG. 1. Elongation responses to experimental light-quality treatments. Average differences in elongation of *Impatiens capensis* plants from clearing and woodland populations exposed either to ambient levels of UV or sheltered from UV-exposure by filters that selectively absorb in the UV region of the spectrum. Relative to the UV-removal treatment, exposure to ambient UV reduces elongation in the woodland population. Columns present mean height adjusted for biomass  $\pm$  1 SE.

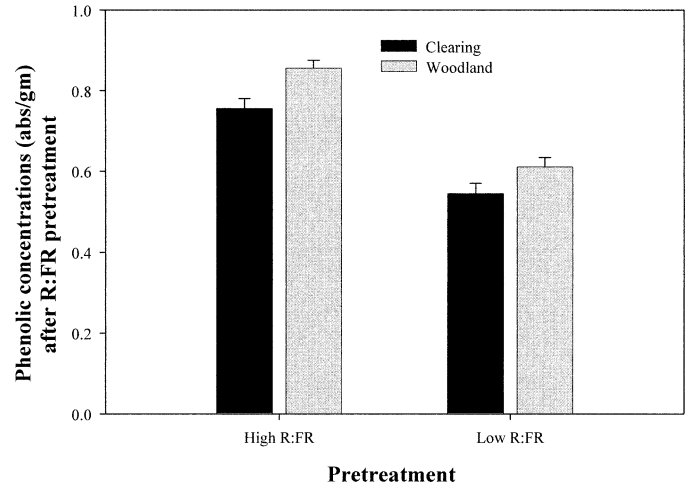


FIG. 2. Plasticity of phenolic production to R:FR conditions. Phenolic concentrations are shown for plants exposed to either high R:FR levels resembling sunlight or low R:FR levels simulating foliar shade. Relative to low R:FR, exposure to high R:FR induced phenolic production. Population differences in average phenolic levels are also visible, with plants from the woodland site having higher average concentrations at this stage. Columns shown mean absorbance/gm of plant material  $\pm$  1 SE.

treatment, providing the first demonstration that plastic reductions in elongation responses to UV are adaptive. The role of UV as a selective agent suggests that the evolution of stem elongation responses may depend on the balance of antagonistic selective forces to either elongate and avoid neighbor shading on the one hand or to decrease elongation to minimize UV damage (see discussion below). In addition, selection to increase phenolic concentrations was observed under ambient-UV conditions, whereas selection to decrease the concentration of these compounds was found in the absence of UV. Selection to decrease phenolics suggests a production cost; that is, allocation to phenolics comes at the cost of allocation to competing functions. Such costs may explain

why many plant species plastically respond to UV exposure rather than constitutively produce high levels of phenolics; plasticity in phenolic production enables plants to experience the protective benefits to these compounds under UV conditions while avoiding the cost of production under non-UV conditions.

Selection analyses coupled with environmental manipulations provide a means to identify the agent(s) of selection acting on a trait (Wade and Kalisz 1991). Because selection acted to decrease elongation under ambient-UV but not in UV-sheltered plants, the current study provides strong evidence that reduced stem elongation is an adaptation to UV rather than some other environmental factor. If decreased

TABLE 3. ANOVA for effects of R:FR pretreatment, UV exposure, genetic line, and population on phenolic concentrations following three weeks in UV.

	Random effects			
	Variance component estimate	Error	Z-value	P-value
Plot(UV)	0.005	0.004	1.37	0.085
Line(pop)	0.006	0.002	3.40	<0.001
R:FR $\times$ line(pop)	0.001	0.001	1.22	0.112
UV $\times$ Line(pop)	0	Inestimable		
R:FR $\times$ UV $\times$ line(pop)	0	Inestimable		
Residual	0.013	0.001	12.88	<0.001
	Fixed effects			
	Numerator df	Denominator df	F-value	P-value
Leaf	1	425.0	8.43	0.004
R:FR	1	299.0	4.91	0.027
UV	1	4.0	1.56	0.279
Population	1	47.5	4.68	0.036
R:FR $\times$ UV	1	355.0	0.41	0.521
R:FR $\times$ pop	1	39.4	1.92	0.174
UV $\times$ pop	1	351.0	0.98	0.324
R:FR $\times$ UV $\times$ pop	1	354.0	0.63	0.427

