Interactive Effects of Spectral Shading and Mechanical Stress on the Expression and Costs of Shade Avoidance

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Abstract: The interacting effects of different environmental cues in determining a plant’s phenotype and performance are poorly understood aspects of phenotypic plasticity. We examined the interacting effects of shading and mechanical stress (MS) on growth, reproduction, and mechanical stability. We subjected 10 grassland genotypes and 10 forest genotypes of Impatiens capensis to two levels of spectral shading and two levels of MS. Shade induced the production of taller, thinner internodes, but this response was inhibited by MS. This interactive effect was stronger in the grassland genotypes than in the forest genotypes, indicating that shade avoidance can be suppressed by MS and that the degree of this suppression differs between habitats. Among genotypes, greater plasticity in shade-induced internode elongation resulted in a larger reduction in the ability of plants to carry their own weight. This suggests that the occurrence of mechanical constraints may strongly contribute to the selection against shade-induced elongation responses in forest understory genotypes. Our results show that combined responses to different environmental cues can result in a fine-tuning of phenotypic expression by plants by maintaining the potential to strongly respond to single environmental cues but also by preventing potential future costs resulting from poor adaptation to other stresses.

Keywords: adaptive plasticity, biomechanics, evolution, phenotypic integration, shade avoidance, thigmomorphogenesis.

Introduction

Although phenotypically plastic responses to variable environments are clearly important to the success of organisms (Bradshaw 1965), the extent to which they interact to produce integrated phenotypes remains poorly understood (Murren et al. 2002; Pigliucci 2003). Variation in the nature of plastic responses has been observed to change with variation in environmental cues in a range of organisms (e.g., Kingsolver 1996; Donohue et al. 2000; Weinig 2000a, 2000b). Under natural conditions, usually more than a single cue varies within and across habitats, and interactive effects on plastic phenotypes are bound to occur (e.g., Schlichting 1989; Sardans et al. 2006).

The evolution of integrated phenotypic responses depends on signal reliability, costs associated with plasticity, and potential constraints impairing plastic responses (Morgan and Smith 1979; Dudley and Schmitt 1995; DeWitt et al. 1998). Plasticity can be considered to be adaptive if the benefits associated with the change in phenotype outweigh the costs (e.g., Bradshaw 1965; Sultan 1995; DeWitt et al. 1998). However, the specific costs and constraints associated with plasticity may depend on the environmental conditions to which plants are subjected in their particular microhabitat. Costs, which are often construed as being necessary to maintain the machinery for plasticity, and constraints, which result from the unreliability of a cue or the inability of an organism to maximally allocate to the response induced by a specific cue, can both limit the evolution of plasticity (sensu DeWitt et al. 1998). In addition, proximate costs may occur if a given trait does not carry any immediate fitness costs but is associated with potential future reduction of survival and reproduction. Selection for different responses to a similar set of cues can therefore be hypothesized to occur among habitats, depending on the information about the present and future environmental conditions that are contained in a specific combination of signals.

Two environmental cues that typically induce opposite responses in plants are canopy shading and mechanical stress (MS). Responses to canopy shading, that is, reductions of both the amount of light and its red-to-far-red (R : FR) ratio, include increased elongation of stem internodes and reductions in stem diameter, lateral branching, and root growth (e.g., Smith 1982; Dudley and Schmitt 1996; Ballaré 1999). These responses are collectively known as the shade avoidance syndrome (Smith 1982). In crowded vegetation, strong shade avoidance responses en-
able plants to place their leaves high in the canopy to shade competitors, and in so doing they can achieve high growth and reproductive rates compared with plants exhibiting a weaker response (Schmitt et al. 1995; Dudley and Schmitt 1996; Ballaré 1999). At least three types of proximate costs or constraints may limit these responses. The production of taller, thinner stems in combination with reduced investment in roots may make plants more susceptible to drought (e.g., Casal et al. 1994; Huber et al. 2004). Resources invested in stem elongation cannot be simultaneously invested in leaves and roots for resource uptake (Ballaré 1999; Cipollini and Schultz 1999). Finally, taller, thinner structures are more prone to mechanical failure (Niklas 1992). This article examines the latter two issues.

In contrast to shading, responses to forms of MS (e.g., wind, touching, or rubbing) generally include inhibition of stem internode elongation and increasing stem diameter and root allocation (e.g., Jaffe and Forbes 1993; Telewski and Pruny 1998). These responses, denoted as thigmomorphogenesis, increase the mechanical strength of plants (Niklas 1992; Anten et al. 2005) but may also reduce growth (Niklas 1993; Cipollini 1999) or be a disadvantage in terms of light competition (Anten et al. 2005).

In natural environments, exposure to MS and shading may not occur independently. Because plants shield each other from wind, the amount of MS is much lower in dense stands than it is in open stands (Goudriaan 1977; Speck 2003; Anten et al. 2005). At the same time, plants extending over the canopy are exposed to higher light availability but also to a more severe MS than plants lower in the canopy. Yet, the interactive effects of MS and spectral shading on plant growth have received little attention (Holbrook and Putz 1989; Henry and Thomas 2002). Henry and Thomas (2002) proposed that, in vegetation where plants compete for light, thigmomorphogenesis should be suppressed because the associated reduction in height growth would lead to reduced fitness, providing data supporting their proposition. However, other studies (Anten et al. 2005; Liu et al. 2007) found no such suppression of thigmomorphogenesis.

