Leaf and tiller dynamics in two competing C₃ grass species: influence of neighbours and nitrogen on morphogenetic traits

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Abstract

The objective of this work was to analyse the competitive interactions between *Bromus willdenowii* Kunth. (BW = prairie grass) and a perennial C₃ grass *Dactylis glomerata* L. (DG = cocksfoot) through morphogenetic traits, during pasture establishment. Four combinations of species (pure BW, pure DG, DG flanked by BW and BW flanked by DG plants), with and without winter nitrogen fertilization, were factorially arranged in a complete random block design. Data were recorded on three tiller age cohorts of the central target plant: the main stem and those that appeared 20 and 30 days after fertilization (daf). Leaf elongation rate (LER) of the older tillers was the only variable affected by nitrogen addition. Annual neighbours, which showed higher LER than perennial ones, caused a decrease in leaf lifespan in the younger tillers and a delay in the leaf appearance rate on the main stem and on 20-daf tillers, and the tiller appearance rate. Annual neighbours controlled leaf and tiller dynamics and therefore restricted the vertical and horizontal space occupation of the target plant. These results reinforce the advantage of using a low seeding rate for the annual species to avoid negative effects on perennial grass establishment and persistence.

Keywords: Bromus willdenowii Kunth., *Dactylis glomerata* L., intra- and interspecific initial competition, tiller cohorts, leaf appearance rate, tiller appearance rate

Introduction

The expansion of agriculture in South America is driving livestock intensification based on cultivated pastures. Detailed attention to the aspects of pasture management, including genotype selection, sowing design and density, and fertilization and defoliation strategies is required to optimize floristic composition and to achieve a state in which legumes and perennial grasses, rather than annuals, dominate the sward (Kemp *et al.*, 2000; Kemp and King, 2001; Laidlaw and Teuber, 2001). These persistent pastures have higher stability and are better-functioning ecosystems owing to their more efficient water use, nitrate capture and cover of the soil surface (e.g. Kemp *et al.*, 2000; Garden and Bolger, 2001; Harris, 2001; Sanderson *et al.*, 2002). They also have a better economic outcome because the initial monetary investment gives an economic return over a longer period of time.

To obtain dense, long-lived pastures, it is necessary to understand canopy growth-regulation mechanisms using knowledge of the competitive interactions between species and the influence of cultural practices on them (Fustec *et al.*, 2005). Establishment is the critical stage of multispecies pastures (Skinner, 2005), because the space that each species will occupy later in the canopy is defined during this phase (Ross and Harper, 1972).

Slow-establishing species, such as the perennial grass *Dactylis glomerata* L. (DG = cocksfoot or orchardgrass), have low seedling competitive ability because of their slow initial belowground growth, which is related to nitrogen capture (Nurjaya and Tow, 2001). Other species, such as the annual (or short-term perennial) *Bromus willdenowii* Kunth = *Bromus catharticus* Vahl (BW = prairie grass), can establish rapidly and exclude more slow-establishing perennial species

(Harris, 2001; Sanderson *et al.*, 2002). The severe competition exerted by annual species reduces pasture stability. In addition, autumn and winter nitrogen fertilization applied to overcome winter herbage shortages may also have negative consequences on the floristic stability of pastures because perennial grasses and supporting N-fixing symbiont species are more likely to be excluded under fertilized conditions (Suding *et al.*, 2005).

In temperate pastures, plants grow as members of a community where intra- and interspecific competition generally affects growth severely (Lemaire and Chapman, 1996). When other events that affect establishment success have taken place, for example development of adventitious roots of sufficient length and diameter to ensure the photosynthetic surfaces receive enough water and nutrients (Ries and Svejcar, 1991), the rate at which the species' morphogenetic programme occurs then determines the initial growth rate. Thus, the morphogenetic aerial dynamics of each species are the main determinants of the success of the establishment of the sward. The morphogenetic traits of leaf appearance rate (LAR), leaf elongation rate (LER), leaf lifespan (LLS) (Lemaire and Chapman, 1996; Lemaire and Agnusdei, 2000) and tiller appearance rate (TAR) (Hirata and Pakiding, 2002) have consequences in competitive interactions between coexisting species (Tallowin *et al.*, 1995) because they determine leaf area development and, subsequently, radiation interception (Belanger, 1996). They also reflect the genetically driven pattern of assimilate allocation between the number and size of tillers (Sugiyama, 1995). This process of vegetative shoot development (Sugiyama, 1995) will be influenced by resource heterogeneity and by the identity, number, size and proximity of neighbours (e.g. Fowler, 1982; Silvertown and Wilson, 2000; Stoll and Weiner, 2000; Sanderson and Elwinger, 2002).

Morphogenetic processes show plasticity in response to environmental signals such as light and nitrogen supply. These responses are often mediated by neighbours (Ballaré *et al.*, 1987; Casal and Smith, 1989; Casal *et al.*, 1990) and vary among tiller cohorts. The competitive ability of annual C_3 species may be enhanced by nitrogen fertilization (Anten and Hirose, 1998; Nurjaya and Tow, 2001). Concomitantly, tillers of higher hierarchy sometimes constrain the response of subordinate tillers because of the preferential carbon and nitrogen allocation to the main shoot meristems, and this is to the detriment of the growth of subordinate tillers (Lemaire and Millard, 1999). As a consequence, tillering is decreased or inhibited under shaded conditions (Casal *et al.*, 1985, 1986; Deregibus *et al.*, 1985; Gautier *et al.*, 1999; Lemaire and Millard, 1999). These morphological and plastic responses play

an important role in determining the competitive ability of the individual within the canopy (Tremmel and Bazzaz, 1993; Lemaire and Millard, 1999).

The objective of the present work was to describe the morphogenetic mechanisms involved in the competitive interactions between annual and perennial C_3 grass species, when nitrogen fertilization practices were carried out during the critical establishment phase of the mixture. These mechanisms are determinants of the initial space occupation and thus condition the future floristic composition of the pasture. We analysed the morphogenesis and leaf and tiller dynamics of three tiller cohorts of individual plants (target) of annual and perennial species flanked in the sowing row by two individual plants (neighbours) of annual and perennial species. The pasture sown is a common mix of grass species used in humid temperate pastoral systems in which the annual grass selected showed higher growth rates during the period of lower temperatures. The annual grass supplemented by nitrogen application will provide valuable green herbage at the end of winter, which is a period of forage shortage. The experiment represents a common situation during undisturbed sward establishment, preceding herbage allowance compatible with the first grazing. We hypothesized that an annual grass neighbour will constrain initial space occupation and the establishment of other species and that owing to their higher LER and light capture, annual neighbours will reduce LAR but increase LER of target plants. We also hypothesized that the magnitude of these effects will be enhanced by nitrogen application and will be greater in the older tillers than in younger ones.