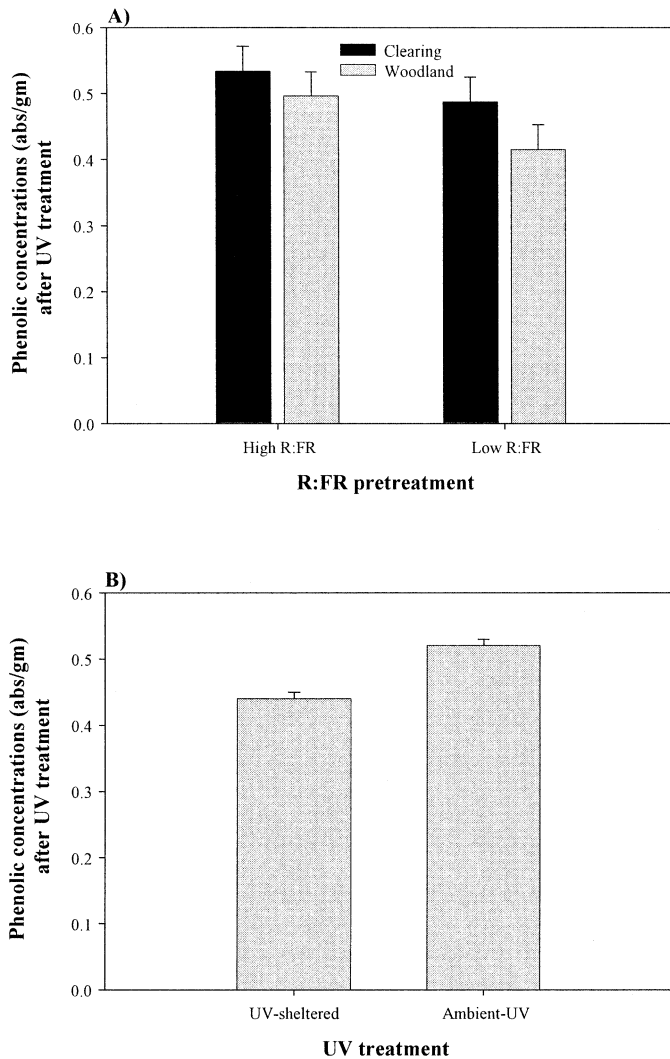


FIG. 3. Plasticity of phenolic production to R:FR and UV exposure. Concentrations of phenolics are shown for plants pretreated with high versus low R:FR and subsequently either exposed to or sheltered from ambient-UV radiation for three weeks. (A) The inductive effect of high R:FR persists after three weeks in UV. Population differences are also shown, and the clearing population has higher phenolic concentrations than the woodland population at this stage. (B) Plants exposed to ambient UV have higher phenolics than those sheltered from UV. Columns again depict mean absorbance/gm of plant material  $\pm$  1 SE.

elongation had proven advantageous in ambient-UV and UV-sheltered environments, then reduced elongation and plant allometry would have to serve a function other than just UV protection and a selective agent other than UV would have to exist in the experimental settings we used. Perhaps it is also worth noting that if decreased elongation were simply a passive growth response to a stressful environmental setting, we would have expected a positive correlation between elongation and fitness under ambient UV; the more stressed plants would be less elongated and have lower fitness. The opposite pattern is observed here; less elongated plants had higher fitness under ambient UV.

Variable selection on phenolic levels across the UV environments supports the role of these compounds as an ad-