As argued above, the adaptive significance of responses to sets of cues depends in part on the degree to which these cues are reliable predictors of the environment. Interesting examples in this respect are herbaceous species growing in either grasslands or woodlands. In grasslands, spectral shading is a reliable cue of light competition with direct neighbors. But this is not the case in woodland habitats, where spectral shading is primarily produced by the overhead canopy. For herbaceous plants growing in the understory, a strong elongation response has therefore been interpreted as being maladaptive, because elongation leads to only minimal increases in light capture (Morgan and Smith 1979; Dudley and Schmitt 1995; Donohue et al. 2000, 2001). Game theoretical models of height growth (Givnish 1982; Falster and Westoby 2003) also foresee such a divergence in plasticity because they predict that height growth is density dependent and will only be selected for at high density when it entails an advantage in light competition.

With regard to MS, plants are more likely to be exposed to larger wind forces in grasslands than in forest understories, where trees generally reduce wind speeds. One might therefore expect stronger selection for responses to wind loading in plants growing in grasslands than in those growing in forests. Such selection pressures would be in the opposite direction of the well-illustrated selection pressures favoring shade-induced elongation responses in plants from grasslands.

Here we analyze the interactive effects of spectral shading and MS on stem properties and growth of Impatiens capensis plants from two habitats: an open grassland habitat and a forest understory. We address the following questions: (1) Is there an interaction between the effects of canopy-type shading and MS on growth and stem properties of this species, or are these effects simply additive? (2) Do grassland genotypes and forest genotypes respond differently to these two cues? (3) Is a greater expression of shade avoidance associated with constraints or proximate costs in terms of growth or whole-plant mechanical stability?

Material and Methods

Plant Material

Impatiens capensis Meerb. (jewelweed) is an annual herb that is found in wetlands and deciduous forests in North America. As such, it occurs across a wide range of canopy habitats, and differentiated grassland and forest understory forms have been observed (Dudley and Schmitt 1995). It has a mixed-mating system, producing both self-fertilized cleistogamous flowers and outcrossed chasmogamous flowers; in many natural populations, however, the majority of seeds are produced by selfing. Genetic differentiation in this species has been demonstrated between populations often growing as close as a few meters apart (Lechowicz and Bell 1991).

In the spring of 2003, seedlings were collected from grassland and forest populations at Weetamoo Woods Tiverton Park in southern New England (41.5°N, 71.2°W). See von Wettberg et al. (2008) for a detailed description of the collection procedure. The forest population was growing in the understory of a mixed Acer rubrum and Fagus americana deciduous forest where canopy openness of the trees was about 6% (von Wettberg et al. 2008). The grassland population grew in a large Carex meadow, which
had a canopy openness of about 80%, at least 50 m from the forest edge. For the current experiment, we used the fifth generation of inbred seeds from 10 families (hereafter genotypes) from each of the two habitats.

The experiment was conducted in the greenhouse facility of Utrecht University (Utrecht, The Netherlands). On August 28, 2006, 24 seeds of each genotype were sown in 1.3-L pots that were 0.12 m in diameter and were filled with a 1 : 1 mixture of sand and potting soil. At this stage, 3 g of slow-release fertilizer (Osmocote, 10% N + 10% P + 10% K + 3% Mg + trace elements) were added to each pot (0.3 g N pot⁻¹). This relatively high-nutrient fertilizer application was used because our objective was to analyze the interactive effects of shading and MS without the potential confounding effects that might occur through nutrient limitation. All seedlings emerged within 2–4 days.

On September 19, 16 plants of each genotype were selected, avoiding the tallest and shortest individuals, and were randomly assigned to each of two shading and two mechanical treatments for a total of four replicates per treatment combination. Each set of 20 (the total number of genotypes) replicate plants was arranged in a 4 × 5-pot grid in which pots were placed 0.35 m apart. The experiment was laid out in four blocks across the greenhouse, with each block containing one replicate 4 × 5 grid of each treatment combination. Grids were placed 1 m apart to minimize shading effects between them. Positions of plants within grids and grids within blocks were changed randomly every week to further minimize possible effects of position in the greenhouse.

