Differential response to wind and shade in mother leaf of *Potentilla reptans*

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**Abstract** Responses to mechanical stress such as wind should be suppressed in dense vegetation since the resultant shorter stature would lead to low fitness. However, this point has been debated i.e. it has been argued that in dense vegetation a sensitivity to mechanical stress might provide an additional cue to shade avoidance. For 10 different genotypes of the clonal plant *Potentilla reptans*, the effects of shade (15% of daylight) with a red far red ratio (R/FR) of 0.3 vs. 50% daylight and R/FR of 1.2 and wind (0 or 40 daily brushes with a duster) on the mother leaf properties were investigated. All genotypes exhibited typical “shade avoidance” responses under shade such as the production of fewer leaves with longer petioles, reductions in petiole diameter, and the production of more rigid petiole tissue and petioles with a higher Young’s modulus. Wind-treated plants produced more leaves with shorter and thicker petioles made of more flexible tissue and lower Young’s modulus. All responses to wind are different from responses to shade. Interestingly the responses to wind

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in leaf of *Potentilla reptans* were opposite in nature to the responses to light.

**Key Words** biomechanics, fitness, plasticity, thigmomorphogenesis, shade avoidance, stoloniferous plant

To acclimate to heterogeneous environments, plants display an important performance trait—phenotypic plasticity. For example, in crowded vegetation, many plants typically have “shade avoidance” responses such as slender stems, strong apical dominance with low branching intensity, and high shoot/ root ratios compared to plants growing in more open conditions. However, when plants are exposed to above-ground mechanical stimuli such as wind touching or rubbing, they typically have shorter and thicker stems lower shoot/root ratios, and they have an increased stiffness and rigidity of stems. These responses termed thigmomorphogenesis by Jaffe increase the resistance of plants to mechanical failure and seem to be in the opposite direction to shade avoidance.

There is an interesting debate about interactive effects of shade and wind on plants. Some studies argue that in dense canopies where plants compete for light responses to wind thigmomorphogenesis should be suppressed as the reduction in height growth would lead to reduced fitness; however, other studies have been arguing the opposite that in dense vegetations plants are protected from wind and that sensitivity to this lower level of wind hence enhanced height growth leads to increased fitness i.e. also plants growing in dense stands should be sensitive to variation wind. According to the best of my knowledge these studies just used erect plants and only at the individual level. No study has yet studied responses of stoloniferous plants to mechanical stress. Stoloniferous plants are more flexible in regulating the number and horizontal placement of leaves, leaf petioles serve as vertical support structures and each leaf is separately supported. These differences from erect plants in architecture probably have different response to mechanical stress. And an analysis at the genotypic level is even more valuable for experimental studies on plasticity because in coarse-grained environments reaction norms are properties of genotypes not individuals and effects on plasticity can only be measured at the genotype level. The aim of the present study was to investigate more precisely the effects of shade 15% of daylight with a red far red ratio R/FR of 0.3 vs. 50% daylight and R/FR of 1.2 and wind 0 or 40 daily brushes with a duster on the leaf properties of 10 genotypes of the stoloniferous plant *Potentilla reptans*. The experiment was conducted in a greenhouse i.e. a wind-protected environment and the control plants can therefore be interpreted as wind-shielded plants. The hypothesis was tested that thigmomorphogenesis was not suppressed under shade. If so, we expect that in dense stands plants subjected to a wind treatment will produce shorter and thicker petioles and more leaves relative to their counterparts. Quantifying these effects is a crucial step toward understanding the adaptive significance of thigmomorphogenesis and shade avoidance responses and their possible interaction.

1 Materials and methods

1.1 Plant material

During spring 1990 to 1997 the stoloniferous rosette-forming herb *Potentilla reptans* L. known 12 genetic identities were collected separately from a natural population in different habitats of the Netherlands and allowed to proliferate successively in the experimental garden of Utrecht University. Established rosettes produce horizontally growing stems i.e. primary stolons which may root at their nodes and thereby give rise to potentially

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independent daughter rosettes\textsuperscript{[12]}\textsuperscript{[12]-[12]}. Unfortunately genotype 6 and 11 were lost during cultured.

