

**PHYSIOLOGICAL MECHANISM OF POPULATION
DIFFERENTIATION IN SHADE-AVOIDANCE RESPONSES
BETWEEN WOODLAND AND CLEARING GENOTYPES OF
*IMPATIENS CAPENSIS*¹**

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Forest understory plants often respond less intensely to reduced ratios of red to far red (R : FR) light, an important signal of foliage shade, than conspecific or congeneric plants from open-canopy sites. Reduced responsiveness to low R : FR in plants from closed-canopy sites could be caused by two physiological mechanisms. First, closed-canopy plants could have less sensitive shade-avoidance responses to low R : FR. Second, the high irradiance response to FR (FR-HIR), which allows seedling de-etiolation under low R : FR, might be stronger or persist longer after de-etiolation in closed-canopy plants, thus counteracting shade-avoidance responses to low R : FR. These hypotheses were tested using diodes that emit red and far-red light to distinguish the responses to altered R : FR of genotypes of *Impatiens capensis* collected from a pair of open- and closed-canopy populations that have previously been shown to differ in sensitivity to R : FR. Genotypes from the open-canopy environment exhibited typical shade-avoidance responses, elongating in response to supplemental FR. However, genotypes from the closed-canopy environment responded to supplemental FR by elongating *less* than under ambient control conditions, indicating a persistent FR-HIR. Thus, the observed population differentiation in response to low R : FR may be linked to population differences in FR-HIR.

Key words: *Arabidopsis*; ecotypic differentiation; high irradiance response; light-emitting diodes; *Nicotiana*; phytochrome; plasticity; shade-avoidance syndrome.

The shade-avoidance syndrome in plants is an ecologically important example of adaptive plasticity (Smith, 1982, 1995; Schmitt et al., 1999, 2003). The ratio of red (~680 nm) to far-red (~735 nm) (R : FR) light is roughly equal in full sunlight, but falls sharply under foliage shade and in light reflected from green vegetation due to the absorption of red light by chlorophyll (Smith, 1981). Plants perceive the decreased R : FR beneath foliage shade via light-stable phytochrome photoreceptors (Smith and Whitelam, 1997; Moller et al., 2002), and respond with a correlated set of photomorphogenic changes that include elongating, flowering earlier, and growing toward high-light patches (Smith, 1982). Plants can perceive reductions in R : FR in the light reflected from nearby vegetation, use it as an accurate indicator of neighbor proximity, and respond to it morphologically even before they are directly shaded (Ballaré et al., 1987, 1990). This phytochrome-mediated shade-avoidance syndrome has been hypothesized to be advantageous because it allows plants to sense and avoid competition for light in dense stands (Casal and Smith, 1989; Schmitt and Wulff, 1993; Schmitt et al., 1999, 2003). This hypothesis has been supported by experiments that have placed elongated and unelongated plants into stands of both

low and high density; elongated plants have higher fitness than unelongated plants at high density, but lower fitness at low densities (Schmitt et al., 1995; Dudley and Schmitt, 1996).

Responsiveness to R : FR varies among species, among populations within species, and within populations (e.g., Fitter and Ashmore, 1974; Morgan and Smith, 1979; Gilbert et al., 1995; Van Hinsberg, 1997; Weinig, 2000a; Gilbert et al., 2001), suggesting that shade-avoidance traits may be evolutionarily labile. Species or genotypes from closed-canopy habitats such as forest understories are often less sensitive to R : FR than species from open habitats (Morgan and Smith, 1979; Corre, 1983; Dudley and Schmitt, 1995; Weinig, 2000b). Morgan and Smith (1979) proposed that such reduced sensitivity could evolve as a result of the overhead canopy generating a constantly low R : FR, which makes direct overhead light an unreliable cue for competition from neighboring plants. Shade-avoidance responses to this unreliable cue under a closed canopy might therefore be maladaptive, resulting in direct selection for decreased sensitivity to R : FR.

It is also possible that in some ecological conditions selection on other phytochrome-mediated processes, such as the shift from growth with seed reserves to autotrophic photosynthetic growth (de-etiolation), may constrain the evolution of shade avoidance. De-etiolation, whereby stem elongation is inhibited, cotyledons expand, and chloroplasts develop, can occur under a range of R : FR conditions because of the antagonistic but complementary activity of light-stable and light-labile phytochromes (Yanovsky et al., 1995; Smith et al., 1997). Under the high R : FR conditions characteristic of open sites, de-etiolation is mediated by light-stable phytochromes, primarily phytochrome B (Smith et al., 1997; Moller et al., 2002). This is the same mechanism that inhibits shade avoidance under high R : FR conditions in light-grown plants. In both cases, activated light-stable phytochromes inhibit stem

¹ Manuscript received 23 July 2004; revision accepted 26 January 2005.