Two levels of shading were applied by exposing plants to either 15% daylight with an R : FR ratio of 0.3 (denoted “shade”) or 50% daylight with an R : FR ratio of 1.2 (denoted “high light”). The shade treatment was meant to simulate the light conditions that plants experience under a forest canopy (low light intensity and an R : FR ratio that changed only minimally with height in the cage), which was created by using cages covered with one layer of a plastic film (Lee Colortran International, Andover, UK; no. 122). This study was thus not designed to determine whether shade avoidance occurred in response to either or both aspects of shading. Light was measured with an LI190 quantum sensor (LiCor, Lincoln, NE) connected to an LI1000 data logger. Red (wavelength, 655–665 nm) and far-red (wavelength, 725–735 nm) light were measured with an LI1800 spectroradiometer. To keep the shade level constant throughout the experiment, the heights of the cages were increased from 0.7 m to 1.5 m as the plants grew taller. We left 0.2 m of open space below the film to allow free air movement; microclimatic measurements revealed no differences in temperature and air humidity between the two shading treatments. Mechanical treatments were applied by flexing plants either 0 (control) or 40 (MS) times daily. This was done by gently grasping the stem at about 80% of its height and bending it back and forth no farther than 30° for a duration of ~90 s. We chose this type of flexing because it simulates the mechanical effect of wind on plants (swaying of the stem) without affecting their microclimate. We chose 40 flexures as our stress level because it has been documented for other species that plant responses differ most strongly in plants that experience between 0 and 10–25 daily flexures and that the added effect of more flexures is almost negligible (Teleswki and Pruyn 1998; Anten et al. 2006).

**Measurements**

Measurements of height, stem diameter, and node number were taken on September 27 and October 4 (denoted hereafter as census 1 and census 2, respectively). Height was measured from the soil level to the youngest meristem. Stem diameter was measured at the middle of the first internode using a digital caliper. Only fully developed nodes (those with an unfolded leaf at their distal end) were counted.

Between October 9 and October 12, we conducted a destructive harvest. On each day, one complete block was harvested. Plant height and the lengths and diameters of the hypocotyl and the first five internodes were measured, and the numbers of internodes, flowers, pedicles, and fruits were counted. Leaves were separately harvested from the upper, middle, and lower one-third of each stem (see eq. [4b]). The rest of the shoots were then destructively separated into stems, branches, and petioles, and the fresh mass of all plant parts was immediately determined. Leaf area was measured using a leaf area meter (LI3100). Stems were immediately packed in wet tissue paper to avoid loss of turgor and were stored at 5°C for mechanical measurements (see below). Roots were carefully washed free of soil substrate. Dry mass of all parts was determined after oven drying for at least 3 days at 70°C.

Two stem mechanical characteristics were measured at the second internode (between the first and second true leaf pairs): the Young’s elastic modulus (E; MN m⁻²), a measure of the rigidity of the tissue, and the breaking stress (σᵥ; MN m⁻²), a measure of tissue strength (Niklas 1992; Gere and Timoshenko 1999). These measurements were performed with a universal testing machine (Instron Model 5542, Canton, OH), using a three-point bending method with force being applied halfway between the nodes. Vertically applied forces (F; N) and resulting deflections (δ; m) were recorded. The value of E could be calculated as follows (Gere and Timoshenko 1999):
where $L$ is the length between the supports (m) and $I$ is the second moment of area ($m^4$; Gere and Timoshenko 1999). The cross-sectional dimensions of the stems were used to calculate $I$, taking into account the fact that they are hollow and roughly circular:

$$I = \frac{1}{4\pi} \left( \frac{r_{\text{out}}^4 - r_{\text{in}}^4}{H} \right),$$

where $r_{\text{out}}$ is the outer radius of the stem and $r_{\text{in}}$ is the radius of the internal hollow part (hereafter, the cavity; Niklas 1992). The break stress ($\sigma_b$) was calculated from the maximum force ($F$) at which the stem ruptured:

$$\sigma_b = \frac{M_{r_{\text{out}}}}{I},$$

$$M = \frac{1}{2} FL,$$

where $M$ is the bending moment (Gere and Timoshenko 1999). Immediately after mechanical testing, the internal and external diameters (to determine $r_{\text{out}}$ and $r_{\text{in}}$) were measured at the closest undamaged part adjacent to the point where the stem broke (for use in the calculations of $E$ and $\sigma_b$; eqq. [1a], [2a]), as well as halfway along the hypocotyl for use in the calculation of whole-plant mechanical stability (see below).

The stem-level traits were used to calculate two measures of mechanical stability at the whole-plant level: the buckling safety factor (BSF), which indicates the ability of plants to carry their own weight, and the maximum lateral wind force that plants resist ($F_{\text{max}}$), which indicates the ability of a plant to resist external (wind) forces. The BSF was calculated as the critical buckling height ($H_c$) of the plant—the height beyond which the stem will deflect from vertical because of a plant’s own weight, but without breaking—divided by its real height ($H_{\text{real}}$):

$$\text{BSF} = \frac{H_c}{H_{\text{real}}},$$

where $H_c$ was calculated using the formula of Greenhill (1881) for a uniform column:

$$H_c = \left( \frac{8EI}{P} \right)^{0.5},$$

with $P$ as the fresh weight (N). This formula treats stems as idealized columns, ignoring tapering and uneven loading. However, its results tend to be comparable to those of more complicated models (Holbrook and Putz 1989; Henry and Thomas 2002). The formula for $F_{\text{max}}$ was

