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Growth and biomass allocation of *Lolium perenne* seedlings in response to mechanical stimulation and water availability

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Wind-induced mechanical stimulation and water shortage are important stresses in arid/semiarid regions. However, little is known about the combined effects of mechanical stimulation and water availability. Since the effects of high water availability on biomass and allocation are opposite to those from mechanical stimulation, it is hypothesized that high water availability suppresses the effects of mechanical stimulation. To test this hypothesis, seedlings of *Lolium perenne* were subjected to two levels of brushing (non-brushing *versus* brushing 60 seconds d^{-1}) and two levels of water availability (200 ml d^{-1} *versus* 400 ml d^{-1}). Brushing had no effects on the total biomass of *L. perenne*, indicating that brushing is not a stressful factor. However, brushing significantly decreased plant height and dramatically increased the root/shoot ratio. The differences in biomass allocation between brushed and non-brushed plants at high water availability were significantly smaller than those at low water availability, showing that the effects of brushing are suppressed by high water availability. These results suggest that *L. perenne* may have evolved some traits to cope with frequent mechanical disturbance.

Key words: brushing, interactive effects, plant height, phytomass, rainfall, root/shoot ratio, tiller

Introduction

In nature, wind and low water availability are important stress factors for plants growing in arid or semiarid areas (Wang *et al*. 2008, 2009). Wind effects on plants are usually direct: e.g. leading to mechanical stimulation or mechanical perturbation, and indirect: e.g. changing microclimate (Grace 1977, Retuerto & Woodward 1993), and the responses to wind stress are usually induced by mechanical stimulation (Grace 1974, Russell & Grace 1978). Thus, mechanical stimulation is an effective manipulation to simulate wind.

In the field, mechanical stimulation is ubiquitous and can be induced by wind, rain, hail, and animal movements (Mitchell 1996). The responses of plants to mechanical stimulation are termed thigmomorphogenesis (Jaffe 1973). Mechanical stimulation can be applied in many ways (Gartner 1994), one of which is brushing plant shoots (Biddington & Dearman 1985, Latimer 1990, Wang *et al*. 2009). The primary response of shoots to mechanical stimulation is to develop dwarfed forms (Beyl & Mitchell 1977, Biddington & Dearman 1985, Anten *et al*. 2005), a decrease in total biomass (Mitchell *et al*. 1977, Heuchert & Mitchell 1983, Biddington & Dearman 1985), or an increase in the root/shoot ratio (Ennos 1991, Crook & Ennos 1994, 1995, Cleugh *et al*. 1998).

Increased water availability usually acts in the opposite direction to mechanical stimulation (e.g. high water availability induces more tillers, higher biomass and lower root/shoot ratio) (Luxmoore & Millington 1971). Wind stress can be ameliorated under conditions in which water availability is not limiting (Retuerto & Woodward 1993), and the effects of mechanical stimulation depend on the levels of other environmental factors (Henry & Thomas 2002, Wang *et al*. 2009). Here we hypothesize that negative effects of mechanical stimulation are alleviated by high water availability. To our knowledge, limited efforts have been made to explore the combined effects of wind and water on plant growth. Moreover, previous results are inconsistent with each other (Wang *et al*. 2008, 2009). To test this hypothesis, seedlings of perennial ryegrass (*Lolium perenne*) were subjected to two levels of mechanical stimulation and two levels of water availability in a factorial design.

Material and methods

Lolium perenne (perennial ryegrass) is a perennial tufted grass, and its height ranges from 30 cm to 90 cm. It is an excellent forage grass and a widely distributed cultivated species, which usually grows in meadow grasslands, wetlands, or roadsides (Liu *et al*. 2002).

The seeds of *L. perenne* were obtained from the Institute of Agricultural Technology of Beijing, China. On 27 May 2006, the seeds were sown in 2 m × 1.5 m × 0.5 m containers and kept in natural daylight at the Ordos Sandland Ecological Station (OSES; 39°02´N, 109°21´E) of the Institute of Botany, Chinese Academy of Sciences. After 22 days, 48 similar-sized seedlings of *L. perenne* were transplanted into 30 cm × 20 cm × 20 cm plastic pots filled with sand. Prior to use, the sand was sieved to remove debris and seeds. Each plant was randomly subjected to one of four treatment combinations, consisting of two kinds of mechanical treatment (brushing *versus* non-brushing) and two levels of water availability (low *versus* high). There were 12 replicates per combination. The mechanical treatment was applied as in Wang *et al*. (2009). Plants were supplied with 200 and 400 ml water per day for the low and high water availability treatment, respectively. To avoid water draining away, all the pots were placed in dishes.