1.2 Experimental methods

Similar-sized rosettes of ten genotypes were taken from this garden population and used as experimental material. For clarity the rosettes of \textit{Potentilla reptans} plants were defined in such a way that the stolons were excluded and the mother rosette is considered as the one that was initially planted. The leaves of a rosette are defined as the petiole and lamina together\textsuperscript{[30]}\textsuperscript{[31]}. The rosettes were transplanted into plastic trays 15 cm × 15 cm × 100 cm\textsuperscript{2} filled with river sand in the greenhouse of this garden\textsuperscript{2} watered daily with tap water\textsuperscript{2} and fertilized weekly with a nutrient solution which had previously been shown to provide non-limiting nutrition for \textit{P. reptans} \textsuperscript{[12]}. This watering and fertilization regime was continued until the end of the experiment. After 4 weeks\textsuperscript{2} offspring ramets of ten genotypes were excavated and size-standardized by removing all but the youngest unfolded leaf from the rosettes\textsuperscript{2} and by cutting roots to a length of 3 cm. Ramets were then planted into plastic pots 13 cm in depth and diameter\textsuperscript{2} filled with river sand supplied with water and nutrients as described above. After 2 weeks\textsuperscript{2} 32 similar-sized plants of each genotype were randomly divided into four groups 8 replicate plants per group of each genotype\textsuperscript{2} and subjected to a combination of canopy shading 15% of daylight with a red\textsuperscript{2} far red ratio R/FR of 0.3 vs. 50% daylight and R/FR = 1.2 and a wind-treatment 0 or 40 daily brushes with a duster\textsuperscript{2} in a 2 × 2 factorial design. Control plants \textsuperscript{2} HLc\textsuperscript{2} were exposed to 50% daylight and R/FR = 1.2 conditions shading by the greenhouse roof with no wind treatment. Wind-treated plants \textsuperscript{2} HLM\textsuperscript{2} were exposed to the same as control light conditions with 40 daily brushes with a duster\textsuperscript{2}.

In two shading treatments\textsuperscript{2} whole plants were grown in cages covered by one layer of a plastic film \textsuperscript{2} Lee Colortran International\textsuperscript{2} Andover\textsuperscript{2} UK\textsuperscript{2} no. 122\textsuperscript{2} which simulating canopy shading give 15% of daylight with R/FR of 0.3 \textsuperscript{2} LLLc\textsuperscript{2} and one \textsuperscript{2} LLM\textsuperscript{2} of the shading treatments additionally gave 40 daily brushes with a duster\textsuperscript{2}.

Light was measured with a LI910 quantum sensor \textsuperscript{2} LiCor\textsuperscript{2} Lincoln\textsuperscript{2} NE\textsuperscript{2} USA\textsuperscript{2} connected to an LI1000 data logger. Red 655 − 665 nm\textsuperscript{2} and far red 725 − 735 nm\textsuperscript{2} light was measured with an LI1800 spectrometer. Wind-treatet plants were individually vibrated by applying 40 brushes for 40 s in the form of a mechanically operated duster\textsuperscript{2} the tip of duster was covered with thick silks to prevent abrasion of epidermal cells\textsuperscript{2} with the same tense and speed\textsuperscript{2} one brush s\textsuperscript{-1}\textsuperscript{2} to simulate the effect of wind\textsuperscript{2} the treatment was given once a day\textsuperscript{2}.

Shade cages were constructed in a way\textsuperscript{2} which allowed air circulation within cages and facilitated exchange of air with the surrounding environment in the greenhouse. Microclimatic measurements revealed no differences in temperature and air humidity between treatments. During the experiment\textsuperscript{2} the position of treatments between control and wind\textsuperscript{2} shadings were changed once a week to minimize the effects of sites.