$$F_{\text{max}} = \frac{\sigma_b I}{H_{l(\text{ave})} r_{\text{out}}},$$

where $H_{l(\text{ave})}$ is the weighted average height of leaves on the plants (Anten et al. 2005). It assumes that wind force acts only on leaves and that all leaves are concentrated at $H_{l(\text{ave})}$, which is calculated as

$$H_{l(\text{ave})} = \frac{\sum h_i A_i}{A_T},$$

where $h_i$ and $A_i$ are the median height and leaf area of segment $i$, respectively, and $A_T$ is the total leaf area of the entire plant. Note that equation (4a) treats wind loading as a static phenomenon, ignoring its dynamic nature (Baker 1995). Generally, simplified mechanical calculations such as equations (3a) and (4a) are sufficient for the qualitative comparisons of mechanical stability between plants of the same species that have very similar basic structures (Holbrook and Putz 1989), as was done here, although they are not sufficient for exact estimates of strength or stability.

Statistics

Mean differences in response parameters were analyzed by ANOVA, with mechanical treatment ($df = 1$), shading ($df = 1$), habitat ($df = 1$), and block ($df = 3$) as fixed factors and genotype nested in habitat. Some variables were log transformed to improve homogeneity of variance.

To calculate whether relative differences in the expression of plasticity resulted in growth-related or mechanical costs, we used the following regression (modified from Weinig et al. 2006):

$$\Delta W_i = \alpha + \beta(\Delta \text{INT}),$$

where $\Delta W_i$ is the log-transformed change in performance of genotype $i$ by shading relative to high light (positive values indicate increased performance and vice versa). As measures of performance, we used whole-plant dry mass as a measure of growth, the number of reproductive structures to indicate reproductive output, and BSF and $F_{\text{max}}$ as measures of mechanical stability. The value $\Delta \text{INT}$, is a measure of plasticity and is calculated as the difference in the log-transformed values of internode length in high light and shade (analysis was performed for the second and third internode). Significance of the intercept $\alpha$ in-
Mechanical Costs of Plasticity

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Figure 1: Plant height at the first (a) and second (b) census, and the mean length of internodes 2–5 above the hypocotyl at final harvest (c) for grassland and forest genotypes of Impatiens capensis subjected to spectral shading and mechanical stress treatments (D indicates stressed plants and C indicates control plants). Only treatment means (n = 10 for number of genotypes) are shown. Statistical analysis is provided in table 1 for height and table 2 for internode length.

Results

Stem Characteristics

In the first two censuses, we found shaded plants to be taller than unshaded ones, whereas MS plants were shorter than the control ones (fig. 1a; table 1). The increase in stem length with shading was stronger for the grassland genotypes than for the forest ones, as indicated by the habitat × light interactive effect (table 1). For the grassland genotypes, the negative effect of MS was stronger for the shaded plants than for the unshaded ones, but this was not the case for the forest genotypes (note the significant habitat × light × MS effect; table 1; fig. 1a).

At the final harvest, stem length increased with shading for all grassland genotypes. Conversely, at this stage, forest genotypes tended to exhibit a reduction in stem length (fig. 1b). The negative effect of MS found during the censuses was maintained at the final harvest. The positive effect of shading on stem length was significantly reduced under MS (table 1).

The lengths of the first five internodes above the hypocotyl were measured only at the final harvest. All of these lengths increased with shading and decreased under MS (fig. 1c; table 2). The increase under shade conditions was greater in the grassland genotypes than in the forest genotypes. This effect decreased under MS, and the interaction was significant for the second and the average of all five internodes (table 2). The suppression of shade-induced internode elongation by MS was more apparent in the grassland genotypes than in the forest genotypes, as indicated by the significant habitat × light × MS interactive effect (table 2). The number of nodes decreased with shading, and this reduction was stronger in the forest genotypes than in the grassland genotypes (table 1).

Stem diameter was smaller in shaded plants than in unshaded plants (fig. 2a; table 1) at both censuses and at the final harvest. The stem diameter was increased by MS at high light, but not in the shade. Grassland genotypes had thicker stems than forest genotypes in all treatments. As noted above, Impatiens capensis plants have hollow stems consisting of a solid stem wall (fig. 2b) and a cavity space (the hollow part; fig. 2c). The greater stem diameter of the grassland genotypes was entirely due to the fact that the stems of those plants had wider cavities (the wall thickness was not different). Conversely, the positive effect of MS on stem diameter was the result of its effect on wall thickness. The effect of the amount of material and its mean distance from the neutral axis of the stem on the stem’s resistance to bending, \( I (m^4) \), decreased about 10-fold when exposed to shading (fig. 2a; table 1). It was also greater in the grassland genotypes than in the forest genotypes. At high light, MS increased \( I \), but this effect was not observed under shade (table 1).