Materials and methods

Environmental conditions

The experiment was carried out in the experimental field of the School of Agriculture, University of Buenos Aires, Argentina (FAUBA; 34°35S, 58°29W). Chemical analysis of the top soil (0–20 cm) indicated a pH (1:2·5) of 5·85, electric conductivity of 0·20 dS m^{-1}, carbon content (Walkley Black) of 12·9 mg g^{-1}, nitrogen content (Kjeldhal) of 1·5 mg g^{-1} and nitrate content (SNEDD) of 1·86 mg kg^{-1}. Extractable phosphorus content (Bray and Kurtz 1) showed a patchy spatial distribution in the top 20 cm, ranging between 5·26 and 10·93 mg kg^{-1}. The experimental period, which lasted from sowing on 27 May 2008 to the last recording on 13 September 2008, was 109 d. The water-deficit stress, which occurred in autumn of 2008, was the cause of late sowing in that year. Data obtained at the FAUBA meteorological station showed that daily temperature was 11·8 ± 2·78°C (mean

minimum and maximum daily temperatures of 6·4 and 18·9°C respectively) during the experimental period (Figure 1). The lowest air temperatures occurred from 25 July to 8 August, after which they showed a slowly increasing trend. Rainfall during the experimental period was 142 mm, 35% lower than the 10-year long-term average. Irrigation was applied to avoid a water deficit estimated as 11 mm, considering a potential and a real evapotranspiration of 128 and 117 mm respectively (Climatology and Phenology, School of Agriculture, FAUBA). Day length increased from a minimum of 9·66 h of light (h d^{-1}) in mid-June to a maximum of 11·79 h d^{-1} at the end of the experimental period (data from 35°S). At harvest time, photosynthetically active radiation (PAR) was measured just above and inside the canopy at soil level at 12:00 h., using a 50-cm-length aluminium data logger bar radiometer [photosynthetic photon flux (PPF) = μMOL m^{-2} s^{-1}] (further details given in Results section). At this time, sward height varied between 20 and 35 cm from ground level.

Plant material and experimental design

Twenty-four experimental units were sown as mini-swards of five alternated rows 0·18 m long, with 0·175-m row spacing (approximately 0·16 m^2). Three rows corresponded to grasses, *B. willdenowii* cv. Fortín Pergamino (BW = prairie grass; annual/biennial species) and *D. glomerata* cv. Porto (DG = cocksfoot/orchardgrass; perennial), and two rows corresponded to *Trifolium pratense* cv. Redgold (TP = red clover), to simulate a typical pasture composition. Prairie grass is native to South American temperate plains (Maddaloni and Ferrari, 2001). It is an annual and biennial species (Scheneiter and Rimieri, 2001), commonly distributed in the Humid Pampa region, where it
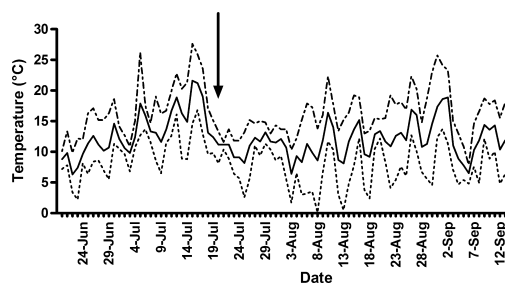
Figure 1 Daily mean (solid line) and minimum and maximum air temperatures (dotted lines) (°C) recorded between seedling emergence and final harvest. The arrow indicates the day of fertilization (19 July). Temperature data were obtained at the meteorological station of Facultad de Agronomía, Universidad de Buenos Aires (FAUBA), Buenos Aires, Argentina.

behaves more like an annual because of the net loss of tillers in summer as it did not produce replacement tillers after the reproductive period (Scheneiter and Rosso, 2005). The mixed pastures sown with cocksfoot are well distributed in the Humid Pampa. We chose prairie grass because of its higher forage provision in the period of lower temperatures compared with the perennial C₃ grass.

In each mini-sward, every grass row was sown with a combination of annual (BW) and perennial species (DG). This species combination corresponded to four sowing arrays of intra- and interspecific competition (BW flanked by BW denoted as BW Bw; DG flanked by DG denoted as DG Dg; BW flanked by DG denoted as BW Dg; and DG flanked by BW denoted as DG Bw; Figure 2).

After emergence, the seedling density was reduced manually to obtain approximately 200 grass seedlings per m^2, with 1·5 cm between plants. This grass seedling distance was used because this spacing is usually attained in Argentine pastures at 2 months after sowing (Gatti *et al.*, 2011). The four sowing–design grass combinations (two target species × two neighbour species) and two levels of fertilization (yes/no) (definition of target plants is given below in section headed Data collection) formed a 2 × 2 × 2 factorial array in a complete randomized block design with three replicates. The blocks controlled two variation sources, topographic position and initial soil P content. To homogenize and enhance P level to 20 mg kg^{-1}, fertilization was carried out by applying between 42 and 68 kg P ha^{-1} in blocks with highest and lowest initial soil P respectively.

On 19 July, during winter, 53 d after sowing and 29 d after emergence, urea fertilizer was applied at a rate of 170 kg N ha^{-1}, an application rate that usually allows a linear response in most C₃ grass species of the Humid Pampa (Fernández Grecco *et al.*, 1995). At this time, plants had one to three tillers per plant and five to six leaves on the main stem (see subsection *Species differences* in the Results section), a size corresponding to the beginning of the plant establishment phase (Ries and Svejcar, 1991; Sanderson *et al.*, 2002).

Data collection

Data were collected after fertilization on the central grass row of the mini-sward to avoid border effects from external grass rows, in a sequence of three central individual plants. The target plant, on which measurements were taken, is the one marked and flanked by two grass neighbours. We did not expect a border effect of the rows with legumes owing to their very slow initial growth and plant biomass in the experimental period with a low mean daily temperature of
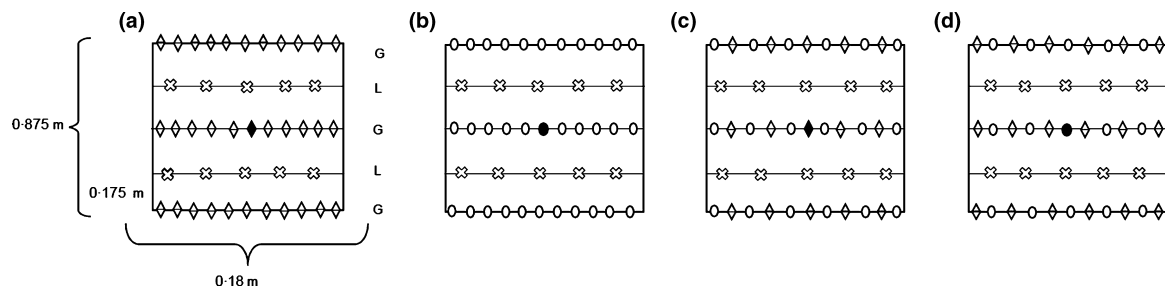
Figure 2 Diagram of the four mini-sward arrangements: (a) BW flanked by BW (BW Bw), (b) DG flanked by DG (DG Dg), (c) BW flanked by DG (BW Dg) and (d) DG flanked by BW (DG Bw). Black symbols identify the target plant: ♦ BW and •
DG; × TP; G grass rows and L legume rows.