The authors thank Mike Hay for helpful technical discussion and for information about his previous diode experiments, John Stinchcombe for invaluable statistical help and discussion of *Impatiens* ecology, and Jacob Weiner and Kelly Gravuer for helpful discussion and careful reading of earlier drafts of this paper. We thank Nannette Reese, Bhoomika Singh, Robin Hopkins, Lauren McGeoch, Joanna Kelly, Emily de Moor, and Afton Brantle for assistance with data collection and maintenance of the experiment, Fred Jackson and Brian Leib for greenhouse assistance, and Ed Mullan for technical assistance. This research was supported by NSF grants DEB-9806858, DEB-0129018, and DEB-DIG 0408015 and by an EPA-STAR graduate fellowship to E.V.

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elongation. However, seedlings emerging under foliage shade must de-etiolate under low R : FR. Leaf litter, which may be more common in closed-canopy sites, also decreases R : FR on the soil surface (Bliss and Smith, 1985). De-etiolation under such conditions is mediated by light-labile phytochrome A, which responds to far-red light with a high irradiance response (FR-HIR) that suppresses shoot elongation, and acts antagonistically to the shade-avoidance response (McCormac et al., 1992; Casal et al., 1997; Smith et al., 1997). This FR-HIR is essential for successful establishment under foliage shade (Yanovsky et al., 1995) or thick leaf litter, and the appearance of phytochrome A, which is far-red light sensitive and light labile, early in angiosperm evolution may have allowed the evolution of forest understory angiosperms (e.g., Mathews et al., 2003). Because open-canopy sites often have both smaller amounts of leaf litter and higher R : FR than closed-canopy sites, selection pressure on the strength of the FR-HIR may vary between open- and closed-canopy sites. Even in temperate regions, where de-etiolation in closed-canopy sites may occur before the closure of the overhead canopy, the need to respond to leaf litter may cause differences in selection pressure between open- and closed-canopy sites.

If selection on the FR-HIR is stronger in closed-canopy sites, it may have correlated effects on the evolution of shade avoidance. For shade-avoidance responses to low R : FR to be expressed in light-grown plants, phytochrome A must degrade rapidly upon exposure of the seedling to light, as is typically observed (Sharrock and Clack, 2002). If phytochrome A persists at high levels in light-grown plants, as a result of transgenic overexpression (Halliday et al., 1997) or mutation (Malooof et al., 2001), its antagonistic action suppresses shade-avoidance responses. Thus, if selection on de-etiolation in closed-canopy sites or sites with thick layers of leaf litter favors phytochrome A persistence and a stronger, more persistent FR-HIR, a correlated suppression of shade-avoidance responses could result.

The relative roles of shade avoidance and phytochrome A-mediated FR-HIR in different populations can be distinguished experimentally by examining plant responses to supplemental FR. If differentiation between open- and closed-canopy forms results from reduced shade avoidance in response to low R : FR, plants from both habitats will elongate more when exposed to supplemental FR (i.e., to low R : FR) than under control conditions, but the extent of elongation will be less in closed-canopy forms. However, if differentiation between open- and closed-canopy forms results from a difference in the balance of FR-HIR and low R : FR shade avoidance, the two forms will respond in opposite ways to supplemental FR. Open-canopy forms will be more elongated under FR supplementation than under control conditions, whereas closed-canopy forms will elongate less under FR supplementation than controls.