The measure of tissue stiffness \( E \) increased with shading and was also greater in the forest genotypes than in the grassland genotypes (fig. 3a; table 1). The increase of \( E \) with shading was greater under MS than in the nonstressed plants. The measure of tissue resistance to rupture, \( \sigma_b \), increased with both shading and MS (fig. 3b; table 1). The flexural stiffness of the stem \( EI \) (the product of modulus and second moment of area) was strongly reduced by shading and was greater in the grassland genotypes than in the forest genotypes (table 1).
Whole-Plant Mechanical Stability

The ratio of $H_c$ to the $H_{\text{real}}$ BSF (eq. [3a]), increased with MS and decreased with shading (fig. 4a; table 1). In the case of shading, the reduction in BSF was mostly the result of a reduction in critical height (data not shown), which in turn can be explained by the plants having a much smaller diameter, which resulted in a lower flexural stiffness ($E I$; see above). The increase in BSF with MS, on the other hand, resulted mostly from a reduced actual height.

The grassland genotypes exhibited a greater BSF than the forest genotypes at high light but a lower BSF under shade (fig. 4a; table 1). The BSF of the grassland genotypes in the shaded, nonflexed treatment was, on average, very close to 1 (1.47); below this value, plants were no longer stable, and one genotype even had a BSF of $<1$. Indeed, shortly before harvesting, we observed that the plants for which we had calculated BSF values close to or $<1$ had started to become unstable (those individuals were staked for the last 2–3 days).

The calculated $F_{\text{max}}$ value was about fivefold greater for the high light plants than for the shaded plants and was 30%–80% greater for the plants exposed to MS than for the unstressed plants. Grassland genotypes were relatively stronger in high light and weaker in the shade than forest genotypes.
genotypes were, but this interaction was weaker than it was in the case of BSF (fig. 4; table 1).

Figure 5 depicts the relationship between the shade-induced changes in internode length ($\Delta$INT) and the concomitant changes in mechanical stability (BSF and $F_{\text{max}}$, $W$), with each point representing a genotype (see eq. [5]). Overall, both the BSF and $F_{\text{max}}$ values were reduced by shading, as demonstrated above. In the case of the BSF, this reduction became significantly greater with increased shade-induced internode elongation, suggesting that greater plasticity in internode elongation came at the cost of a plant’s ability to carry its own weight (table 3). The correlation was significant across as well as within habitats, with the genotypes of both habitats seemingly falling along the same line. By contrast, changes in $F_{\text{max}}$ were not correlated with changes in internode length, suggesting that increased plasticity in internode elongation did not come at the expense of the ability of plants to resist external forces.

**Growth and Reproduction**

Whole-plant dry mass was considerably lower in the shaded plants than in the high light plants and was somewhat higher for the grassland genotypes than for the forest genotypes (fig. 6; table 1). No significant effect on mass was observed with MS.

The stem mass ratio (SMR) increased with shading. Grassland genotypes had overall larger SMR values and also exhibited a greater increase with shading. This shade-induced increase in stem allocation was suppressed by MS in the grassland genotypes but not in the forest genotypes (fig. 6b), as indicated by the significant habitat $\times$ light $\times$ MS interaction (table 1). In the forest genotypes, the leaf mass ratio increased with shading, but this was not the case in the grassland genotypes. Root mass ratios were strongly reduced by shading but were not affected by any other factor. Branch allocation was also negatively affected by shading, but much more so in the grassland genotypes than in the forest genotypes.

At the genotype level, shade-induced changes in biomass did not correlate with shade-induced changes in internode length (table 3; fig. 5; eq. [5]). That is, genotypes that exhibited greater plasticity in internode elongation did not exhibit a greater growth reduction. When considering mass allocation, genotypes that were more plastic exhibited greater increases in stem allocation but greater reductions in branch and leaf allocation. Changes in root mass ratio with shading did not correlate with changes in internode elongation (analyses not shown).

The reproductive output—the sum of the total number of flowers, pedicles, and fruits—was significantly reduced by shading (table 1) but was not affected by MS. The shade-induced reduction was more apparent in the forest genotypes than in the grassland genotypes (table 1). Grassland genotypes had lower reproduction than forest genotypes did. The shade-induced reduction in reproduction was not correlated with plasticity in internode elongation (fig. 5; table 3).

**Discussion**

Under natural conditions, plants are subjected to simultaneously acting environmental cues that can be associated with opposite selective forces. The integrated phenotype of plants results from a response to these often-contrasting environmental cues. Depending on the microhabitat conditions to which plants are exposed in their natural en-

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**Table 2: Results of ANOVA ($P$) for the separate internode data with habitat ($h$), shading treatment ($s$), and mechanical stress treatment ($m$) as fixed factors and genotype nested in habitat ($g(h)$)**