The experiment was carried out in the greenhouse at the OSES, where the mean temperature and humidity (Thermo Datalogger, Campbell Inc., USA) were 27.6 °C and 63%, respectively. During the experiment, each plant was supplied with 10 ml 0.08% nutrient solution (Peters Professional: 20% N, 20% P_2O_5, 20% K_2O, Scotts Company, Ohio, USA) once every 2 weeks. The experiment was conducted from May to September 2006.

At the end of the experiment, the heights and tiller numbers of all plants were determined, the plants were harvested and divided into leaves, sheaths and roots. Dry masses of all the plants were determined after oven drying for at least 72 h at 70 °C.

A two-way ANOVA (general linear model, SPSS 13.0; SPSS, Chicago) was used to test for differences in response parameters, with brushing and water treatment as fixed factors. When significant interactive effects of brushing and water treatment were detected, we used a one-way ANOVA to investigate the effect of water treatments on the differences between brushing and non-brushing treatment groups. The differences (D) were determined using the following equation:

$$D = [(X_b - X_{nb})/X_{nb}] \times 100\ (\%) \qquad (1)$$

where X_b are the variables for plants under brushing treatment, X_{nb} are the ones under non-brushing treatment.

Before the data analysis, equality of the variance was checked using Levene's test and normality of the data was tested with the Shapiro-Wilk test. Plant height was distributed normally, and variances were not homogeneous. To achieve homogeneity of the variances, plant height was square-root transformed. The other variables were distributed normally and their variances were homogeneous, thus they were not transformed.

Results

Brushing caused the plants to be short, allocate less biomass to leaves and more biomass to roots, and have a greater root/shoot ratio (Table 1; Figs. 1 and 2). Increased water availability allowed the plants to be taller, yield more biomass, and allocate more biomass to sheath (Table 1; Figs. 1 and 2). Also, there were significant brushing × water effects on the root/shoot ratio, and on the biomass allocation to leaves, to sheaths and to roots (Table 1). The one-way ANOVA analysis showed that high water availability significantly reduced the differences in the root/shoot ratio ($F_{16} = 6.192$, $p = 0.024$) and in the biomass allocation to: root ($F_{16} = 6.339$, $p = 0.023$), sheath ($F_{16} = 4.843$, $p = 0.043$) and leaf ($F_{16} = 6.912$, $p = 0.018$) between brushing and non-brushing treatment plants.

Discussion

Under high water availability, the differences in the root/shoot ratio between brushing and non-brushing treatments were much smaller than those under low water availability. These results support our hypothesis that high water availability can suppress the effects of brushing. Additionally, these findings indicate that high water availability induces mechanically-disturbed plants to generate more above-ground biomass, especially sheaths, which can harden the support structure and resist more loadings on the shoots. Similar interactive effects have been detected in *Corispermum mongolicum* (Chenopodiaceae) (Wang *et al*. 2009), but not in *Hedysarum laeve* (Fabaceae) (Wang *et al*. 2008). Combined effects of mechanical stimulation and other environmental factors have previously been found in some studies (Retuerto & Woodward 1993, Henry & Thomas 2002), but other studies did not find such interactions (Anten *et al*. 2005, Liu *et al*. 2007). Thus, it appears that the degree to which plants respond to mechanical stimulation and the direction of the response depend on the presence of other factors.

Brushing reduced the plant height by 30%. This is consistent with previous results (Biddington & Dearman 1985, Anten *et al*. 2005). In nature, decreased height can reduce the loading of wind. Wind speeds increase drastically with

Table 1. Effects (*F* values followed by degrees of freedom in parentheses) of brushing and water availability and their interactions on the parameters of *Lolium perenne*. Significance levels: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$; ns = $p > 0.05$.