The harvest was conducted 30 days after the start of the treatments. Plants were cut at ground level and divided into petioles\textsuperscript{2} leaf lamina and stolons. The total mother leaf number was counted\textsuperscript{2} the length and basal diameter of the longest petiole of each plant were measured to the nearest 0.1 mm with a digital ruler and calliper\textsuperscript{2} mother leaf lamina area was measured with a leaf area meter \textsuperscript{2} LI 3100\textsuperscript{2} LiCor\textsuperscript{2} Lincoln\textsuperscript{2} NE\textsuperscript{2}. Dry masses of above petioles and lamina were determined after oven drying for at least 72 h at 70°C. In addition\textsuperscript{2} the Young’s elastic modulus \textsuperscript{2} E\textsuperscript{2} MN m\textsuperscript{-2}\textsuperscript{2} of the longest petiole of each plant was measured on the basal part of the petiole. The petioles were enclosed in plastic bags with moist filter paper and immediately put into a freezer at about 8°C\textsuperscript{2} brought to the laboratory within 30 min of collection\textsuperscript{2} during the transport the temperature in the transport box was kept at the temperature at 8°C\textsuperscript{2} and all measurements were completed within 1 h after cutting. In the actual measurement of Young’s modulus\textsuperscript{2} we used an Instron universal testing machine \textsuperscript{2} 5542\textsuperscript{2} Instron\textsuperscript{2} Canton\textsuperscript{2} MA\textsuperscript{2} USA\textsuperscript{2}. Sections of the basal part of the petiole were placed on two fixed pins\textsuperscript{2} which were separated by a given distance L\textsuperscript{2} (in cm\textsuperscript{2}). The petiole sections were bended by a third pin\textsuperscript{2} placed exactly between the two fixed pins. This third pin moved

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with a controlled speed given in mm min\(^{-1}\). Both the imposed force \( P \) in Newtons and the deflection \( \delta \) in mm were instantaneously measured by the equipment. The Young’s modulus \( E \) in MPascal was automatically calculated from the linear part of the force-deflection curve. For a beam with free ends, the relation between deflection and force is given by\(^{390}\)

\[
\delta = \frac{P L^3}{48EI}
\]

where \( E \) is the Young’s modulus and with \( I \) the second moment of area

\[
I = 16/175 a^3 b
\]

That is, the cross section of the petiole was assumed to have a parabolic form with a long axis \( a \) and width \( 2b \)delete x-symbol both \( a \) and \( b \) measured in mm\(^{390}\).

1.3 Statistical Analysis

A three-way ANOVA was used to test for differences in response parameters with shade \( df = 1 \), wind \( df = 1 \) and genotype \( df = 9 \) as fixed factors. Data transformation was based first on Levene’s test for quality of variance second on the Shapiro-Wilk test of normality.

2 Results

2.1 The longest petiole length

The treatments had significant effects on petiole length [Fig. 1][Table 1]. Shade increased length regardless of wind while the wind-treatment decreased it regardless of shade. All genotypes displayed the same responses in petiole length to shade and wind. No significant interactive effects on petiole length were observed between low light and wind and between low light and genotype. There was a significant interactive effect on petiole length between wind and genotype suggesting differences in thigmomorphogenic responses between the 10 genotypes [Table 1].

![Graph of petiole length changes](http://www.ecologica.cn)

Fig. 1 Average longest petiole lengths and their changes in four treatments for ten genotypes. HLe = High light control, HLn = High light and wind, LLe = Low light control, LLn = Low light and wind. The change in length due to light effects is calculated by [HLc-HLe]/HLc, [HLe]/HLn, [HLe-HLn]/HLn and for thigmomorphogenic effects the calculation is given by [LLe-HLe]/LLe, [HLe-HLe]/HLe. Bars indicate standard errors \( n = 8 \) the same below.

2.2 Petiole diameter

The shade and wind treatments had significant effects on petiole diameter [Fig. 2][Table 1]. Low light decreased the petiole diameter in all genotypes while wind increased it except for genotype 2, 3 and 10 under the high light treatment. No significant interactions were observed between low light and wind, between low light and genotype or between wind and genotype.

2.3 Petiole Young’s modulus

Both treatments had a significant effect on petiole Young’s modulus [Fig. 3][Table 1]. Low light increases Young’s modulus except for genotype 4, 5 and 9 while wind decreases it except for genotype 4, 5 and 12 under low light. No significant interactions were observed between low light and wind.
genotype between wind and genotype.

