A Comparative Analysis of the Temperature Response of Leaf Elongation in *Bromus stamineus* and *Lolium perenne* Plants in the Field: Intrinsic and Size-mediated Effects

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- **Background and Aims** Growth of grass species in temperate-humid regions is restricted by low temperatures. This study analyses the origin (intrinsic or size-mediated) and mechanisms (activity of individual meristems vs. number of active meristems) of differences between *Bromus stamineus* and *Lolium perenne* in the response of leaf elongation to moderately low temperatures.

- **Methods** Field experiments were conducted at Balcarce, Argentina over 2 years (2003 and 2004) using four cultivars, two of *B. stamineus* and two of *L. perenne*. Leaf elongation rate (LER) per tiller and of each growing leaf, number of growing leaves and total leaf length per tiller were measured on 15–20 tillers per cultivar, for 12 (2003) or 10 weeks (2004) during autumn and winter.

- **Key Results** LER was faster in *B. stamineus* than in *L. perenne*. In part, this was related to size-mediated effects, as total leaf length per tiller correlated with LER and *B. stamineus* tillers were 71% larger than *L. perenne* tillers. However, accounting for size effects revealed intrinsic differences between species in their temperature response. These were based on the number of leaf meristems simultaneously active and not on the (maximum) rate at which individual leaves elongated. Species differences were greater at higher temperatures, being barely notable below 5 °C (air temperature).

- **Conclusions** *Bromus stamineus* can sustain a higher LER per tiller than *L. perenne* at air temperatures >6 °C. In the field, this effect would be compounded with time as higher elongation rates lead to greater tiller sizes.

**Key words:** *Bromus stamineus*, *Lolium perenne*, temperature response, leaf elongation, tiller size, intrinsic effects, number of growing leaves, low temperature, field study.

**INTRODUCTION**

Leaf growth in the Gramineae is predominantly unidirectional, parallel to the longitudinal axis of the leaf (Volenc and Nelson, 1981). When water and soil nutrients are not limiting, leaf elongation rate (LER) correlates strongly with temperature (between 2 and 24 °C in C3 grasses: Williams and Biddiscombe, 1965; Peacock, 1976; between 13 and 36 °C in C4 species: Watts, 1971; Bendah Salah and Tardieu, 1995). Therefore, in temperate-humid regions, growth of grass crops and forages is often restricted by limited rates of leaf area development (Ong and Baker, 1985). Hence, the current efforts directed towards genetic selection for better growth at low temperatures (Kemp et al., 1989; Reed et al., 2004). Genetic differences in C3 species in leaf area expansion at low temperature have been documented, e.g. between Mediterranean and temperate Festuca arundinacea ecotypes (Cooper, 1964; Robson, 1967), and between high- and low-altitude *L. perenne* ecotypes (Ollerenshaw and Baker, 1982). However, information outside these two well-known species is scarce. Besides their potential agronomic value, information on less studied species can provide insight into the mechanisms determining LER, and is thus important for the development of models of leaf elongation (Nelson, 2000).

*Lolium perenne* is the most common grass component of perennial pastures in temperate regions. However, alternative species are currently being evaluated, particularly where marked seasonal climatic fluctuations occur. In New Zealand (Stewart, 1992) and Australia (Fulkerson et al., 2000), species such as *Bromus willdenowii*, *B. stamineus* and *F. arundinacea* can be more productive than *L. perenne* in winter and summer. Compared with the other species, *B. stamineus* has been studied very little. It is native to Chile and is similar in seasonal growth pattern, vernalization requirements and flowering time to *B. willdenowii*, a species native to Argentina. However, to our knowledge, there is no comparative information on the temperature response of leaf growth.

The comparison of LER responses can be complicated (obscured) by tiller size effects. This is because of positive relationships between tiller size and LER, as revealed by...
grazing experiments with control of sward state for several C3 forage grasses (Bircham and Hodgson, 1983; Mazzanti et al., 1994; Agnusdei et al., 2007). The origin of this effect is not clear, although it seems to be related to an influence of the length of the sheath tube through which the growing leaf emerges on the length of the leaf growth zone (Casey et al., 1999; Kavanová et al., 2005). Coordination between the lengths of the sheath tube and of the leaf growth zone (Arredondo and Schnyder, 1999) is thought to be mediated by photo-morphogenetic effects (Skinner and Simmons, 1993). The existence of such size effects can complicate the analysis of differences in LER, as, for instance, differences between genotypes can be due to intrinsic, genetic effects, and/or size differences between the species.