In distinguishing these hypotheses, it is also important to ask whether responses to overhead and lateral low R : FR light are the same in open- and closed-canopy forms. If understory plants can distinguish between overhead shading from the canopy and light reflected from neighboring plants in the understory, they can avoid responding to overhead shading but still respond preemptively to incipient shading in the understory. These two R : FR signals can be distinguished by the tissue that perceives them. Light transmitted through the overhead canopy, with reduced photosynthetically active radiation (PAR) and a reduced R : FR, will primarily strike horizontally

oriented tissue such as leaves, while some light striking neighboring plants in the understory will be reflected and refracted onto vertically oriented tissue such as stems (Ballaré et al., 1987, 1990; Novoplansky et al., 1990). Yet, both stem and leaf tissues have the ability to induce shade-avoidance responses because subsets of the phytochrome photoreceptors responsible for mediating the responses are expressed in both tissues (Sharrock and Clack, 2002). Sensitivity in leaves and stems has been documented in experiments that shine FR-emitting diodes, which reduce the R : FR ratio, onto stem and leaf tissue (e.g., Robin et al., 1994a, b; Heraut-Bron et al., 1998, 1999, 2001; Hay et al., 2001), as well as inferred from experimental treatments that use filters to reduce red light in overhead radiation (e.g., Ballaré et al., 1990; Dudley and Schmitt, 1995; Weinig, 2000a). Molecular dissection of the expression of phytochromes has shown that they are expressed in both stem and leaf tissue, but that the total expression level and the balance of the different forms varies (Sharrock et al., 1995, 2003; Adam et al., 1997; Goosey et al., 1997; Sharrock and Clack, 2002). This suggests the possibility for independent evolution of response to lateral R : FR cues (Schmitt et al., 1999).

Here we investigate shade avoidance and FR-HIR responses to tissue-specific light in the annual plant *Impatiens capensis*, which grows in a wide range of canopy habitats. Field observations and reciprocal transplant experiments have documented differentiation between shade and sun forms in *I. capensis* and the related *I. pallida* (Schemske, 1984; Schmitt, 1993; Donohue and Schmitt, 1999; Donohue et al., 2000a, b, 2001), including reduced plasticity to shade (Dudley and Schmitt, 1995). Selection favors greater plasticity of internode elongation in open sites than woodland sites (Donohue et al., 2000a). De-etiolation under low R : FR may also be under selection in field conditions, especially in woodland sites, where it is important for penetration of variable layers of leaf litter and for early growth before the forest canopy closes.

To separate shade avoidance and FR-HIR responses, we directed red and far-red light-emitting diodes (Robin et al., 1994a, b; Heraut-Bron et al., 1998, 1999, 2001; Hay et al., 2001) at both stem and leaf tissue of de-etiolated seedlings of genotypes from a pair of previously studied open- and closed-canopy populations of *Impatiens capensis* (e.g., Schmitt, 1993; Dudley and Schmitt, 1995). The results suggest that differences in FR-HIR between closed- and open-canopy genotypes contribute to differences in shade avoidance between the genotypes.

MATERIALS AND METHODS

Study species—*Impatiens capensis* Meerb. (Balsaminaceae) is an annual, self-compatible herb of North American deciduous forests and wetlands (Gleason and Cronquist, 1963; Leck, 1979, 1996). With its mixed mating system, *I. capensis* commonly produces self-fertilizing cleistogamous flowers as well as outcrossing chasmogamous flowers (Waller, 1979), allowing the production and maintenance of inbred lines.

Genotype collection—In summer 1995, seedlings were collected from a 40 × 40 m permanent grid (Schmitt and Gamble, 1990; Argyres and Schmitt, 1991) in the center of a population in the understory of an oak-hickory forest and in an open seasonal stream bed population 1 km away at Brown University's Haffenreffer reserve (Bristol, Rhode Island, USA). These seedlings were grown in a greenhouse to start a collection of inbred lines. These lines were maintained by single-seed descent for six generations, and thereafter by bulk collection for another 10 generations. Seeds were collected from cleistoga-

TABLE 1. Proc Mixed results for shade-avoidance traits in *Impatiens capensis* differentially affected by the three diode types across populations. Diode type, source population (Pop), and diode position were fixed effects, with genotype nested within source population as a random effect. Only traits with significant diode type by source population effects are shown. Interaction terms greater than 0.20 were removed from the models. All three-way interactions were not significant and were removed. All tests of fixed effects are type III. Height, first internode length, second internode length, and leaf areas were analyzed as residuals of the final trait value regressed on its initial value at the beginning of the experiment.