Fig. 2  Average longest petiole diameters and their changes in four treatments for ten genotypes

Fig. 3  Average petiole Young’s modulus and their changes in four treatments for ten genotypes

2.4 Leaf number

The number of leaves in the mother rosette was reduced by shading. Wind on the other hand tended to increase the number of leaves in the mother rosette and this effect was much stronger in the shading than in the non-shading treatment as indicated by the significant wind and light interaction [Table 1].

Table 1  Results P values of analysis of variance with genotype [df = 9] and wind [df = 1] and light [df = 1] as factors for the genotype, wind interaction and genotype × light interaction df = 9 for the wind × light interaction df = 1

<table>
<thead>
<tr>
<th>Item</th>
<th>Genotype (df = 9)</th>
<th>Wind (df = 1)</th>
<th>Light (df = 1)</th>
<th>Genotype × wind (df = 9)</th>
<th>Genotype × light (df = 1)</th>
<th>Wind × light (df = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leaf number</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.541</td>
<td>0.291</td>
<td>0.001</td>
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<tr>
<td>Mother leaf area</td>
<td>0.000</td>
<td>0.034</td>
<td>0.000</td>
<td>0.953</td>
<td>0.074</td>
<td>0.009</td>
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<tr>
<td>Mother leaf dry mass</td>
<td>0.000</td>
<td>0.063</td>
<td>0.000</td>
<td>0.656</td>
<td>0.000</td>
<td>0.013</td>
</tr>
<tr>
<td>Specific leaf area [SLA]</td>
<td>0.000</td>
<td>0.007</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.040</td>
</tr>
<tr>
<td>The longest petiole length</td>
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<td>0.000</td>
<td>0.042</td>
<td>0.624</td>
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<tr>
<td>The longest petiole diameter</td>
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<td>0.000</td>
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<tr>
<td>The longest petiole mass</td>
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<td>0.001</td>
<td>0.123</td>
<td>0.749</td>
<td>0.877</td>
</tr>
<tr>
<td>Petiole Young’s modulus</td>
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<td>0.000</td>
<td>0.000</td>
<td>0.313</td>
<td>0.469</td>
<td>0.257</td>
</tr>
</tbody>
</table>

SLA = mother leaf area per unit mother leaf biomass

2.5 Leaf area and leaf mass in the mother rosette

Low light significantly decreased both the leaf area and leaf dry mass in the mother rosette however the degree of a reduction in leaf dry biomass was more than that in leaf area [Fig. 4 Table 1] thus plants had a higher SLA [leaf area per unit leaf mass] under low light than under high light [Fig. 5 Table 1]. Under high light the wind treatment significantly decreased the leaf area of the mother rosette and marginally decreased the leaf dry mass but there was no consistent effect of the wind-treatment on the leaf area and leaf mass of the mother rosette under low
light. There were significant interactions in SLA between wind and light between genotype and light and between wind and genotype suggesting that with respect to these traits the ten genotypes differed in their response to shade and wind.

3 Discussion

Our results demonstrate that the effects of shade and wind on morphology were independent and additive. For both the full light plants and the plants grown under shade wind-treated plants had shorter thicker petioles with lower Young’s modulus than untreated plants which suggests that thigmomorphogenesis did not differ between high light and low light. These results are consistent with those of Mitchell and Anten et al. and contrary to those of Ashby et al. and Henry and Thomas who suggested that thigmomorphogenesis is suppressed when plants grow in dense stands which they interpreted as an adaptive response because a shorter stature would confer a large disadvantage under those shade conditions. It is well known however that wind exposure generally increases with plant height consequently also for plants grown in the shade an inappropriate stem elongation increases the risk of mechanical failure. If maintaining the capacity for plasticity is costly the optimal plastic response will be a compromise between the reaction norm expressing the optimal phenotype in each environment and the reaction norm with the lowest cost. The adaptive value of plasticity to canopy shading may thus be diminished because of stem and/or petioles buckling or uprooting.