The response of leaf elongation to moderately low temperature was studied in two cultivars of both L. perenne and B. stamineus in the field. The mechanisms of eventual differences were explored with the aims of (a) disentangling intrinsic differences from tiller size-mediated effects; and (b) distinguishing differences in the activity of individual intercalary meristems from differences in the number of active meristems. It was observed that B. stamineus produced leaf tissue substantially faster than L. perenne, in part simply due to the larger size of B. stamineus tillers (‘size effect’). However, intrinsic differences between B. stamineus and L. perenne were also present, not in the maximal rate of activity of individual meristems, but in the number of leaves growing simultaneously on a given tiller.

**MATERIALS AND METHODS**

*Experimental site*

Experiments were carried out near Balcarce, Argentina (37°45’S, 58°18’W). The climate of the area is temperate-humid: long-term average annual temperature is 13.7°C, varying from 20.1°C in January to 7.3°C in July, with a 280-d frost-free period from October to early May.

Soil was a loam Mesic Fine Typical Argiudol, with an A horizon of 25 cm, 62 g kg⁻¹ organic matter and pH 6.2. The textural B horizon presented no impediments to plant growth. The experiment was planned for growth conditions to be as close to optimal as possible. For this, nutrient supply and water availability were kept at optimal levels at all times. All plots were irrigated twice a week to soil saturation, and received periodic dressings of fertilizers (3 g P m⁻² year⁻¹ applied in autumn as super-phosphate; 5 g N m⁻² month⁻¹ applied as urea). Likewise, weeds and pests were controlled.

*Trial 1: 2003*

Four cultivars were evaluated: B. stamineus D. ‘Zamba’ and ‘Gato’, and L. perenne L. ‘Horizon’ and an experimental cultivar of L. perenne. Each cultivar was planted on 1 March 2002 in two dense rows, each 1 m long, in a completely randomized design. Accumulated herbage was cut monthly at 50 mm above ground level from 1 June 2002 until 10 May 2003. Thereafter, plants grew undisturbed.

On 5 May 2003, 20 tillers with four leaves per cultivar (ten per row), placed in the middle of the canopy, were randomly selected and were marked with a plastic ring (cohort 1). Selected tillers were of similar physiological status, as indicated by a similar number of green leaves and a similar position in the canopy. However, they did differ in size. Further, eight daughter tillers of cohort 1 per cultivar were marked with a plastic ring on 31 July 2003 (cohort 2).

At this time, tiller density was 6000 tillers m⁻² for B. stamineus (both cultivars), and 25 000 and 13 000 tillers m⁻² for L. perenne ‘Experimental’ and ‘Horizon’, respectively. Leaf length was measured periodically between 2 June 2003 and 25 August 2003. As leaf blades on reproductive tillers grow faster than those on vegetative ones (Peacock, 1976; Parsons and Robson, 1980; Kemp et al., 1989), only data from tillers in which the pseudostem did not elongate actively are reported; thus avoiding measures on tillers undergoing reproductive differentiation in the apical meristem (Parsons and Robson, 1980). During August of 2003, B. stamineus tillers suffered severe symptoms of foliar disease, and these data were not used.

*Trial 2: 2004*

For this study, B. stamineus ‘Zamba’ and L. perenne ‘Horizon’ were evaluated. Both were seeded on 22 September 2003 into 35 cm deep wooden boxes containing a 1:1 mixture of sand and the Typical Argiudol soil described above, at a density of 434 seeds m⁻² (4.1 g seed m⁻² for B. stamineus, 1.6 g seed m⁻² for L. perenne) that were nearly equally spaced. As in 2003, accumulated herbage was cut at 50 mm at monthly intervals from 1 December 2003 till 27 April 2004. Thereafter, plants grew undisturbed. On 27 April 2004, 18 tillers per cultivar were marked with a plastic ring, and leaf length measured between 1 May 2004 and 30 June 2004. Again, selected tillers were of similar physiological status (similar number of green leaves and similar position in the canopy). On 15 May, tiller density was 12 000 and 10 000 tillers m⁻² for B. stamineus and L. perenne, respectively. All tillers remained vegetative throughout this trial.