$11\cdot8 \pm 2\cdot78°C$ (Figure 1). Tiller-appearance cohorts were marked with plastic rings and recorded weekly. The leaf dynamics were monitored weekly on three of these tiller cohorts: the main tiller and those that appeared 20 and 30 days after fertilization (20-daf and 30-daf tillers respectively). Data recording ended at the beginning of the reproductive period of the prairie grass (13 September; 85 d after emergence and 56 daf), because it is considered that competition for resources is directly linked to the vegetative development of plants (Lemaire and Millard, 1999). Study variables were length and date of appearance of each leaf (tip of visible leaf), beginning of ageing (decrease in green lamina length owing to leaf senescence) and death of successive leaves (senescence initiation on leaf tips). Tips of new leaves were cut off to identify those already recorded (Duru and Ducrocq, 2000). Between eight and four measurement intervals were studied for the leaves, according to the tiller age cohort.

Estimation of morphogenetic variables

Leaf appearance rate (LAR = leaves tiller^{-1} per day), which is defined as the number of leaves that appeared per unit of time, was calculated from the slope of the linear regression between the total number of leaves appeared and time (Hume, 1991). Phyllochron [growing degree days (GDD) per leaf], the thermal time until a leaf appears across the sheath of the previous leaf, is used to describe and understand the development of grasses (Wilhelm and McMaster, 1995). This parameter was calculated as the inverse of the slope of the linear regression between number of leaves and thermal time (Fustec *et al.*, 2005), with a basal temperature of 4°C (Lemaire and Agnusdei, 2000). Leaf lifespan (LLS = GDD per leaf) was estimated as the number of growing days from the beginning of leaf emergence to the beginning of leaf death. As several leaves expanded simultaneously within each tiller, individual leaf elon-

gation rates were integrated for each tiller. Gross leaf elongation rate (^{gross}LER = mm tiller^{-1} per day) and leaf senescence rate (LSR = mm tiller^{-1} per day) per tiller were estimated as the daily average of the sum of positive or negative differences, respectively, of green leaf length between successive measurements. Net elongation rate (^{net}LER = mm tiller^{-1} per day) was calculated as the difference between ^{gross}LER and LSR; the rates were expressed as millimetre per tiller per day (Gastal *et al.*, 1992; Mazzanti *et al.*, 1994; Torres *et al.*, 2000; Virkajärvi and Järvenranta, 2001; Agnusdei *et al.*, 2007; Berone *et al.*, 2007a,b, 2008). Tiller appearance rate (TAR = tillers plant^{-1} per day) was calculated from the slope of the linear regression between total number of tillers and time (Hume, 1991). Relative tiller appearance rate (RTR = tillers tiller^{-1} per day) was estimated as the ratio of average TAR to the number of tillers on the previous date (Hirata and Pakiding, 2002; Bultynck *et al.*, 2004; Berone *et al.*, 2008). Site filling (F_S = tillers leaf^{-1}) was calculated as the slope of the linear regression between logarithms of the number of tillers per plant and the number of leaves appeared on the main shoot (Davies, 1974; Neuteboom and Lantinga, 1989; Bahmani *et al.*, 2000).

Statistical analysis

Factorial analyses of variance with a significance level of 5% were performed following a randomized complete block design with three factors: target species (two levels) × neighbour species (two levels) × fertilization (two levels) (*n* = 3; 24 mini-swards). All traits were assessed for normality using the Shapiro–Wilk test. The differences between tiller cohorts were analysed with a split-plot array (three levels). As there were usually interactions involving tiller cohorts, the analysis was split for each cohort; partitions by factor were carried out when other interactions existed. Info-Stat Professional Package version 1.1 (Universidad

Nacional de Córdoba, Argentina) was used to perform statistical analyses.

Results

Microcanopy photosynthetically active radiation (PAR) interception

At harvest time, PAR interception was significantly affected by the identity of the neighbour (species × neighbour interaction, $P = 0.013$) and nitrogen fertilization ($P = 0.042$). DG PAR interception was similar, irrespective of neighbour identity (92·8%) and fertilization. BW PAR interception was 9% higher when it was flanked by a neighbour of the same species (92·7% when the neighbour was BW vs. 85·3% when the neighbour was DG) and 7% higher when it was fertilized (85·7% N⁻ vs. 92·2% N⁺).

Number of leaves on the main stem and tillers per plant at N-fertilization time

At N fertilization, 53 d after sowing and 29 d after emergence (dae), BW had 81% more tillers than DG

(BW: 3·17 vs. DG: 1·75; $P = 0.0002$) and 21% more leaves on the main stem (BW: 6·25 vs. DG: 5·17; $P = 0.001$) (data not shown). There was no significant effect of neighbour identity on leaf or number of tillers per plant ($P = 0.39$ and $P = 0.79$ respectively).

### Morphogenetic variables

Phyllochron and leaf appearance rate

During the post-fertilization period, the main tiller of target plants of both grass species showed short and similar phyllochrons (56·9 and 60·5 GDD leaf⁻¹ respectively) ($P = 0.29$; Table 1; Figure 3a). Phyllochron at the main tiller was not affected by fertilization ($P = 0.77$) but increased as a consequence of BW vicinity (62·5 and 54·9 GDD leaf⁻¹ when the neighbour was BW and DG respectively; $P = 0.03$). The successive tiller cohorts showed increased phyllochron, this trend being more marked in *D. glomerata* (from 56·9 to 108·9 GDD per leaf) than in *B. willdenowii* (from 60·5 to 86·2 GDD per leaf). There was no effect of neighbour species in the 30-daf tiller cohort ($P = 0.84$). Fertilization did not affect phyllochron ($P \geq 0.50$).

Table 1 Results of analysis of variance comparing the effects of species [two levels: *Dactylis glomerata* (DG) and *Bromus willdenowii* (BW)], neighbours (two levels: DG and BW), winter fertilization (two levels: with and without nitrogen) and their interactions, upon morphogenetic leaf variables ($n = 3$).