Trait	Type	Tissue	Pop	Type × Pop	Type × Tissue	Tissue × Pop	Line
First internode	$F_{2,107} = 0.59$ $P = 0.556$	$F_{1,107} = 0.01$ $P = 0.092$	$F_{1,6,18} = 1.67$ $P = 0.243$	$F_{2,107} = 5.19$ $P = 0.007$	$F_{2,107} = 0.65$ $P = 0.522$	$F_{1,107} = 0.45$ $P = 0.505$	LRR = 10.8 $P < 0.001$
Second internode	$F_{2,107} = 1.06$ $P = 0.349$	$F_{1,107} = 0.02$ $P = 0.880$	$F_{1,6,22} = 5.97$ $P = 0.049$	$F_{2,107} = 5.67$ $P = 0.005$	$F_{2,107} = 0.85$ $P = 0.429$	$F_{1,107} = 0.00$ $P = 0.955$	LRR = 7.9 $P = 0.003$
Total height	$F_{2,106} = 0.04$ $P = 0.961$	$F_{1,106} = 0.01$ $P = 0.916$	$F_{1,6,07} = 0.56$ $P = 0.482$	$F_{2,106} = 3.73$ $P = 0.027$	$F_{2,106} = 0.73$ $P = 0.482$	$F_{1,106} = 0.23$ $P = 0.634$	LRR = 27.6 $P < 0.001$
Total leaf area	$F_{2,107} = 0.95$ $P = 0.390$	$F_{1,107} = 1.12$ $P = 0.292$	$F_{1,6,16} = 0.36$ $P = 0.571$	$F_{2,107} = 3.21$ $P = 0.044$	$F_{2,107} = 0.45$ $P = 0.640$	$F_{1,107} = 1.01$ $P = 0.318$	LRR = 14.3 $P < 0.001$

mous flowers, placed in 96-well trays, and stratified at 4°C in water for 4 months. Seeds were planted after radicles began to emerge but before hypocotyl elongation began.

Eight genotypes, four from the open-canopy population and four from the forest understory population, were used in this study. These genotypes have previously been shown to differ in elongation responses to low R : FR (Dudley and Schmitt, 1995; Donohue and Schmitt, 1999).

Greenhouse conditions—Twenty seeds of each of eight genotypes were planted on 7 August 2002 in the research greenhouse of Brown University. Each seed was placed in a 10.16-cm pot with Metromix 350 (Scotts-Sierra Horticultural Products Co., Marysville, Ohio, USA) potting soil. Pots were placed approximately 10–15 cm apart on the bench, at a sufficient distance to minimize shading of young plants. Plants were initially top-watered until seedlings became established, and then bottom-watered daily.

Diodes—Red (680 nm) and far-red (735 nm) diodes were purchased from Quantum Devices Inc. (Barneveld, Wisconsin, USA), as used by Hay et al. (2001). These diodes provided light with less than 1% of energy outside 10 nm of the listed wavelength. Diodes produced 3.3 mW of energy at 50 mA and 2.5 V. Each diode was powered by two AAA batteries. Diodes were attached to flexible aluminum rods so that they could be adjusted to shine on a single leaf or stem internode. Diodes were aimed directly and perpendicularly at tissues, because reflected light at large angles may alter the effect of far-red light on elongation (Seavers and Smith, 1997). Control devices were constructed of the same aluminum material and adjusted to shade plants in a fashion similar to the experimental devices, but had no diode. Although these diodes direct all of their light within 20° of the direction in which they are aimed, aluminum foil was wrapped around the diodes like a lampshade to reduce scattering of light. Aluminum foil reflects over 95% of light at infrared and red wavelengths. Diodes were turned on at 0900 hours and turned off at 1600 hours daily. Throughout the course of the experiment, diodes were adjusted to remain focused on stem or leaf tissue. Three of the diodes burned out and were replaced within 24 h.

Harvesting and processing—On 23 August 2002, all plants were harvested. Plants were separated into stem and leaf tissues. A few plants had begun to produce cleistogamous flowers, but the majority of plants had not begun either to flower or to form branches at nodal secondary meristems. Reproduction was scored, but reproductive tissues were not separated from nearby leaf tissue because of their very small size. All leaves were collected individually, stored flat in plastic bags, and photocopied within 3 d. Photocopies of leaves were scanned into a digitized format, and leaf areas were calculated using NIH Image (<http://rsb.info.nih.gov/nih-image/>). Leaf and stem tissue were dried for 2 d at 60°C and weighed. Our response variables were total height, first internode length, second internode length, and total leaf area. Total height and the lengths of all internodes were analyzed as residuals of their final size

regressed on their initial size, because preliminary analyses showed the traits were significantly affected by initial size (Graham, 2003).