<table>
<thead>
<tr>
<th>Parameter Transformation</th>
<th>$h$</th>
<th>$s$</th>
<th>$m$</th>
<th>$g(h)$</th>
<th>$h \times s$</th>
<th>$h \times m$</th>
<th>$s \times m$</th>
<th>$h \times s \times m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internode length:</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 Log</td>
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<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.7430</td>
<td>.0760</td>
<td>.5560</td>
</tr>
<tr>
<td>2 Log</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.1977</td>
<td>.0050</td>
<td>.9200</td>
</tr>
<tr>
<td>3 Log</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
<td>.3330</td>
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<td>.0001</td>
<td>.0001</td>
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<td>.4320</td>
<td>.3800</td>
<td>.0220</td>
</tr>
<tr>
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<td>.0001</td>
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<td>.0001</td>
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<td>.8720</td>
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<td>.0001</td>
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<td>.4790</td>
<td>.0230</td>
<td>.0450</td>
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</tbody>
</table>

| Internode diameter:      |     |     |     |        |             |             |             |                 |
| 1 Log                    | .0001 | .0001 | .0246 | .0001 | .2232 | .2456 | .0044 | .0524 |
| 2 Log                    | .0001 | .0001 | .0148 | .0001 | .3559 | .2362 | .8478 | .2513 |
| 3 Log                    | .0001 | .0001 | .0023 | .0001 | .9629 | .5802 | .8377 | .0882 |
| 4 Log                    | .0001 | .0001 | .0443 | .0001 | .9119 | .1356 | .8685 | .1724 |
| 5 Log                    | .0001 | .0001 | .2880 | .0001 | .5386 | .2789 | .7029 | .6002 |

Note: Block effects not shown. Values in bold and underscore indicate significant ($P < .05$) and marginally significant ($1 < P < .05$) effects, respectively. Log = data were logarithmically transformed; normal = untransformed data were used.
Are There Interactions between the Effects of Spectral Shading and MS?

Spectral shade induced typical shade avoidance responses (Smith 1982), including production of longer, thinner internodes and a reduced allocation to branches. These responses were more strongly exhibited by the grassland genotypes than by the forest genotypes; these responses have also been observed for other grassland and forest populations of this species (Dudley and Schmitt 1995; Donohue et al. 2000). Donohue et al. (2000, 2001) reported a selective advantage of greater shade avoidance in grasslands and a disadvantage in forest understories, suggesting that there is an adaptive divergence in plasticity between populations from these habitats. Interestingly, forest genotypes did exhibit some degree of shade avoidance. Whereas seedling densities in forest understories are usually low and strong intraspecific competition seems to be rare (Schmitt et al. 2003), these densities vary in time and space and can sometimes be high. In addition, understory plants may experience competition from other, later-emerging species (Winsor 1983), suggesting that some degree of shade avoidance is adaptive. Gene flow between the grassland populations and the forest populations, which occurs when they are in close proximity to one another, could also have contributed to the maintenance of plasticity within the forest population. Amplified fragment length polymorphism genotyping of these populations, as a part of a larger genotyping effort (von Wettberg et al. 2008), found the $F_{ST}$ value between these two populations to be 0.20, suggesting that these populations are not admixed but that they do exchange genes.

There were clear indications that the expression of shade environment, not only will genotypes of the same species be exposed to a different set of cues but also the consequence of a given response may differ. Different selection regimes can therefore be hypothesized to affect the expression of integrated phenotypes. In this article, we showed how the environmental cues spectral shading and MS elicit opposite phenotypic responses in plants and that a response to one cue reduces the response to the other, thereby constraining the phenotypic expression of plants. Interestingly, not only does the responsiveness to the individual cues differ between habitats, but so does the interactive effect of both cues. This shows that past habitat-specific selection pressures strongly affect the production of integrated phenotypes to present environmental conditions.
avoidance can be constrained by MS: shade-induced internode elongation was less apparent under MS. To our knowledge, this has not been shown before (but see Liu et al. [2007] for root allocation), and it suggests that the expression of shade avoidance depends on the presence of other environmental factors such as the presence or absence of MS. It also suggests that, physiologically, thigmomorphogenesis (the plastic response to MS; see “Introduction”) and shade avoidance do not act independently. Indeed, there are indications that the two responses could involve partially overlapping signal transduction pathways (Braam et al. 1996). For example, in Arabidopsis thaliana, 67% of the genes that were upregulated at least twofold by MS were also upregulated in darkness (Lee et al. 2005). An overlap would also be expected because both thigmomorphogenesis and shade avoidance involve changes in the rates of cell elongation and/or cell division (Braam et al. 1996). It is worth noting, however, that these two cues are different in the sense that one (shade) entails a reduction in resources, whereas the other (MS) does not. It would be interesting to investigate whether these different effects on resource status of the cues associated to shading and MS may contribute to the fact that, despite the partially overlapping signal transduction pathways and the upregulation of the same genes, MS and spectral shading elicit opposite developmental responses.

Overall, our finding that shade-induced internode elongation can be suppressed by MS contradicts the argument that this would be maladaptive because plants would be unable to escape shade caused by less inhibited neighbors (Henry and Thomas 2002). On the basis of this argument, one would expect this suppression to be less apparent in the grassland genotypes than in the forest genotypes because light competition in the grassland is generally more severe, thereby selecting for maintenance of shade-induced elongation responses, even under MS conditions (Morgan and Smith 1979; Dudley and Schmitt 1995). Our findings appear to be in the opposite direction: the suppression of shade avoidance by MS was stronger in the grassland genotypes.