aptation to UV stress. Higher concentrations of phenolics were advantageous under ambient UV, yet maladaptive when plants were sheltered from UV. This result differs from that of a prior study in *Impatiens*, in which selection on phenolics was nonsignificant (Dixon et al. 2001). The difference in results between the two experiments may arise due to slight differences in the pretreatment conditions and attendant differences in initial concentrations of phenolics when plants were transferred to the UV treatments. More specifically, the filters used in the R:FR pretreatments reduced photosynthetically active radiation (PAR) by 60 percent in the prior experiment but only 30 percent in the current experiment. The lower irradiance during the R:FR pretreatment in the prior experiment may have increased the effects of light-regulated developmental pathways other than those mediated by phytochromes (Dixon et al. 2001) and impeded induction of phenolics by high R:FR. Alternatively, higher PAR during the pretreatments in the current experiment may have provided the carbon resources necessary for the production of phenolic compounds. Regardless of the underlying mechanism, only in the current experiment did the high R:FR pretreatment significantly increase phenolic production relative to the low R:FR treatment. The resulting increase in the range of phenotypic variation enhanced the ability to detect selection on phenolics in the current experiment. Consistent with the patterns of selection observed here, previous studies in *Arabidopsis thaliana* have shown that the fitness of phenolic-deficient mutants is lower than that of wild-type plants under UV conditions (Li et al. 1993; Landry et al. 1995), and that transgenic plants overexpressing the phenylpropanoid pathway, by which phenolics are produced, have lower fitness than wild-type plants in non-UV conditions (Bieza and Lois 2001). Our results demonstrate that natural genetic variants in phenolic expression undergo similarly strong selection.

The adaptive nature of plasticity in phenolic production is analogous to other important plant responses to environmental stress, such as the induction of plant defensive compounds. In wild radish, defensive compounds enhance fitness when plants are under attack by natural enemies (Agrawal 1999), but reduce fitness in the absence of enemies as a result of production costs (Agrawal et al. 1999). The compounds are therefore adaptive in the inductive environment but maladaptive under noninductive conditions, favoring the evolution of plasticity. However, it is likely that the magnitude of production costs will vary with environmental quality such that costs are reduced in higher-quality environments (Cipollini et al. 2003). Correspondingly, constitutively high production of phenolics and plasticity might equally be expected to evolve in species with predictably high-quality environments.

Differences in biomass accumulation between the R:FR pretreatment groups were consistent with prior studies testing allocation trade-offs associated with stem elongation. In low-density conditions such as those of our initial planting, nonelongated plants are expected to accumulate more biomass than elongated ones that allocate preferentially to stem growth at the cost of resource-harvesting organs such as leaves and roots (Smith 1982; Cipollini and Schultz 1999; Maliakal et al. 1999; Weinig 2000b). Suppressed (nonelongated) seedlings are also more responsive than elongated

TABLE 4. ANOVA for effects of R:FR, UV, line, and population on biomass accumulation after (A) three and (B) six weeks in the ambient-UV and UV-removal treatments.

A. Biomass after three weeks in UV				
Random effects				
	Variance component estimate	Error	Z-value	P-value
Plot(UV)	0.012	0.012	0.93	0.176
Line(pop)	0.028	0.010	2.78	0.003
R:FR $\times$ line(pop)	0.001	0.007	0.02	0.494
UV $\times$ line(pop)	0	Inestimable		
R:FR $\times$ UV $\times$ line(pop)	0	Inestimable		
Residual	0.151	0.016	9.44	<0.001
Fixed effects				
	Numerator df	Denominator df	F-value	P-value
Leaf	1	439.0	126.65	<0.001
R:FR	1	46.9	32.46	<0.001
UV	1	215.0	4.25	0.041
Population	1	49.8	0.03	0.853
R:FR $\times$ UV	1	219.0	0.87	0.352
R:FR $\times$ pop	1	46.4	0.25	0.617
UV $\times$ pop	1	216.0	0.70	0.404
R:FR $\times$ UV $\times$ pop	1	217.0	0.98	0.323
B. Biomass after six weeks in UV				
Random effects				
	Variance component estimate	Error	Z-value	P-value
Plot(UV)	0	Inestimable		
Line(pop)	7.878	2.836	2.78	0.003
R:FR $\times$ line(pop)	0.703	1.769	0.40	0.346
UV $\times$ Line(pop)	1.355	1.857	0.73	0.233
R:FR $\times$ UV $\times$ line(pop)	0	Inestimable		
Residual	33.454	2.599	12.87	<0.001
Fixed effects				
	Numerator df	Denominator df	F-value	P-value
Leaf	1	461.0	67.30	<0.001
R:FR	1	43.7	28.82	<0.001
UV	1	44.1	0.97	0.330
Population	1	50.1	0.91	0.344
R:FR $\times$ UV	1	369.0	0.22	0.640
R:FR $\times$ pop	1	41.7	11.15	0.002
UV $\times$ pop	1	44.1	0.91	0.345
R:FR $\times$ UV $\times$ pop	1	370.0	0.13	0.721