Variable	Unit of growth (tiller cohort)	Species effect Actual values and P		Neighbour effect Actual values and P		Fertilization effect Actual values and P		Interaction Type and P	References
Phyllochron (GDD leaf⁻¹)	Main stem	DG = 56·9	ns	DG = 54·9	*	N⁻ = 59·2	ns	ns	Figure 3a
		BW = 60·5		BW = 62·5		N⁺ = 58·2			
	20-daf tiller cohort	DG = 83·3	ns	DG = 71·2	*	N⁻ = 79·0	ns	ns	
		BW = 76·5		BW = 88·6		N⁺ = 80·8			
	30-daf tiller cohort	DG = 108·9	ns	DG = 98·8	ns	N⁻ = 101·9	ns	ns	
		BW = 86·2		BW = 96·2		N⁺ = 93·2			
LAR (leaves tiller⁻¹ d⁻¹)	Main stem	DG = 0·14	ns	DG = 0·14	*	N⁻ = 0·13	ns	ns	Figure 3b
		BW = 0·13		BW = 0·13		N⁺ = 0·14			
	20-daf tiller cohort	DG = 0·16	ns	DG = 0·18	**	N⁻ = 0·16	ns	ns	
		BW = 0·17		BW = 0·15		N⁺ = 0·16			
	30-daf tiller cohort	DG = 0·13	ns	DG = 0·14	ns	N⁻ = 0·15	ns	ns	
		BW = 0·18		BW = 0·16		N⁺ = 0·15			
LLS (GDD leaf⁻¹)	Main stem	DG = 477·7	**	DG = 425·7	ns	N⁻ = 412·2	ns	ns	Figure 3c
		BW = 337·7		BW = 389·8		N⁺ = 403·3			
	20-daf tiller cohort	DG = 316·8	*	DG = 326·9	**	N⁻ = 279·8	ns	sp*neig*	
		BW = 270·2		BW = 260·0		N⁺ = 307·2			
	30-daf tiller cohort	DG = 260·8	*	DG = 260·8	*	N⁻ = 232·1	ns	ns	
		BW = 229·0		BW = 229·0		N⁺ = 257·7			

ns, non-significant differences. GDD, growing degree days; LAR, leaf appearance rate; LLS, leaf lifespan. Results presented are means and P values: **$P < 0.01$; *$P < 0.05$.
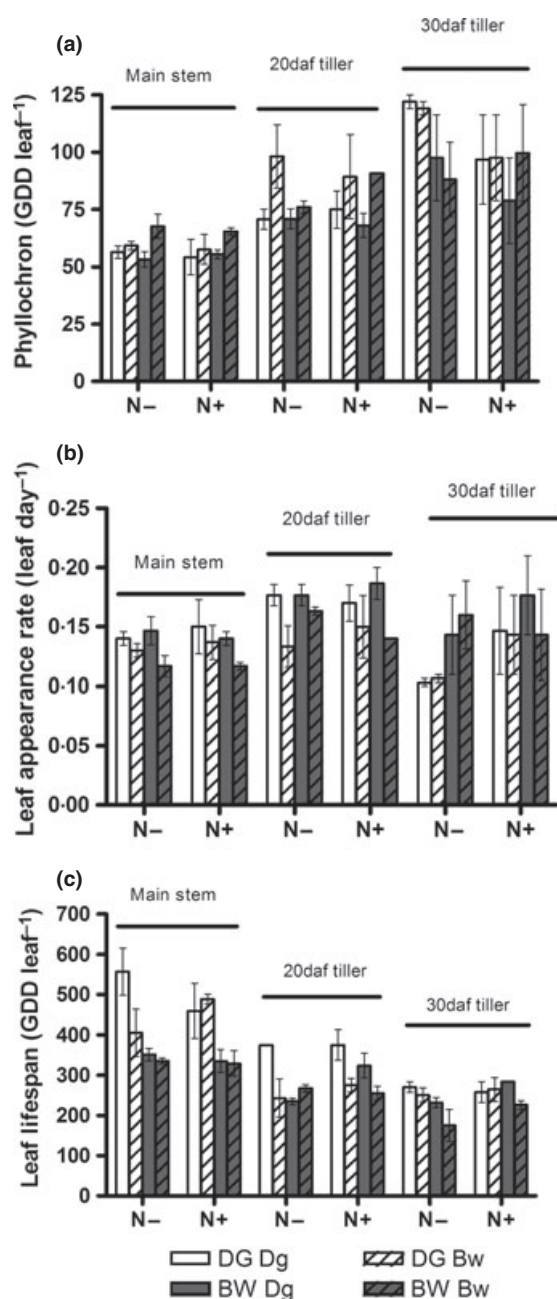
Figure 3 (a) Phyllochron (growing degree days (GDD) leaf^{-1}), (b) leaf appearance rate (LAR = leaf d^{-1}) and (c) leaf lifespan (LLS = GDD leaf^{-1}) evaluated in four different situations of intra- and interspecific competition of the perennial *Dactylis glomerata* (DG) and the annual *Bromus willdenowii* (BW) in non-fertilized (N^{-}) and fertilized (N^{+}) treatments. Data are means ± standard error of three tiller age cohorts (main stem and marked tiller cohorts appeared 20 and 30 days after fertilization = daf). First abbreviation: name of target species; second abbreviation: name of neighbour species (e.g. DG Dg).

The LAR was similar in both species, not affected by nitrogen addition ($P > 0.77$) and higher when the neighbour was *D. glomerata* than when the neighbour was BW. Again, neighbour effects were recorded in older tillers, but not in the 30-daf tiller. Every day, 0·14 leaves appeared on the main tiller when the neighbour was DG and 0·13 when the neighbour was BW and 0·18 and 0·15 leaves per day, respectively, on the 20-daf tiller ($P = 0.04$ and 0·009 respectively; Table 1; Figure 3b). LAR did not decrease in the younger tillers, in contrast with the increase in phyllochron reported above (Figure 3a).

Leaf lifespan

Leaf lifespan of *D. glomerata* was longer than that of the annual BW species (main stem: DG: 477·7 vs. BW: 337·7 GDD per leaf; $P = 0.0004$) and decreased in the younger tillers as compared with the main tiller. No effects of fertilization were shown ($P = 0.77$). Twenty-daf tillers showed a species × neighbour interaction ($P = 0.025$; Table 1); *D. glomerata* LLS was 31% lower when plants were flanked by BW plants than when they were flanked by *D. glomerata* ($P = 0.018$), whereas BW was not significantly affected by the neighbour identity ($P = 0.39$). The LLS of 30-daf tillers of both species decreased by 12% when the neighbour was BW ($P = 0.047$; Figure 3c).

Leaf elongation rate per tiller

In all tiller cohorts, the gross leaf elongation rate at til-ler level, ^{gross}LER (the daily increase in total length of all growing leaves per tiller), of BW was 70–72% greater than that of *D. glomerata* ($P \leq 0.0007$). Fertil-ization increased ^{gross}LER by 32% in the main stem and in the 20-daf tillers ($P \leq 0.0071$), but not in the youngest one ($P = 0.79$). The identity of the neigh-bouring plants did not affect the ^{gross}LER of any tiller cohort ($P > 0.85$; Table 2; Figure 4a).

Leaf senescence rate was lower than ^{gross}LER and greater in BW than in *D. glomerata* ($P < 0.0001$). Also, BW showed an increasing trend through 75–162% for the main stem and the 20-daf tiller respectively. LSR did not show significant effects of fertilization ($P > 0.08$) or neighbour identity in any tiller cohort ($P > 0.45$; Table 2; Figure 4b). As a consequence, ^{net}LER was greater in BW than in DG ($P < 0.0004$), increased from the older to the younger tillers (68–95%) and increased ≈ 35% in the fertilized treatment ($P > 0.004$). These nitrogen responses were significant in the main stem ($P = 0.0003$) and in the 20-daf tillers ($P = 0.032$), but not in the 30-daf tillers ($P = 0.10$; Table 2; Figure 4c).