Statistical analysis—The experimental treatments were performed in a 2 × 3 design, with focal tissue (stem or leaf) fully crossed with diode type (red, far-red, and control). Because of poor germination, only three plants per genotype were in each diode treatment, and two in each control treatment. Because of subsequent mortality of seedlings, we had only one control plant for the three genotypes in one of the tissue treatments. In total, there were 128 plants in the experiment.

To discover whether any traits were differentially affected by diode type in the two populations or the tissue locations, we tested for significant interactions between diode type and population and between diode type and tissue treated with Proc Mixed (Littell et al., 1996) in SAS v. 8.0 (SAS Institute, Cary, North Carolina, USA). Diode treatment, population, and tissue were fixed effects, and genotype nested within population was a random effect. We included genotype as a class variable in these analyses to account for effects of genetic variation within these populations (e.g., Schmitt, 1993; Dudley and Schmitt, 1995; Donohue and Schmitt, 1999; Donohue et al., 2000a, b, 2001). All interactions, including three-way interactions, were initially included in the models. Because all three-way interactions were not statistically significant ($P > 0.2$ for all), they were removed. We retained second-order interactions in the model regardless of statistical significance, because these interactions were in many cases the tests of our hypotheses. Denominator degrees of freedom for F tests were determined by Satterthwaite approximation (the “DDFM = SATTERTH” option in Proc Mixed). When we detected statistically significant diode treatment × population or diode treatment × tissue treatment interaction terms, we used means contrasts to determine which of the three diode treatments differed from each other between populations or tissue treatments. Means contrasts for interaction terms were performed using the “slice” option of the “Ismeans” statement of Proc Mixed (Schabenberger et al., 2000; Herrera et al., 2002).

To conclude that there is not a difference between treatments or populations in our analysis, we depend on the null hypothesis not being rejected. To determine if our design gives us the power to detect population differences, such as those detected by Dudley and Schmitt (1995), we performed a power test using G*Power software (Buchner et al., 1997). Instead of performing a post-hoc power test, we used the means and standard deviations reported by Dudley and Schmitt (1995) to determine our effect size.

RESULTS

Elongation—The effects of diode treatment on the first and second internode and total height were consistent between the two tissues treated but not between the two source populations (Table 1, Fig. 1A–C). Stems and leaves did not differ in their response to diode treatment, as indicated by the absence of a