seedlings to subsequent R:FR cues of competition (Weinig and Delph 2001), potentially due to their greater resource base. During the initially low-density conditions of the experiment, the suppressed plants pretreated with high R:FR accumulated more biomass relative to the plants pretreated with low R:FR. After three to four weeks in the UV treatments, plants were visibly crowded, and plants in all treatments experienced selection to increase height. The greater biomass accumulation of the suppressed plants enabled them to grow taller than the elongated plants and sustain an overall size advantage when crowded later in the experiment.

In natural populations, expressed patterns of shade-avoidance should reflect a balance between selection to reduce susceptibility to UV damage on the one hand and prevent complete shading by neighbors. Less elongated plants fare better than tall plants when exposed to UV, although greater elongation enables plants to overtop neighbors and increase both leaf exposure and fitness in crowded settings. Plants should therefore express a shade-avoidance phenotype until the damaging fitness effects of UV exposure counterbalance

the favorable effects of leaf exposure and greater photosynthesis.

Experimental plants apparently adaptively modified their phenotypes in response to the light treatments. Plants exposed to ambient UV had lower biomass than those sheltered from UV, but prior exposure to low R:FR did not exacerbate the negative effect of UV, which is contrary to the result expected if UV-susceptibility were an opportunity cost of phytochrome-mediated shade avoidance. The absence of a R:FR  $\times$  UV interaction presumably reflects the ongoing plasticity of plants to their environment, resulting in adaptive reductions in stem elongation as well as shifts in phenolic distributions and increases in other unmeasured, UV-protective responses when plants are moved to UV from the low R:FR pretreatments. Although the low R:FR pretreatment resulted in maladaptive trait expression (elongated plants with lower initial levels of phenolics relative to high R:FR plants), subsequent exposure to UV for six weeks prior to harvest provided sufficient time for plants pretreated with low R:FR to modify their phenotype in an adaptive manner. Observed ac-

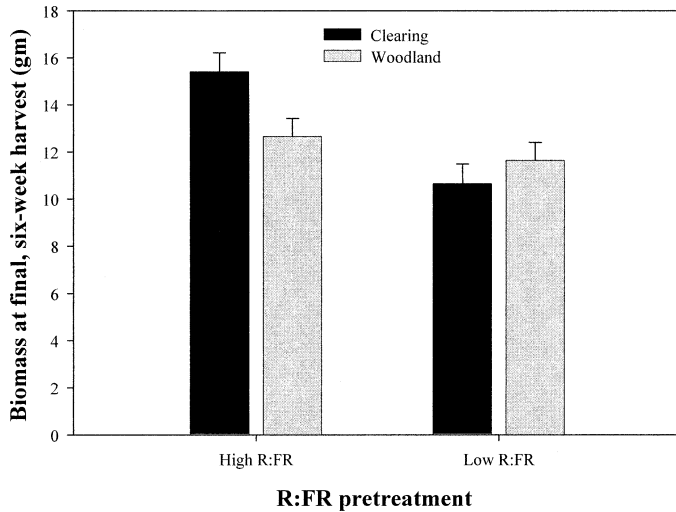


FIG. 4. Population and R:FR effects on biomass accumulation at the final, six-week harvest. Plants exposed to high R:FR accumulated more biomass than did those pretreated with low R:FR. Populations also differed in size, with plants from the clearing population accumulating more biomass than those from the woodland population under high R:FR. Columns show means  $\pm$  1 SE.

climation is consistent with biochemical studies, in which UV-B exposure rapidly elevates transcript abundance of genes in the phenylpropanoid pathway (Beggs and Wellman 1994). Given that plants in natural populations immediately modulate elongation on exposure to UV and have several weeks to acclimate (e.g., produce protective secondary compounds) to UV prior to senescence, individuals overtopping their neighbors and emerging from low R:FR into high UV conditions are not expected to be more damaged by UV exposure than those initially experiencing high R:FR.