Table 2 Results of analysis of variance comparing the effects of species [two levels: *Dactylis glomerata* (DG) and *Bromus willden-owii* (BW)], DG and BW), neighbours (two levels: DG and BW), winter fertilization (two levels: with and without nitrogen) and their interactions, upon morphogenetic tiller and plant variables ($n = 3$).

Variable	Unit of growth (tiller cohort; plant)	Species effect Actual values and P		Neighbour effect Actual values and P		Fertilization effect Actual values and P		Interaction Type and P	References
^{Gross}LER (leaf mm tiller^{-1} d^{-1})	Main stem	DG = 14·9 BW = 25·4	**	DG = 20·3 BW = 19·9	ns	N$^-$ = 17·3 N$^+$ = 22·9	**	ns	Figure 4a
	20-daf tiller cohort	DG = 13·9 BW = 23·8	**	DG = 18·9 BW = 18·8	ns	N$^-$ = 16·3 N$^+$ = 21·5	**	ns	
	30-daf tiller cohort	DG = 11·6 BW = 19·9	**	DG = 15·9 BW = 15·7	ns	N$^-$ = 15·6 N$^+$ = 16·7	ns	ns	
LSR (leaf mm tiller^{-1} d^{-1})	Main stem	DG = −5·6 BW = −9·8	**	DG = −7·4 BW = −7·9	ns	N$^-$ = −8·3 N$^+$ = −7·0	ns	ns	Figure 4b
	20-daf tiller cohort	DG = −3·1 BW = −8·1	**	DG = −5·2 BW = −5·9	ns	N$^-$ = −5·5 N$^+$ = −5·7	ns	ns	
	30-daf tiller cohort	DG = −1·9 BW = −2·4	ns	DG = −2·0 BW = −2·3	ns	N$^-$ = −2·5 N$^+$ = −1·8	ns	ns	
^{Net}LER (leaf mm tiller^{-1} d^{-1})	Main stem	DG = 9·3 BW = 15·6	**	DG = 12·9 BW = 11·9	ns	N$^-$ = 10·2 N$^+$ = 14·6	**	ns	Figure 4c
	20-daf tiller cohort	DG = 9·6 BW = 15·7	**	DG = 12·4 BW = 12·9	ns	N$^-$ = 11·0 N$^+$ = 14·2	*	ns	
	30-daf tiller cohort	DG = 8·8 BW = 17·1	**	DG = 13·7 BW = 12·1	ns	N$^-$ = 11·3 N$^+$ = 14·5	ns	ns	
TAR (tillers plant^{-1} d^{-1})	Entire plant	DG = 0·24 BW = 0·21	ns	DG = 0·27 BW = 0·17	*	N$^-$ = 0·23 N$^+$ = 0·21	ns	ns	Figure 5a
RTR (tiller tiller^{-1} d^{-1})	Entire plant	DG = 0·026 BW = 0·033	**	DG = 0·027 BW = 0·032	**	N$^-$ = 0·030 N$^+$ = 0·029	ns	ns	Figure 5b
F_S (tillers leaf^{-1})	Entire plant	DG = 0·28 BW = 0·20	**	DG = 0·26 BW = 0·21	ns	N$^-$ = 0·25 N$^+$ = 0·22	ns	ns	Figure 5c

ns, non-significant differences; LSR, leaf senescence rate; RTR, relative tiller appearance rate; TAR, tiller appearance rate. Results presented are means and P values: **$P < 0.01$; *$P < 0.05$.

Tiller appearance rate

The TAR was similar in both species ($P = 0.36$) but was 59% higher with *D. glomerata* than with BW neighbour plants (0·27 and 0·17 tillers plant^{-1} per day respectively) ($P < 0.01$; Table 2; Figure 5a). The relative tiller appearance rate (RTR = tillers tiller^{-1} per day) was also higher in *D. glomerata* than in BW ($P = 0.0016$) and higher in the vicinity of *D. glomerata* ($P = 0.006$) than in the vicinity of BW (Table 2; Figure 5b). While TAR showed effects of neighbour identity and RTR showed effects of species and neighbour identity, the rate of site filling (F_s) showed significant differences only between species ($P = 0.008$). F_s was 40% higher in *D. glomerata* than in BW (DG: 0·28 vs. BW: 0·20 tillers per leaf) (Table 2; Figure 5c). Fertilization did not significantly affect TAR, RTR or F_S ($P > 0.25$; Table 2; Figure 5).

Discussion

Species effects on morphogenetic variables in the main stem

Morphogenetic differences related to specific differential growth characteristics were found. The perennial *D. glomerata* had lower leaf elongation rates (^{gross}LER and ^{net}LER = Figure 4a,c) and longer leaf lifespan (LLS = Figure 3c) than the annual BW. However, the two species showed similar leaf appearance (LAR = Figure 3b) and, unexpectedly, similar tiller appearance rate (TAR = Figure 5a).