Suppression of shade avoidance under MS might act as a preventive strategy to prevent mechanical damage by high wind speeds. High winds are unpredictable but tend to be much more common and severe in grasslands than in forest understories. Shade avoidance suppression may also act as a mechanism that regulates height growth. In stands of herbaceous plants, the taller individuals are often...
Figure 5: Calculated relative change in buckling safety factor (BSF; a, b), maximum lateral force that plants can resist ($F_{\text{max}}$; c, d), growth (e, f), and reproduction (g, h) due to shading, as a function of the relative shade-induced increase in the length of the second internode (eq. [5]) for grassland genotypes and forest genotypes of Impatiens capensis subjected to spectral shading and mechanical stress treatments. Mechanically stressed plants are represented in a, c, e, and g; control plants are represented in b, d, f, and h. Each point indicates a genotype mean. Solid lines indicate linear regressions in which grassland genotypes and forest genotypes were combined. Significance of correlation coefficients is provided in table 3.
very similar in height but can differ widely in total mass and leaf area and thus in growth potential (Weiner and Thomas 1992; Nagashima et al. 1995). Game theoretical models also predict this type of height convergence (Givnish 1982). Although it is important to maintain leaves in the top of the canopy, there is little benefit from growing too far out above the canopy in terms of light capture. The proximate costs in terms of mass investment, and especially the mechanical risk, however, might be substantial. The latter is because wind speeds increase dramatically above the boundary layer of the canopy (Goudriaan 1977; Speck 2003). Suppression of stem elongation by MS through this increased wind exposure might be a mechanism that facilitates height convergence.

### Table 3: Significance levels of slopes of intercepts in the linear regression of relative changes in buckling safety factor (BSF), maximum lateral force ($F_{\text{max}}$), final biomass, and reproductive value as a function of relative change in the length of either the second or third internode above the hypocotyl

<table>
<thead>
<tr>
<th>Trait, internode, treatment</th>
<th>Intercept</th>
<th>$P$</th>
<th>Slope</th>
<th>$P$</th>
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<tr>
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<tr>
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<td></td>
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<tr>
<td>MS</td>
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<td>0.010</td>
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<td></td>
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<tr>
<td>3:</td>
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<tr>
<td>MS</td>
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<td>0.0001</td>
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<tr>
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<tr>
<td>3:</td>
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<tr>
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<tr>
<td>Growth:</td>
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<tr>
<td>Total mass:</td>
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<tr>
<td>2:</td>
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<tr>
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<td>2:</td>
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Note: See eq. (5) and associated text. Values in bold and underscore indicate significant ($P<0.05$) and marginally significant ($0.1<P<0.05$) effects, respectively. MS = mechanical stress.

Our results do not indicate that increased expression of shade avoidance was associated with proximate costs in terms of biomass increment (growth) or reproduction, but they do suggest that there might be constraints in terms of mechanical stability. In both light treatments, genotypes that were more plastic exhibited the same or higher growth rates than less plastic ones. In addition, shade-induced reductions in either growth or reproduction were not correlated with plasticity. These results are consistent with other studies (e.g., Weinig and Delph 2001; Weischedel et al. 2006) that compared growth of different genotypes that differ in plasticity in stem or petiole elongation. They are, however, contrary to studies that showed that stem elongation stimulated either through mutation (e.g., Casal et al. 1994; Schmitt et al. 1995) or by application of hormones (Cipollini and Schultz 1999) resulted in reduced growth and reproduction. Cipollini and Schultz (1999) argued that stem elongation could produce costs in terms of growth if the resources invested in the stems cannot be invested in resource-harvesting structures (leaves and roots). We found that expression of a stronger shade avoidance response was indeed associated with a greater increase in allocation of mass to stems. Greater allocation to stems occurred at the expense of allocation to branches and leaves, but not to roots. Although not associated with immediate costs, decreased allocation to light-harvesting structures and branches will eventually result in a reduced carbon acquisition and fewer meristems available for reproduction, respectively, both of which can constrain lifetime reproductive output (e.g., Geber 1990). It also bears mentioning that we did not measure total seed production (we only counted the number of reproductive organs at one time point), which in this autoballochoric species is almost impossible. Effects that might have arisen through difference in seed mass per reproductive organ were thus not included.

Our results did show clear mechanical constraints, because an increased expression of shade avoidance was associated with a reduction in BSF, reflecting the safety against global buckling of the stem (although without breaking) under a plant’s own weight (see “Material and Methods”). Shading generally caused a reduction in BSF, but this effect was stronger in the more plastic genotypes (fig. 5). Some of the shaded plants of the more plastic grassland genotypes were calculated to have a BSF of <1, indicating that they had grown taller than their $H_r$ values and had become unstable (Niklas 1992; this was also observed—see “Results”). Field data on Impatiens capensis revealed that plants that were induced to elongate early in ontogeny were more likely to buckle and that buckling was associated with reduced fitness (H. Huber, unpub-
lished data). Consistent with these observations for *I. capensis*, it has been observed that when individuals of *Cirsium palustre*, a grassland species that exhibits strong shade avoidance, grow in the forest understory, they also tend to buckle, with buckled individuals exhibiting lower survival and reproduction rates (Pons and During 1987). Although buckling usually does not kill a plant, the leaves will be closer to the soil, where they are usually strongly shaded and more vulnerable to pathogen attack (Niklas 1992). Thus, mechanical constraints may provide one explanation for why strong shade avoidance would be selected against in a forest understory (Donohue et al. 2000, 2001): the constant low-light signal would otherwise induce such plants to elongate beyond their $H_s$ values.