The results of the current study are consistent with the growing understanding of plant developmental systems and the potential for adaptive responses. Experimental evidence for an unidentified UV-B receptor suggests that plants may detect and adaptively respond to UV-B exposure (Ballaré et al. 1995), in addition to other aspects of light quality that supply information regarding ambient environmental con-

ditions or future changes. The developmental mechanisms underlying induction of phenolics by R:FR and UV also appear largely independent. Studies with the model systems *Arabidopsis thaliana* (Batschauer et al. 1996) and *Lycopersicon esculentum* (Ballaré et al. 1995) suggest that different photoreceptors perceive R:FR and UV light. Therefore, at an early developmental stage, perception of and attendant phenolic induction by different aspects of light quality appear independent. However, other studies have shown that biosynthetic intermediates of the phenylpropanoid pathway can block the induction of key enzymes in that pathway (Bolwell et al. 1988), suggesting that the evolution of phenolic production elicited by alternative light cues may be constrained by downstream convergence in the developmental pathway. In the natural lines of *I. capensis* used here, we observed that plasticity of phenolic induction to R:FR was genetically uncorrelated with plasticity of the same line to UV, and the R:FR pretreatments did not affect the relative induction of phenolics by UV.

This study illustrates the value of connecting developmental, physiological, and evolutionary studies. Physiological studies have described both the effects of R:FR and UV on phenolic production (Beggs and Wellman 1994) and how light quality varies in the natural environment (Smith 1982; Ballaré et al. 1990). With this information, it was possible to identify an ecologically relevant manipulation that expanded the phenotypic distribution of phenolics independent of UV conditions and enabled a test of adaptive plasticity. One assumption of phenotypic manipulations is that the experimental manipulation accurately reproduces the costs and benefits of the focal phenotype. In the current experiment, we assume that the costs associated with phenolic production are the same regardless of whether UV or R:FR induces them. This is likely a valid assumption if synthesis of the compounds rather than maintenance of the underlying developmental mechanisms accounts for the observed production cost. Moreover, tests of adaptive plasticity are notoriously difficult because plastic organisms continually modify their phenotype in response to environmental variation (Dudley and Schmitt 1996), and phenotypic manipulations offer unique initial insights into the evolutionary dynamics of this

TABLE 5. Standardized selection gradients,  $\beta'$ , for phenolics and height (A) including phenolics produced in response to the two-week R:FR pretreatment and (B) including phenolics induced by three weeks in UV. In these analyses, relative biomass of plants after six weeks in the UV treatments is used as the estimate of fitness and is regressed on standardized height, leaf length, and phenolics. For both models, height and leaf length were measured at the completion of the R:FR pretreatments. Significant selection gradients are shown in bold.

Effect	Ambient UV				UV removal			
	$\beta'$ high R:FR	<i>P</i>	$\beta'$ low R:FR	<i>P</i>	$\beta'$ high R:FR	<i>P</i>	$\beta'$ low R:FR	<i>P</i>
Height	<b>0.33</b>	<b>0.043</b>	<b>-0.36</b>	<b>0.004</b>	<b>0.42</b>	<b>0.014</b>	0.02	0.908
Leaf	0.24	0.134	0.69	<0.001	0.12	0.495	0.40	0.02
Phenolics <sub>R:FR</sub>	-0.20	0.104	-0.05	0.720	<b>-0.33</b>	<b>0.038</b>	<b>-0.40</b>	<b>0.016</b>

Effect	$\beta'$ Ambient UV		$\beta'$ UV-removal	
	$\beta'$	<i>P</i>	$\beta'$	<i>P</i>
Height	-0.21	0.032	0.18	0.094
Leaf	0.40	<0.001	0.34	0.003
Phenolics <sub>UV</sub>	<b>0.34</b>	<b>0.003</b>	0.01	0.928

strategy. The work also demonstrates how evolutionary studies using naturally occurring genetic variants are important for determining the ecological relevance of photomorphogenic responses identified in model systems. Other studies have tested the importance of phenolics to UV-protection by comparing the fitness of wild-type plants under UV conditions with that of mutants and transgenics carrying genetic modifications of the phenylpropanoid pathway (Li et al. 1993; Landry et al. 1995; Bieza and Lois 2001). However, few studies have used wild species to test the relevance of natural, within-population variation to individual fitness under current UV conditions (Mazza et al. 2000).

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