In this study, which comprised the pasture establishment phase, the LAR of young plants (Figure 3b) of both species was faster than that recorded previously for adult plants (phyllochron of ≈ 60 GDD per leaf in this experiment vs. 91–204 GDD per leaf; e.g.
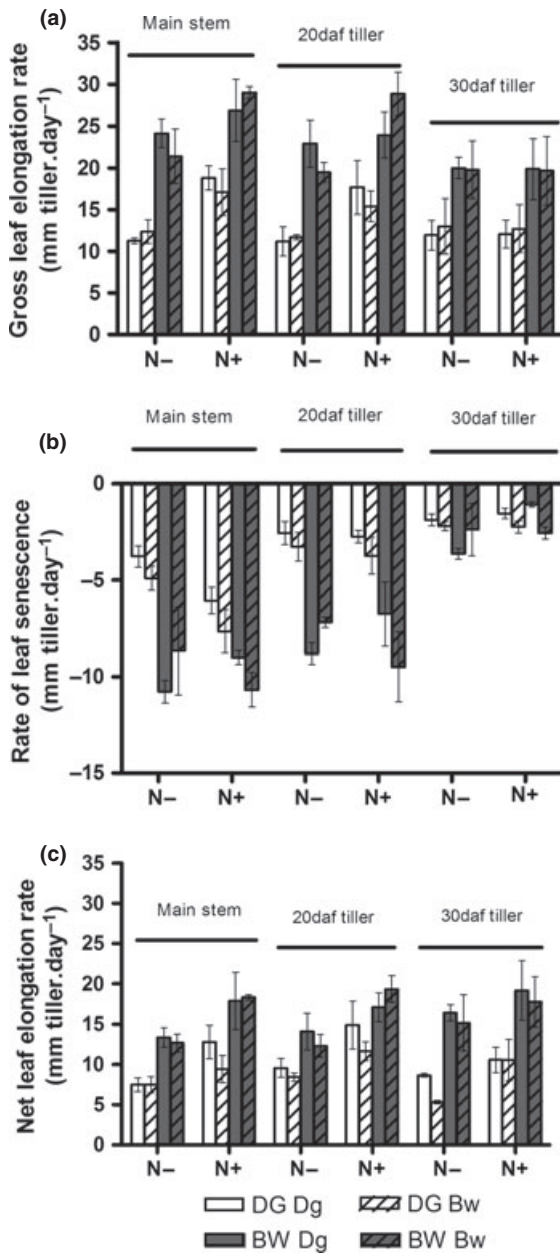
Figure 4 (a) Gross leaf elongation rate (^{gross}LER), (b) leaf senescence rate (LSR) and (c) net leaf elongation rate (^{net}LER) per tiller (mm tiller^{-1} d^{-1}) evaluated in four different situations of intra- and interspecific competition of the perennial *Dactylis glomerata* (DG) and the annual *Bromus willdenowii* (BW) in non-fertilized (N$^-$) and fertilized (N$^+$) treatments. Data are means ± standard error of three tiller age cohorts (main stem and marked tiller cohorts appeared 20 and 30 days after fertilization = daf). First abbreviation: name of target species; second abbreviation: name of neighbour species (e.g. DG Dg).
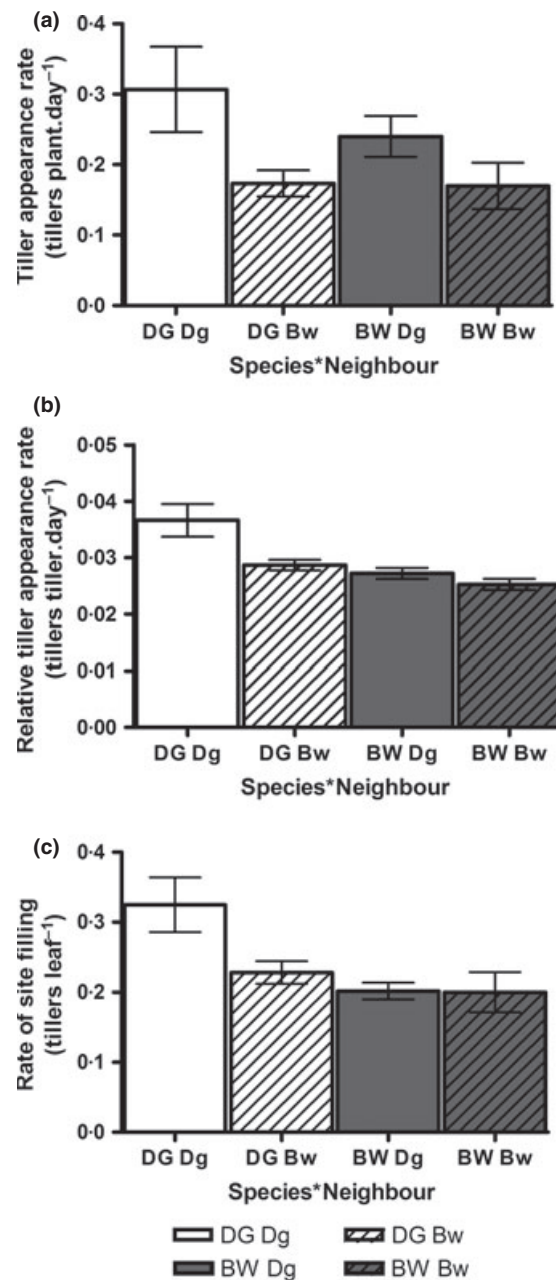
Figure 5 (a) Tiller appearance rate (TAR = tillers plant^{-1} d^{-1}), (b) relative tiller appearance rate (RTR = tillers tiller^{-1} d^{-1}) and (c) rate of site filling (F_S = tillers leaf^{-1}) comparing only the effects of the four different situations of intra- and interspecific competition of the perennial *Dactylis glomerata* (DG) and the annual *Bromus willdenowii* (BW). Fertilization effects ≥ 0·05 (see Table 2 for details). Data are means ± standard error. First abbreviation: name of target species; second abbreviation: name of neighbour species (e.g. DG Dg).

Hume, 1991; Calviere and Duru, 1995; Duru and Ducrocq, 2000; Rawnsley et al., 2002). This pattern is compatible with a fast vertical and horizontal space occupation in young plants, allowing for greater light interception (Hume, 1991).

Vertical space occupation is related to two leaf morphogenetic mechanisms: LAR and LER. As phyllochron (Figure 3a) and LAR (Figure 3b) were similar in both species, only higher ^{net}LER allowed the annual species a better occupation of the space and a more efficient interception of light, probably affecting the vertical profile of light compared with the perennial D. glomerata.

Horizontal space colonization is typically linked to tillering (Lemaire and Millard, 1999). Similar phyllochron and LAR imposed the same upper limit to tillering in both species (Davies, 1974; Bahmani et al., 2000; Matthew et al., 2000; Nelson, 2000). However, tillering depends not only on LAR but also on the rate of site filling (F_S = tiller leaf^{-1}; Figure 5c) (Davies, 1974; Gautier et al., 1999; Bahmani et al., 2000). The higher F_S of the perennial species reflected a significant increase in RTR (Figure 5b), but, unexpectedly, not a significant increase in TAR (Figure 5a) or final tiller density (data not shown). This pattern may have been associated with the fact that BW compensated for its lower F_S with a prior faster leaf area display, leading to a higher initial tiller density at 29 d after emergence (at the time of fertilization) with 81% more tillers in BW. This pattern led to a similar horizontal space occupation (similar final tiller density) but to different tiller dynamics between the two species. These findings agree with those of Gautier et al. (1999), who found that long-leaved populations of Lolium perenne L. produced the first tiller at a lower developmental stage than short-leaved ones. The annual species, therefore, had a better vertical space occupation (higher LER) and a faster horizontal colonization than the perennial D. glomerata.

Several trade-offs were detected among morphogenetic traits. Interspecific differences in the morphogenetic variables ^{gross}LER and ^{net}LER (Figure 4a,c) and LLS (Figure 3c) were the main determinants of a higher F_S in the perennial than in the annual life form. In the present study, we found a negative relationship between LER (or leaf size) and F_S. DG showed lower ^{net}LER and higher F_S than BW (Figures 4c and 5c), which confirmed previous findings (Hume, 1991; Bahmani et al., 2000; Nelson, 2000). This negative LER-FS correlation might be due to a negative correlation between leaf and tiller growth and/or to a light-mediated effect on tiller bud initiation and, hence, on F_S. Both correlations may reflect the existence of some competition between daughter tillers and adjacent leaf meristems for available carbon (C) (Bahmani et al.,

2000). We also found a positive relationship between LLS and F_S, both of which were higher in the perennial than in the annual species (Figures 3c and 5c). This pattern is probably mediated by the negative correlation of both variables with LER and was also related to (i) the overlapping of periods between leaf production and loss of and reduction in CO$_2$ fixation rate and (ii) the increase in nutrient residence time and leaf-area persistence within the plant (Chabot and Hicks, 1982; Aerts, 1995; Reich et al., 1999; Mediavilla and Escudero, 2003; Navas et al., 2003) in the perennial as compared to the annual species. So, the perennial D. glomerata, with longer LLS than the annual BW, would have less available C and greater potential priority of that C to satisfy the demand of axillary buds (higher F_S), but lower for tiller growth, that is, lower LER. These patterns are in agreement with those found in different Lolium species. The perennial L. perenne has an allocation priority to daughter tillers during vegetative growth (Robson et al., 1988), whereas the annual Lolium temulentum shows meristematic activity concentrated in the terminal buds (Ryle, 1970). In this experiment, there was a negative genotypic relationship between LER and F_S and between LLS and LER and a positive relationship between LLS and F_S, during the early and late sward establishment phases.