*Leaf measurements*

On each tiller the green blade length of individual fully expanded leaves was measured from the tip to its own ligule, or of growing leaves from the ligule of the previous fully expanded leaf with a 1 mm graduated ruler. From these measurements, leaf elongation rate per tiller (LERₜ) and per growing leaf (LERₗₜ, where n is the leaf number with n = 1 for the youngest leaf) were calculated. The number of growing leaves was counted on each date.

Total blade length per tiller (LL) at the beginning of each measurement period was recorded. In all cases, LL was highly correlated to the length of the youngest fully expanded blade (data not shown). In undisturbed temperate C3 grasses, the lengths of mature blades and of sheaths are strongly correlated (Durand et al., 1999). Therefore, LL was used as a proxy for tiller size, because sheath lengths are
difficult to measure accurately in the field without disturbing the plant’s canopy too much, because their base is located below-ground.

**Climate measurements**

Daily air temperature at 1.5 m height was recorded with a portable meteorological station (LI-1200S, Li-Cor Inc., Lincoln, NE, USA). Mean, minimum and maximum air temperatures are shown in Fig. 1, along with the duration and average temperature of each measurement period.

**Statistical analysis**

Analyses of variance were performed using the SAS GLM procedure (SAS Institute, Cary, NC, USA) to analyse species and cultivar effects on the responses. Means were separated using Tukey’s test at the $P = 0.05$ significance level.

Simple and multiple linear regression were used to model $LER$ responses to tiller size ($LL$) and temperature (continuous variables) using the SAS GLM procedure. Convergence (null hypothesis: intercepts are equal) and parallelism (null hypothesis: slopes are equal) were tested via dummy variables (Berenson *et al.*, 1983).

**RESULTS**

In both years, *B. stamineus* showed higher $LER_T$ and had greater $LL$ than *L. perenne* ($P < 0.05$, Table 1 and Fig. 2).

No difference was observed between *L. perenne* cultivars, but *B. stamineus* cv. ‘Gato’ tended to show higher $LER_T$ ($P < 0.10$) and greater $LL$ than ‘Zamba’ ($P < 0.05$, Table 1 and Fig. 2).

Likewise, averaging cultivars and years, two to three leaves per tiller were elongating simultaneously in *B. stamineus*, while in *L. perenne* the number of growing leaves varied between one and two (Fig. 2). Thus, in *B. stamineus*, $LER_{L1}$, $LER_{L2}$ and $LER_{L3}$ contributed, on average, 45, 41 and 14% to $LER_T$, respectively. Conversely, in *L. perenne*, $LER_{L1}$ and $LER_{L2}$ explained, on average, 75 and 25% of $LER_T$, respectively (Table 1).
tiller cohorts’, because (daughter) tillers from cohort 2 were smaller than those from cohort 1 (see Materials and Methods). It is worth mentioning that, in both species, the variation in tiller size between individual tillers and between cohorts was much higher than the variability in tiller size between dates.

Strong, linear, positive correlations between $LL$ and $LER_T$ were observed for each cultivar, in both species, and at each measurement period (Fig. 3; in all cases $P < 0.05$). Essentially the same relationships were observed for $LER$ of each individual leaf (data not shown). This means that, for a given temperature, different $LER_T$ and $LER_L$ were recorded in tillers of similar physiological status, and that this variability correlated with the size of those tillers, measured either as $LL$ or as the length of the mature youngest fully expanded leaf. Two features were evident in all data sets: the similar behaviour of cultivars and cohorts within species, and the contrasting responses of $B. stamineus$ and $L. perenne$.

A significant temperature (i.e. measurement period) $\times$ $LL$ (i.e. tiller size) interaction was detected in all cultivars ($P < 0.05$) in multiple regression models where $LER_T$ was a linear function of tiller size, temperature and their interaction. The biological nature of such an interaction is evident from Fig. 4; the response of $LER_T$ to $LL$ differed between measurement periods, and the variability was closely associated with air temperature of the period. In all cultivars, the slope of $LER_T$ against $LL$ increased with temperature. The slope was similar for cultivars of the same species (parallelism: $P > 0.10$), but of greater magnitude in $B. stamineus$ than in $L. perenne$ (parallelism: $P < 0.05$). No difference in intercept was observed between cultivars or species (convergence: $P > 0.10$). In fact, intercepts differed significantly from zero ($P < 0.05$) only for $L. perenne$ for a few measurement periods.