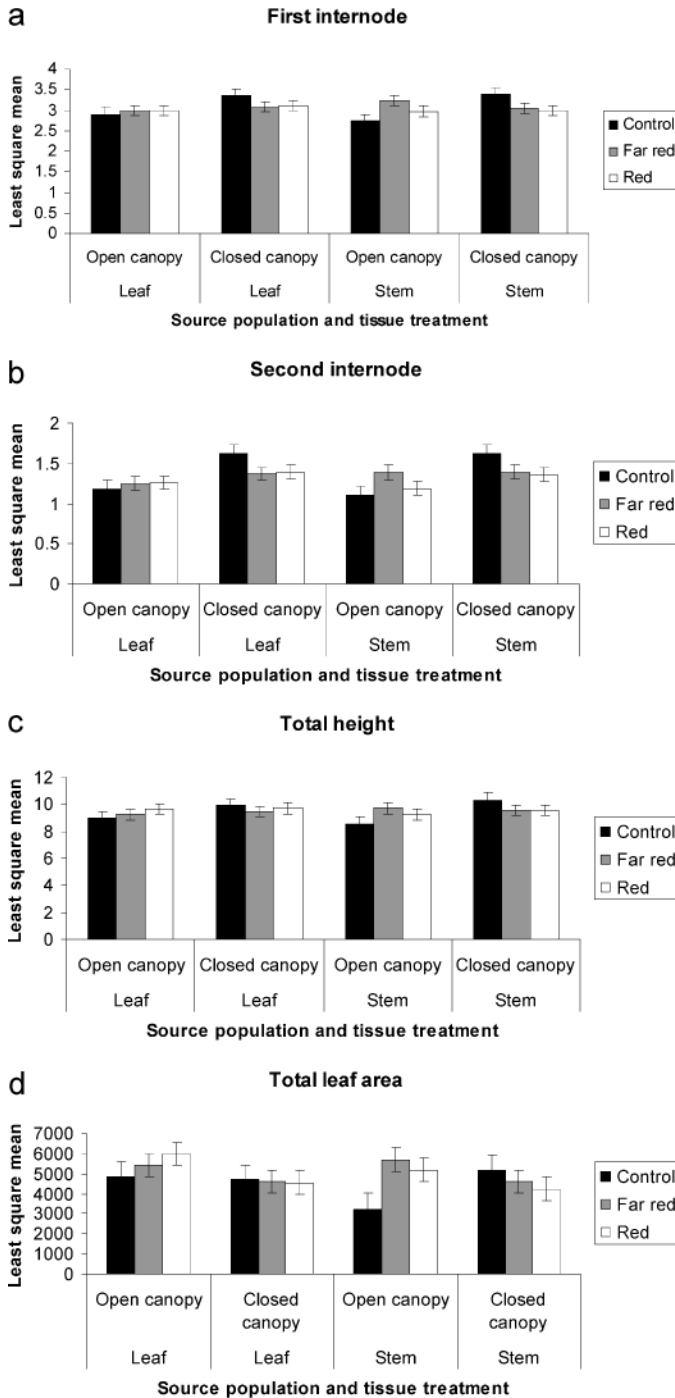


Fig. 1. Shade-avoidance traits in *Impatiens capensis* differentially affected by treatment with far red, red, or control diodes. Analyses were performed on residuals of traits to account for differences in initial size. Analyzing the residuals brought the traits into agreement with the assumptions of analysis of covariance. Post-hoc least square means were used to identify significant differences between specific diode treatments within source populations and the two tissue treatments. (a) First internode, (b) Second internode, (c) Total height, (d) Total leaf area.

significant main effect of treatment position or significant interactions of treated tissue with source population or diode type (Table 1). Our power analysis suggests that this is not due to a lack of power: our power to detect effects (effect size

= 1.7667) as large as those reported by Dudley and Schmitt (1995) is 0.9840 at alpha = 0.05 ($F = 5.987$, lambda = 24.970), and our power to detect an effect half the size is 0.9602 ($F = 4.494$, lambda 15.679). However, there was a difference in the response of plants to far-red or red light, in comparison to the control treatment, depending on their source population. When compared to control treatments, plants from the two source populations responded to supplemental far-red light in opposite directions, as indicated by decomposition of the significant diode treatment \times source population effect (Table 1).

We used the “slice” option to decompose the interaction. Plants in the control treatment had significantly shorter internodes in the open-canopy population than the closed-canopy population, and tended to be shorter (Fig. 1A–C; 1st internode $F_{1,16.7} = 7.69$, $P = 0.0132$; 2nd internode $F_{1,19.4} = 14.11$, $P = 0.0013$; total height $F_{1,10.7} = 3.41$, $P = 0.0928$; 1st internode difference -0.5407 , $P = 0.0132$; 2nd internode difference -0.4796 , $P = 0.0013$; total height difference -1.3071 , $P = 0.0928$). In the open-canopy population, the far-red-treated first internodes were longer than the control-treated internodes, while the second internodes tended to be shorter (1st internode difference -0.2775 , $P = 0.0401$; 2nd internode difference -0.1698 , $P = 0.0686$), indicating shade avoidance in response to supplemental FR. In the closed-canopy population, both the far-red- and red-treated internodes were significantly or marginally significantly shorter than those in the control treatment (1st internode control-FR difference 0.2943, $P = 0.0211$, control-R difference 0.3155, $P = 0.0136$; 2nd internode control-FR difference 0.2474, $P = 0.0053$, control-R difference 0.2518, $P = 0.0046$).

Leaf traits—The effects of diode treatment on leaf area were consistent between the two tissues treated, but not between the two source populations (Table 1, Fig. 1D). No difference was found in the effects of treating the stem or the leaves, as indicated by the absence of a significant main effect of treatment position and of significant interactions of treated tissue with source population or diode type (Table 1). Plants from the open-canopy population responded to added far-red light by increasing leaf area compared to control treatments, while plants from the closed-canopy population did not respond significantly to added far-red light. This difference in response is manifested by a significant interaction between diode treatment and source population (Table 1). Individual leaves showed the same patterns as total leaf area (data not shown).

Both red and far-red light treatments increased leaf area in the open-canopy population (Fig. 1D), but not in the closed-canopy population. The differences in the responses to diode treatment between the populations resulted in a significant interaction between diode type and source population (Table 1). The “slice” option indicated that all these interactions were driven by differences between responses to diode type in the open-canopy population (total leaf area $F_{2,107} = 3.53$, $P = 0.0327$). Examination of the least square means showed that, in the open-canopy population, the plants treated with red and far-red diodes had greater total leaf area (total leaf area, control-FR difference -1477.95 , $P = 0.0190$, control-R difference -1473.25 , $P = 0.0182$).