Neighbour effect on morphogenetic variables in the main stem

Neighbour species-specific traits, not just neighbour plant biomass, determined the target plant performance because different neighbour species imply different suites of traits and different competitive abilities (Tremmel and Bazzaz, 1993). When the annual BW was the neighbour species, it had greater ^{gross}LER and ^{net}LER (Figure 4a,c) and hence larger tiller size (data not shown), which induced changes in morphogenetic variables like LAR, TAR and RTR in both species.

Previous studies have shown that annual neighbours affect the ontogeny, morphology and vertical space occupation of target plants through an increase in phyllochron (Bos and Neuteboom, 1998; Gautier et al., 1999; Lemaire and Millard, 1999; Nelson, 2000) (Figure 3a) and a decrease in LAR (Figure 3b), probably due to light changes (Gautier et al., 1999; Bahmani et al., 2000; Lemaire, 2001). LAR plays a strategic role (Lemaire, 2001) because it is the key variable in morphogenesis, having direct influence on other components of plant and sward structure (Lemaire and Agnusdei, 2000). As LAR controls the production of tiller buds (Davies, 1974; Bahmani et al., 2000; Matthew et al., 2000; Nelson, 2000), a BW neighbour resulted in fewer sites for tillering, thus decreasing both TAR (Figure 5a) and RTR (Figure 5b).

In spite of the proposed effect on light, neighbour identity did not affect ^gross^LER and ^net^LER (Figure 4a, c) of target plants. There are two possible explanations related to competition for light and/or plant age. The first explanation is that during the first 85 d after plant emergence, competition for light did not reach extreme intensities because canopy light interception was ≤93%. Decreasing LAR was the only mechanism of tillering regulation checked. In the present work, we were unable to corroborate any of the following three stages of the competition processes for light: F_s reduction, a true indicator of plasticity (as site usage: Huber *et al.*, 1999), plant photomorphogenetic responses and the self-thinning law (e.g. Skinner and Nelson, 1992; Lemaire and Millard, 1999; Nelson, 2000; Lemaire, 2001). The second mechanism explaining the lack of neighbour effects on leaf growth is related to plant age. The plant age and/or the phenological stage might modify the expression of the shade avoidance response, which generally involves both an enhancement of leaf growth and a suppression of tiller development (Mónaco and Briske, 2000). Young, rapidly growing plants of *Schizachyrium scoparium* (≈60 d old) had enhanced leaf growth in response to the low red/far red ratio but did not suppress tiller initiation, whereas older plants (≈120 d old) suppressed tiller initiation but did not enhance leaf growth. Our results in C_3 plants of intermediate age (85 d old) were in accordance with those of the older plants.

Therefore, during pasture establishment, LAR, TAR and RTR control leaf and tillering dynamics and vertical and horizontal space colonization by a light-mediated effect exerted by an annual neighbour of tall architecture. However, the annual neighbour did not modify the ^gross^LER, ^net^LER (Figure 3a,c) or species F_s (Figure 4c), probably because in the experimental period it was not possible to fulfil all the stages of the processes of competition for light and/or because age may have changed plant responses to shading.

Fertilization effect on morphogenetic variables in the main stem

As expected, nitrogen fertilization increased both ^gross^LER and ^net^LER (Figure 4a,c) in the main tiller but did not affect LAR (Figure 3b), TAR (Figure 5a), leaf senescence rate (LSR = Figure 4b) or LLS (Figure 3c). The effect of fertilization would have resulted in an immediate increase in the cell number of the tillers present at the moment of fertilization, that is, the older tillers (Volontec and Nelson, 1983; Gastal and Durand, 2000), without residual effects on the subsequent tiller cohort. The lack of LAR response to fertilization in the perennial grass species is in accordance with the results of Cruz and

Boval (2000), but not with those of Lattanzi *et al.* (1996) in the annual *Lolium multiflorum* cv. Grasslands Tama, who found phyllochron decreases with nitrogen addition.

Variation of species, neighbour identity and fertilization effects on different tiller cohorts

All the leaf-level morphogenetic variables differed between the three tiller cohorts. Phyllochron (Figure 3a) increased with the appearance of successive cohorts because of leaf area expansion and canopy closure, especially in the cases of annual neighbour plants (Table 1). Hume (1991) also found higher phyllochron in BW new tillers of swards with high herbage mass. Despite the increase in phyllochron towards the younger tillers, the lack of changes in LAR may be due to the increase in air temperatures following fertilization. The mean air temperature increased from 12·5 to 12·9°C in the study period (Figures 1 and 3b). Changes in the day length might not have affected the morphogenetic traits because there was only 1 h of difference between dates of appearance of the three tiller cohorts evaluated [between 19 July (9·66 h d^{-1}) and 23 August (10·68 h d^{-1})]. Sato (1980) found leaves of greater length but lower tiller density per plant when day length was 14 h d^{-1} compared with 9 h d^{-1} (Figure 3a,c).

In spite of the reported increase in phyllochron, LLS decreased from the older to the younger tillers (Figure 3c), contrary to the expected positive relationship between phyllochron and LLS (Duru and Ducrocq, 2000). The inverse relationship between phyllochron and LLS may be due to an ontogenic delay and an early senescence of leaves of the younger tillers, subjected to more limiting light signal. The timing of senescence is accelerated owing to a decrease in the red/far red ratio (Rousseaux *et al.*, 2000; Wingler *et al.*, 2006), which may be considered an early warning system (Nelson, 2000) lead by an annual neighbour initially on the plants of DG (Figure 3c; Table 1). These changes occur long before the decrease in PAR interception of the canopy and intense light competition begin (Casal *et al.*, 1986; Ballaré *et al.*, 1987; Gautier *et al.*, 1999). Although PAR interception of the canopy was about 93% of incident light, the growth of younger subordinated tillers occurred upon the perception of a greater decrease in the light quality owing to their basal position, as shown by their lower LLS. Such shortening of leaf longevity of the younger tiller cohort may be due to the trade-off between allocating nitrogen to maintain the photosynthetic activity of existing leaves or to build new leaves located in better illuminated strata, in order to optimize the photosynthetic capacity and the carbon balance of the whole

plant (Chabot and Hicks, 1982; Gastal and Lemaire, 2002; Wingler *et al.*, 2006). In the same sense, nitrogen effects on ^{gross}LER and ^{net}LER (Figure 4a,c) were evident only in the older tillers, main stem and 20-daf tiller cohort. Added nitrogen may have been incorporated into better-illuminated plant tissues, being unavailable to the 30-daf tiller cohort. Both changes in allocation patterns, with tiller age, correspond to a change from a predominantly horizontal to a more vertical strategy of growth (Lemaire and Millard, 1999).