	Brushing	Water	Brushing × water
Trait			
Height[a]	17.708*** (1,44)	9.031** (1,44)	1.014^{ns} (1,44)
Tiller number	0.051^{ns} (1,44)	2.908^{ns} (1,44)	0.855^{ns} (1,44)
Total plant mass	0.036^{ns} (1,32)	9.685** (1,32)	0.741^{ns} (1,32)
Biomass allocation (%)			
Leaf	7.986** (1,32)	0.091^{ns} (1,32)	6.397* (1,32)
Sheath	1.423^{ns} (1,32)	18.368*** (1,32)	5.234* (1,32)
Root	8.435** (1,32)	2.468^{ns} (1,32)	9.731** (1,32)
Root/shoot ratio	8.652** (1,32)	2.456^{ns} (1,32)	11.287** (1,32)

[a] Data were square-root transformed before analysis.

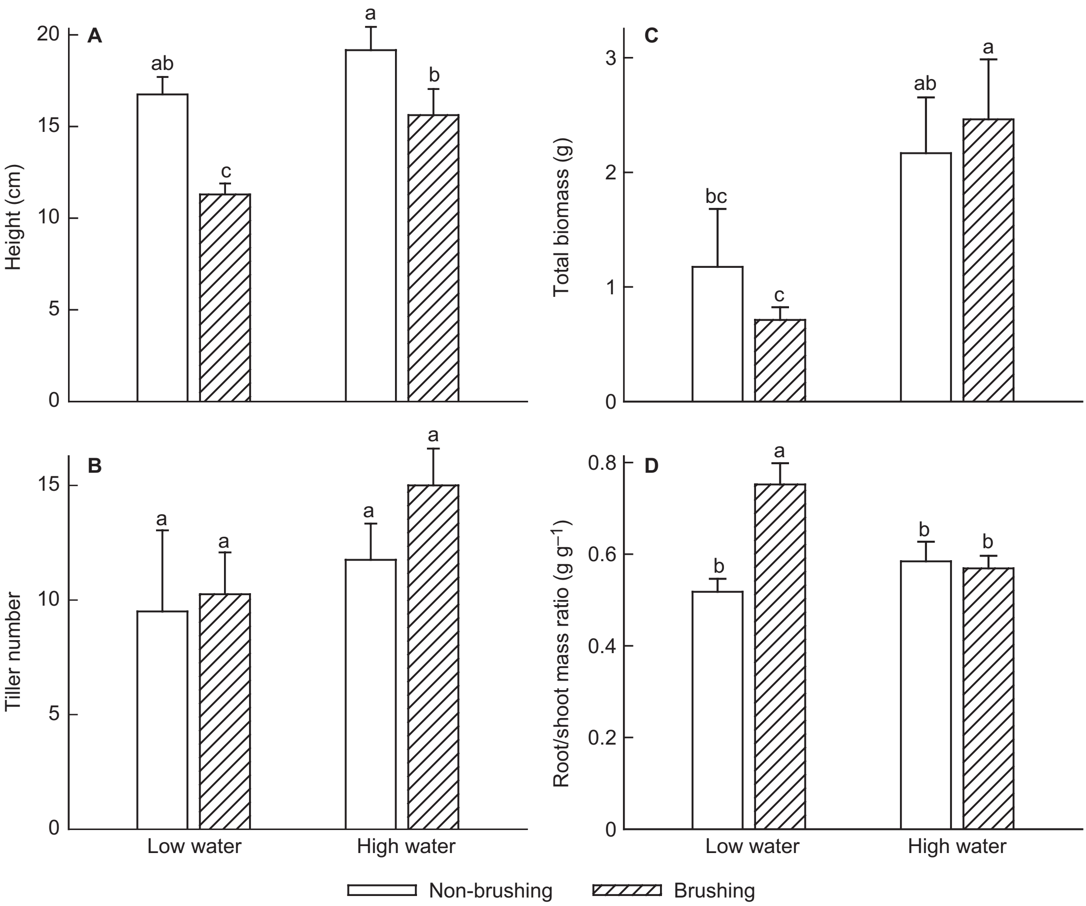

Fig. 1. (**A**) The height, (**B**) tiller number, (**C**) total biomass, and (**D**) root/shoot mass ratio of *Lolium perenne* under low water and high water conditions. Data are means + 1 SE. The bars with different letters are significant at $p < 0.05$.