These results indicate no evidence of differences in $LER_T$ between cultivars nor cohorts within each species once differences in tiller size (i.e. $LL$) are considered. Conversely, (a) slopes of $LL$ vs. $LER_T$ were greater in
B. stamineus than in L. perenne (Fig. 4), indicating that the higher LER_T of B. stamineus cannot be entirely ascribed to larger tillers, and (b) the magnitude of this intrinsic effect depended on the temperature, becoming greater as temperature increased. Indeed, base temperatures determined by extrapolation to LER = 0 were similar in both species (3–0.5 °C; Fig. 4).

The response of intercepts and slopes of LL vs. LER_T to temperature were combined into one model relating LER_T to temperature and LL. The resultant surface response shows that the difference between species widens as temperature and/or tiller size increased (Fig. 5). The relationships were used to estimate LER_T of tillers measured during 2004. The 2004 tillers were smaller than the range of sizes in 2003, from which the model was derived, but predicted values agreed well with observed data during May. However, the model overestimated LER_T during June by ~2 mm d⁻¹ in both species (Fig. 2).

**DISCUSSION**

Leaf length production per tiller at mean daily temperatures between 5 and 13 °C (absolute range: 0–19 °C, Fig. 1) was 140% faster in cultivars of B. stamineus than in L. perenne. Further, B. stamineus had larger tillers than L. perenne. We are aware of no published comparable data. Nonetheless, a comparison between two other species from the same genera showed that B. wildenowii Kunth was 231% faster than Lolium sp. (Hume, 1991). The higher LER_T of B. stamineus was not associated with higher (maximum) rates of length production by individual intercalary meristems nor with a lower base temperature,
Notably, within each species, cultivars showed a unique relationship between tiller size (measured as total blade length per tiller, $L_{max}$) and maximal leaf elongation rate of individual leaves ($LER_{max}$) for two consecutive time periods with different mean temperatures (A, 6–9 June, 6-9°C; B, 10–18 June, 10-4°C) for cultivars of $L. \text{perenne}$ (open circles, ‘Horizon’; open triangles, ‘Experimental’) and $B. \text{stamineus}$ (filled circles, ‘Zamba’; filled triangles, ‘Gato’). Each point represents data for an individual tiller. Lines are linear regression for $B. \text{stamineus}$ (solid line) and $L. \text{perenne}$ data (dotted line).

but with a greater number of leaves elongating simultaneously.

Accounting for size effects in LER

In all four cultivars and at each measurement period, both $LER_{T}$ (Fig. 3) and $LER_{max}$ (Fig. 6) correlated positively with tiller size (measured as total blade length per tiller, $LL$). Notably, within each species, cultivars showed a unique relationship between $LL$ and $LER_{T}$. This suggests that cultivars had no differential capacity for foliar elongation, i.e. the higher $LER_{T}$ of $B. \text{stamineus}$ ‘Gato’ compared with ‘Zamba’ was strongly associated with its larger tillers (Table 1 and Fig. 2).

Size effects have received little consideration in leaf growth studies. They are, nonetheless, not a new finding. Positive relationships between $LL$ and $LER_{T}$ have been reported in several forage grasses (Bircham and Hodgson, 1983; Mazzanti et al., 1994; Agnusdei and Assuero, 2004). Further, Kavanová et al. (2006) found that tiller size affected only the size (length) of intercalary meristems, but not the amount of leaf tissue produced per unit meristem length. Often, the length of intercalary meristems correlates closely with the length of the sheath tube through which growing leaves emerge (Arredondo and Schnyder, 2003; Kavanová et al., 2006; and references therein). A photo-morphogenetic control of meristem size has repeatedly been hypothesized (Begg and Wright, 1962; Skinner and Simmons, 1993; Skinner and Nelson, 1994). Substrate supply effects on meristem activity have also been suggested (Williams and Farrar, 1990).

The correlation between $LL$ and $LER_{T}$ means that differences between species in $LER_{T}$ would, in part, be explained by size effects, as tillers were larger in $B. \text{stamineus}$ than in $L. \text{perenne}$. However, differences between species persisted when size effects were accounted for (Fig. 4), indicating that $B. \text{stamineus}$ tillers responded more strongly to temperature than $L. \text{perenne}$ tillers, even when compared at similar sizes. Thus, both intrinsic and size-mediated effects interacted to determine a higher $LER_{T}$ of $B. \text{stamineus}$. As a consequence, the difference between species in the temperature response of $LER_{T}$ depended on tiller size (Fig. 5).