DISCUSSION

Our results suggest that evolution of FR-HIR de-etiolation responses may be an important component of population differentiation of shade avoidance. Application of far-red light from light-emitting diodes caused stem elongation relative to control and red light treatment in the open-canopy population, but inhibited elongation in the closed-canopy population. The responses in the open-canopy population are indicative of a shade-avoidance response mediated by light-stable phytochromes, while the responses in the closed-canopy population are indicative of an FR-HIR mediated by light-labile phytochrome A. This result suggests that the mechanism of population differentiation in shade-avoidance sensitivity between these two populations of *Impatiens* is not a reduced sensitivity mediated by a light-stable phytochrome R : FR in the closed-canopy population. Rather, it suggests that in closed-canopy populations, low R : FR induces a high irradiance response, while it induces shade-avoidance responses in open-canopy populations. This persistent FR-HIR in woodland populations may have evolved as a response to direct selection for shade avoidance. However, it could also have been shaped as a correlated response to selection on seedling de-etiolation.

De-etiolation conditions may be a significant constraint on FR-HIR evolution. Forest understory sites often have a considerable layer of leaf litter through which *Impatiens* seedlings must emerge. Penetrating the litter can consume considerable resources from a seed and lowers the R : FR perceived by seedlings (Bliss and Smith, 1985). In greenhouse experiments, seedlings emerging through leaf litter have longer hypocotyls and first internodes (J. Stinchcombe and J. Schmitt, unpublished manuscript; E. von Wettberg and J. Schmitt, unpublished manuscript). Selection analysis from one of these experiments shows that longer hypocotyls are selected for in the presence of leaf litter (J. Stinchcombe and J. Schmitt, unpublished manuscript). In competition experiments in a natural population, the early growth of *Impatiens* was found to be important for success in competition against perennial herbs (Winsor, 1983). This early growth is limited to a few weeks early in the spring after *Impatiens* emerges but before the forest canopy closes and perennial herbs emerge (excepting spring geophytes, which emerge concurrently with *Impatiens* but inhabit mostly drier microsites in New England; E. von Wettberg, unpublished data). Therefore, seedlings that de-etiolate early can maximize their light acquisition while light is plentiful.

Under the open-canopy but heavy leaf litter conditions that exist in woodland *Impatiens* habitats in early spring in southern New England, a high irradiance response to low R : FR (or supplemental FR) will induce rapid de-etiolation after the leaf litter is penetrated and will suppress elongation in response to low R : FR from the emerging leaf canopy. In streambank and other open-canopy habitats, where the litter layer is usually thinner (E. von Wettberg, unpublished data) and early-season densities of seedlings of both *Impatiens* and other herbaceous species are typically higher (Schmitt et al., 2003), extra sensitivity to R : FR may allow seedlings to elongate preemptively to avoid neighbor shading. Because taller plants can benefit disproportionately from asymmetric competition for light with shorter neighbors (Schmitt et al., 1987a, b; Weiner, 1990), plants from open-canopy populations may benefit from extra sensitivity to shade if it places them at the top of the size hierarchy.

Application of both far-red and red light increased leaf area relative to the control treatment in the open-canopy genotypes but not in the closed-canopy genotypes. Because both light types had this effect in the open-canopy population, this may be a response to light intensity rather than light quality. An interactive effect between light quality and intensity is a possibility, but our design does not allow us to test this. Although intense light normally reduces relative leaf area rather than increasing it, the limited area covered by the diode treatment may not have this effect. We did not measure leaf thickness or individual leaf weight in this experiment.

We found no evidence for the hypothesis that shade plants retain sensitivity to neighbors in stem tissue but lose sensitivity to overhead shade. Thus, population differentiation has not involved differential responsiveness to lateral and overhead shade, as suggested by Schmitt et al. (1999). Instead, the balance between high irradiance suppression of stem elongation under low R : FR and shade-avoidance stem elongation under lowered R : FR is an important aspect of population differentiation. Thus the balance between shade-avoidance responses mediated by light-stable phytochromes and FR-HIR responses mediated by phytochrome A may be an important target of selection in species and populations adapting to different canopy environments.

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