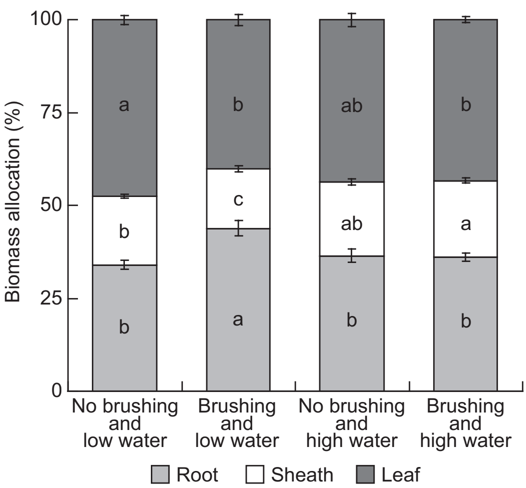

Fig. 2. Mean biomass (± 1 SE) allocation to leaf, sheath and root of *Lolium perenne*. Different letters indicate significant differences at $p < 0.05$.

increasing height above the vegetation (Bertness & Callaway 1994, Speck 2003). Moreover, the bending moment exerted by a given wind force scales linearly with plant height. Increased mechanical stress, such as brushing that plants experience when they grow above the surrounding vegetation level, induces a reduction in height, which is an adaptive strategy. The lack of response of tillers to the range of treatments imposed is striking.

Langer (1963) concluded that growth of axillary buds to form tillers depended on the availability of adequate carbohydrate resources as determined by the relative rates of photosynthesis and respiration. In this study, brushing had no effect on the total biomass, which is consistent with our recent experiment with *C. mongolicum* (Wang *et al.* 2009), but not with *H. laeve* (Wang *et al*. 2008). Probably the minimum amount of stress that results in significant growth reduction is species specific (Autio *et al*. 1994). For ryegrass, this stress may be inadequate to lead to the growth reduction exhibited by *H. laeve*, since the seedlings of ryegrass grow larger during the treatment. Also, as shown by Heuchert and Mitchell (1983) and Autio *et al*. (1994), plant responses to mechanical stimulation depend on the season. In our series of studies, the experiment period may be of importance in the case of *H. laeve*, but not in the case of ryegrass and *C. mongolicum*. Thus, it is important to note that the nature or extent of the responses depends on the species as well as the physiological stage of the plant when it is stimulated (Jaffe 1973). The regression analysis showed that the variation in total biomass was mainly associated with tiller number ($r^2 = 0.682$, $p < 0.001$, $n = 36$). Obviously, although the height decreased under brushing, the tillers and the total biomass were not affected, which indicates that for perennial ryegrass, brushing is not a stressful factor. Brushing significantly affected the biomass allocation but in a different way that in *C. mongolicum* (Wang *et al*. 2009). The increase in root biomass in response to brushing is advantageous because it increases the magnitude of the mechanical forces required to dislodge a plant from its substrate. In the field, the mechanical stimulus may come from a variety of sources. Shaking or brushing of plants can be caused by passing of animals or vehicles, or by animals seeking to relieve an itch (Jaffe 1973). For herbage it is likely that a mechanical stimulus is caused by grazing animals. It can be assumed that this kind of biomass allocation pattern reduces uprooting of plants by grazing animals. Thus, responses to brushing may be adaptive because brushing is a cue indicating the presence of grazers and the potential risk of being grazed upon.

One possible proximate explanation for these responses to brushing is that this kind of mechanical stimulation causes cavitations of the water columns in the xylem, disrupting the pathway for transport, and therefore leading to water stress (Parkhurst & Pearman 1972). However, in our experiment, the effects of brushing were suppressed by high water availability, which means that the water stress induced by water translocation interruption is not a major cause of inducing brushing effects. Grace (1982) found that mechanically stimulated plants had lower water potentials than the controls. Brushing may increase evaporation and increased water availability may help to compensate increased water loss by evaporation. Thus, the brushing effects can be suppressed by high water availability. Another likely explanation is that the effects of mechanical stimulation on plant growth are mediated by phytohormones (Jaffe & Biro 1979, Mitchell 1996, Anten *et al*. 2006), which has been widely accepted. At present, precise mechanisms for the responses to brushing are not known. In our recent series of experiments, the variable findings may be related to vibrating means and target species. In addition, interactions and interdependences between many responses partly explain the different effects among species (Biddington & Dearman 1985), and the thigmotactic effects should not be extrapolated from one species to another (Niklas 1998).

In summary, mechanical stimulation is ubiquitous, but its effects can be suppressed by high water availability. In addition, our experiment shows that brushing is not a stressful factor affecting growth of perennial ryegrass. It can be concluded that perennial ryegrass is adapted to moist habitats with a high frequency of mechanical disturbance.

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