The origin of the intrinsic difference between $B. \text{stamineus}$ and $L. \text{perenne}$

The magnitude of the difference in $LER_{T}$ between species increased as temperatures increased (Figs 4 and 5). Indeed, extrapolating the observed response to lower temperatures suggests that $B. \text{stamineus}$ and $L. \text{perenne}$ differ little below 5°C, and have similar base temperatures for leaf growth, although this needs confirmation by studies in a controlled environment. These results support the observations that $B. \text{stamineus}$ has good potential for herbage production during the cool season in temperate regions (e.g. Stewart, 1992), and is promising for the use in areas such as the humid Pampas of Argentina. However, importantly, the results suggest that any growth advantage would be restricted to areas/periods with mean daily air temperatures above 6°C. As base temperatures are approached, the advantage of $B. \text{stamineus}$ in $LER_{T}$ diminishes (Fig. 5).

Tillers in the 2004 trial were 2-5 ($B. \text{stamineus}$) to 1-8 ($L. \text{perenne}$) times smaller than in 2003 (Table 1). Therefore, $LER_{T}$ was predicted and observed to be lower in 2004 than in 2003 (Fig. 2C). The good agreement between predictions and observations during May suggests that most of the reduction in $LER_{T}$ was size mediated in both species. However, overestimation during June suggests that other factors are involved. A better understanding of the extent of tiller density-mediated compensations (Bircham and Hodgson, 1983; Mazzanti et al., 1994) in mitigating differences in accumulation of leaf area per tiller growth is necessary.

Tiller growth depends on the inter-relationship of the apical meristem, producing leaf primordia, with intercalary meristems, responsible for leaf elongation (Briske, 1991; Skinner and Nelson, 1994). Therefore, it is relevant to distinguish which meristematic mechanisms are involved in the higher response to temperature in $B. \text{stamineus}$ tillers. Mechanisms reported previously to enhance growth at low temperature stressed the importance of intercalary meristem activity to explain differences in $LER_{T}$ (assuming the number of expanding leaves per tiller remained unchanged), e.g. Mediterranean vs. temperate ecotypes of $F. \text{arundinacea}$ (Robson, 1967), and high- and low-altitude ecotypes of $L. \text{perenne}$ (Ollerenshaw and Baker, 1982). The present results are exactly opposite: once tiller size effects are accounted for, both species show remarkably similar maximal rates of LER (Fig. 6). Conversely, the absolute number of growing leaves was consistently 70% greater in $B. \text{stamineus}$ (Table 1 and Fig. 2). This suggests that interspecific differences were not based upon differential capacities of intercalary meristems to produce leaf tissue.
Rather, the number of simultaneously active leaf meristems was the major difference.

Understanding the causes of the higher capacity for foliage elongation at low temperature has inter-related implications and applications. For instance, such differences could be exploited for breeding. Further, they should be important in modelling leaf area expansion, as they would change plant morphology and sward structure. Reduced growth of individual leaves at low temperature is known to be due to changes in meristem activity rather than meristem length (Durand et al., 1999), which are in turn associated with changes in rheological properties of cell walls (Thomas et al., 1989). Conversely, very little is known about what co-ordinates the activity of successive leaf intercalary meristems. Skinner and Nelson (1994) noted that two leaves of F. arundinacea grow at one time and suggested that co-ordination would be associated with cell division in a leaf meristem starting or ending as the same process ends or starts in the immediately previous or the subsequent leaf. The two leaves growing simultaneously would be on opposite sides of the shoot apex. However, the B. stamineus results imply that cell division occurred in more than two blades at a time. The exact co-ordination between intercalary meristems might thus depend on the species considered.

In conclusion, B. stamineus showed a greater capacity to elongate leaf tissue at moderately low temperatures than L. perenne, which was based on having more than two simultaneously active intercalary meristems, not on the maximal rate at which individual meristems produced leaf length. This advantage, however, depends on temperature, differences being small below 5 °C. To reveal intrinsic differences between species, size effects were accounted for. The fact that size-mediated and intrinsic effects on LER may act via different control mechanisms emphasizes the importance of accounting for tiller size effects before inferring the determinants of differences in leaf growth responses.

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LITERATURE CITED