Shade plants were calculated to be able to resist lower $F_{\text{max}}$ values, but this reduction was not correlated with plasticity in internode elongation. Thus, contrary to the BSF results, this suggests that increased expression of shade avoidance does not come at the expense of the maximum wind drag that plants can resist. However, because wind speeds increase with height above the soil (Goudriaan 1977; Speck 2003), the more plastic genotypes, being taller, are probably exposed to larger forces, and at equal $F_{\text{max}}$ values they will be more prone to stem fracture. Indeed, in the field, the more elongated *I. capensis* plants were more likely to experience stem rupture (H. Huber, unpublished data).

Overall, taller stature will lead to lower mechanical stability unless it is associated with a concomitant increase in stem diameter, tissue strength, or tissue rigidity (Niklas 1992). Grassland genotypes did have an intrinsically greater stem diameter than forest genotypes when compared at the same plant height, which in terms of mechanical stability compensated at least in part for their greater height. This greater stem diameter was entirely the result of grassland genotypes having stems that were more hollow than those of the forest genotypes, that is, the cavity diameter was greater while the wall thickness was similar. A hollow stem is more efficient in terms of resistance to bending because the material is placed farther away from the neutral axis, such that the same strength and flexural stiffness can be achieved with less material (Niklas 1992; Gere and Timoshenko 1999). Thus, a plant requires less biomass per unit stem length to achieve the same mechanical stability, which may be advantageous when there is strong competition for light. In general, these results indicate that genotypic variation in shade avoidance can be associated with a concomitant variation in geometric stem design, which to our knowledge has not been demonstrated before.

Figure 6: Total standing biomass (a) and the fractions of this mass in stems (b), leaves (c), and branches (d) for grassland and forest genotypes of *Impatiens capensis* subjected to spectral shading and mechanical stress treatments (*D* indicates stressed plants and *C* indicates control plants). Only treatment means ($n = 10$ for number of genotypes) are shown. Statistical analysis is provided in table 1.
Costs of and constraints on genotypic variation in response to MS were hard to determine because this variation was relatively small (there were no significant genotype × MS interactive effects on internode or stem lengths in an ANOVA in which habitat was excluded as a factor). Various studies (e.g., Niklas 1998; Cipollini 1999) have reported that mechanically stressed plants exhibited lower growth and a lower flower production. They argued that resources allocated to mechanical strength cannot simultaneously be allocated to leaves or reproduction and that there are thus fitness costs associated with responses to MS. We did not find reductions in either growth or reproduction under MS, which is consistent with the results of Anten et al. (2005). It should be noted, however, that our method of applying MS (stem flexing) probably does not exactly simulate the type of stress experienced by plants in the field. Under wind loading, plants in dense stands may also experience leaf and branch abrasion from contact with neighbors, which has been shown to result in reduced branch and leaf growth (Rudnicki et al. 2001).

There can be a strong competitive cost of responses to MS in crowded plant populations; in a competition experiment, mechanically stressed plants were shaded and had lower survival and reproduction rates than their un-stressed competitors (Anten et al. 2005). In terms of benefits, we found that mechanically stressed plants exhibited greater whole-plant mechanical stability (BSF and $F_{\text{max}}$ values), as was reported elsewhere (Niklas 1992; Anten et al. 2005). This indicates that plasticity in response to MS is adaptive, because in open habitats, plants that are exposed to MS can increase their resistance to wind at relatively small costs. In dense vegetation, on the other hand, plants are wind shielded. This reduced level of MS induces additional stem elongation, strengthening a plant’s ability to compete with its neighbors (Anten et al. 2005; Liu et al. 2007). In addition, as noted above, sensitivity to MS may prevent plants from growing out above the canopy.

Concluding Remarks

The magnitude of the costs of and constraints on plasticity strongly determines the range of conditions under which plastic plants can prevail. If costs and constraints are large, strong plasticity will be selected against, especially if it produces an inaccurate fit with the environment (Sultan and Spencer 2002). This article suggests that the existence of mechanical constraints on the expression of shade avoidance, particularly the increased likelihood of global stem buckling, is one of the reasons why plastic genotypes are less successful in forest understory. A higher likelihood of mechanical failure of highly elongated genotypes thus contributes to the selection against shade avoidance in forest understory genotypes. This leads to the evolution of locally adapted populations that are characterized by different degrees of shade-induced elongation responses in populations occurring in sites with different canopy openness values. Suppression of shade avoidance under MS, as well as intrinsic differences in stem geometry between genotypes with different plasticity, however, may help to mitigate some of these potential costs and allow plants under open conditions to maintain high degrees of shade-induced plasticity.

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