In our experiment wind-treated plants had a lower Young’s modulus than the plants not subjected to such a treatment. This result is consistent with those of Goodman and Ennos more flexible stems i.e. petioles with a lower Young’s modulus bend more easily in the wind and hence convey a smaller bending moment to their bases which can increase the resistance to wind. On the contrary plants grown under shade had a higher Young’s modulus compared to plants grown under high light except for genotype 512. Furthermore these shaded plants had quite

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a longer petiole length and a smaller diameter. So we see that to maintain mechanical stability i.e. to prevent the petiole from falling over the plants increased their Young’s modulus. That is for example plants grown in canopy shading are longer and therefore might have more parenchyma cells [Puijalon et al. 2006 14th New Phytologist Symposium[1]]. Because of the larger amount of parenchyma cells the mass density is lower. And because of this low mass density one would expect a lower Young’s modulus. However if the turgor pressure in this cells is increased relative to the petioles grown in light then this increase in pressure can lead to an increase in Young’s modulus and therefore maintains the mechanical stability. This could explain our above results in other words if changes in petiole length are related with changes in the petiole anatomy but not necessarily to the expense of mechanical stability[21].

Leaf architecture plays a major role in the light-harvesting efficiency of herb canopies petiole length even plays a dual role in leaf light harvesting[33][9][10] For leaves organized in a rosette an increase in petiole length can decrease the clumping i.e. it can decrease the mutual overshadowing of the leaves. However an increase in petiole length may also lead to an increase in the lamina angle with respect to the horizontal and hence it can lead to a decrease in the average light intensity on the leaves. That is according to beam theory lamina deflection from the horizontal increases with the cube of petiole length[34][1][11] suggesting that modest changes in petiole length may have a large impact on light intensity. In herbaceous plants the petioles of stoloniferous plants and the internodes of erect plants can be regarded as analogous organs and then their strong increase in length due to shading can be viewed as a mechanism to try to enhance light harvesting[25]. Due to their low shade tolerance[1][1][4][12] rapid stem and/or petiole elongation may be especially pronounced in colonizing annual species like Abutilon and P. reptens growing under crowded conditions with strong vertical light gradients so that there is a low light availability at the bottom and increasingly higher light supply in the upper parts of canopy[35]. In this study canopy shading low R/FR produced taller thinner petioles Table 1 indicating a trade-off between petiole height and diameter growth[11][32]. In this experiment wind-treatment had an effect on plants that was opposite in nature to that of canopy-shading producing shorter thicker petioles Table 1. Plant responses to wind most likely increase the mechanical stability of plants[11][34][9][10]. That is petioles have to carry their own weight as well as the weight of leaves. So anyhow a minimum investment in carbohydrates is needed to guarantee a certain strength of the petiole to prevent buckling or breaking[34]. Plants grown in crowded conditions experience a certain wind-shielding while plants grown under open field conditions are subjected to an extra loading in terms of wind and/or precipitation[36][37] requiring an extra investment in carbohydrates to resist mechanical failure. In open field situation there is a very strong increase in wind speed with height above the vegetation[11][34][39]. So plants with short stem and/or petiole therefore experienced less mechanical stress[32]. In the experiment it was found that the mean petiole diameter of winded plants was thicker than that of untreated plants except for genotype 2[9][3][10] under high light. Fig. 2. This result is consistent with most literature on mechanical stress effects mechanical stimuli tend to result in increased stem diameter growth relative to height growth[9][3][32] but the observed effects are contrary to those of Henry and Thomas[11[11].

Shading reduced the number of leaves in the mother rosette. Mother SLA leaf area per unit leaf biomass was higher in low light plants than in high light plants. The result is consistent with the low leaf thickness typically exhibited by shade leaves and is thought to minimize internal shading of chloroplasts[11][3][11].

Overall changes in leaf properties hints at important trade-offs between light capture and stability this apparent

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compromise possibly reflects conflicting requirements in terms of light capture and mechanical stability for biomass investments in petiole length and diameter[1]and in leaf thickness and leaf area. The morphological characteristics are generally employed in selection for genotypes of more adaptive variable environments such compromises may need further consideration at a genotype level[57].

References