Conclusions

Management decisions concerning life forms, species and/or cultivar selection, and sowing design and densities define the early competitive relationships between pasture components in the row, resulting in differences in pasture structure and floristic composition. In this work, annual neighbour plants, with greater LER, caused a delay in LAR in the older tillers. This delay limited the potential number of tillers and TAR of plants by limiting light arrival at the base of the canopy. Also, they diminished the LLS of younger subordinated tillers that were growing under conditions of reduced light quality owing to their basal position. Positive nitrogen fertilization effects on LER were evident in both species, but only in those tillers that were growing actively at the time of fertilization.

This early and irreversible damage imposed by annual neighbour plants on LAR and TAR, both associated with leaf and tiller dynamics, limited horizontal and vertical space occupation during the critical establishment phase. Persistence of perennial C₃ grass is of more concern than that of annual C₃ grasses because it can severely constrain pasture stability. Annual species can persist in pastures by re-establishment from seeds; in subsequent years, grazing management practices might affect re-establishment of prairie grass. The shortening of leaf longevity (LLS reduction) of the younger tiller cohorts by an annual neighbour and the increase in LER, evident only in the older tillers present around fertilization time, were two mechanisms associated with the optimization of plant nitrogen use and carbon balance and with a change to a more vertical strategy of growth, usually seen under restricted light.

These results reinforce the advantage of sowing, at low seeding densities, annual grass species to compensate for the irreversible negative effects of annual neighbour competition on perennial grass establishment, which is the component of the pastures that guarantees a lower primary production variability. Otherwise, a better space colonization by grass plants may restrict the spread of weeds. This work allowed us to clarify some morphogenetic mechanisms involved in early competitive interactions between annual and perennial grasses in the sowing rows of temperate pastures.

Acknowledgments

References

AERTS R. (1995) The advantage of being evergreen. *Trends in Ecology and Evolution*, **10**, 402–407.

AGNUSDEI M.G., ASSUERO S.G., FERNÁNDEZ GRECO R.C., CORDERO J.J. and BURGHI V.H. (2007) Influence of sward condition on leaf tissue turnover in tall fescue and tall wheatgrass swards under continuous grazing. *Grass and Forage Science*, **62**, 55–65.

ANTEN N.P.R. and HIROSE T. (1998) Biomass allocation and light partitioning among dominant and subordinate individuals of *Xantium canadense* stands. *Annals of Botany*, **82**, 665–673.

BAHMANI I., HAZARD L., VARLET-GRANCHER C., BETIN M., LEMAIRE G., MATTHEW C. and THOM E.R. (2000) Differences in tillering of long and short leaved perennial ryegrass genetic lines under full light and shade treatments. *Crop Science*, **40**, 1095–1102.

BALLARÉ C.L., SÁNCHEZ R.A., SCOPEL A.L., CASAL J.J. and GHERSA C.M. (1987) Early detection of neighbour plants by phytochrome perception of spectral changes in reflected sunlight. *Plant Cell and Environment*, **10**, 551–557.

BELANGER G. (1996) Morphogenetic and structural characteristics of field-grown timothy cultivars differing in maturity. *Canadian Journal of Plant Science*, **76**, 277–282.

BERONE G.D., LATTANZI F.A., COLABELLI M. and AGNUSDEI M. (2007a) Análisis del crecimiento por macollo en dos cultivares de *Bromus stamineus* y dos de *Lolium perenne*. [Tiller growth analysis in two cultivars of *Bromus stamineus* and two of *Lolium perenne*.]. *Revista Argentina de Producción Animal*, **27**, 203–204.

BERONE G.D., LATTANZI F.A., COLABELLI M. and AGNUSDEI M. (2007b) A comparative analysis of temperature response of leaf elongation in *Bromus stamineus* and *Lolium perenne* plants in the field. Intrinsic and size-mediated effects. *Annals of Botany*, **100**, 813–820.

BERONE G.D., LATTANZI F.A., AGNUSDEI M.G. and BERTOLOTTI N. (2008) Growth of individual tillers and tillering rate of *Lolium perenne* and *Bromus stamineus* subjected to two defoliation frequencies in winter in Argentina. *Grass and Forage Science*, **63**, 504–512.

BOS H.J. and NEUTEBOOM J.H. (1998) Morphological analysis of leaf and tiller number dynamics of wheat (*Triticum aestivum* L.): responses to temperature and light intensity. *Annals of Botany*, **81**, 131–139.

BULTYNCK L., TER STEEGE M.W., SCHORTEMEYER M., POOT P. and LAMBERS H. (2004) From individual leaf elongation to whole shoot leaf area expansion: a comparison of three *Aegilops* and two *Triticum* species. *Annals of Botany*, **94**, 99–108.

CALVIERE I. and DURU M. (1995) Leaf appearance and senescence patterns of some pasture species. *Grass and Forage Science*, **50**, 447–451.

CASAL J.J. and SMITH H. (1989) The function, action and adaptative significance of phytochrome in light-grown plants. *Plant, Cell and Environment*, **12**, 855–862.

CASAL J.J., DEREGIBUS V.A. and SÁNCHEZ R.A. (1985) Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam. vegetative and reproductive plants as affected by differences in R/FR irradiation. *Annals of Botany*, **56**, 553–559.

CASAL J.J., SÁNCHEZ R.A. and DEREGIBUS V.A. (1986) The effect of plant density on tillering: the involvement of red/far red ratio and the proportion of radiation intercepted per plant. *Environmental and Experimental Botany*, **26**, 365–371.

CASAL J.J., SÁNCHEZ R.A. and GIBSON D. (1990) The significance of changes in the red/far red ratio, associated with either neighbour plants or twilight, for tillering in *Lolium multiflorum* Lam. *New Phytologist*, **116**, 565–572.

CHABOT B.F. and HICKS D.J. (1982) The ecology of leaf life spans. *Annual Review of Ecology and Systematics*, **13**, 229–259.

CRUZ P. and BOVAL M. (2000) Effect of nitrogen of some morphogenetic traits of temperate and tropical perennial forage grasses. In: Lemaire G., Hodgson J., de Moraes A., Nabinger C., de F. and Carvalho P.C. (eds) *Grassland ecophysiology and grazing ecology*, pp. 151–168. Wallingford, UK: CAB International.

DAVIES A. (1974) Leaf tissue remaining after cutting and regrowth in perennial ryegrass. *Journal of Agricultural Science, Cambridge*, **82**, 165–172.

DEREGIBUS V.A., SÁNCHEZ R.A., CASAL J.J. and TRLICA M.J. (1985) Tillering responses to enrichment of red light beneath the canopy in a humid natural grassland. *Journal of Applied Ecology*, **22**, 199–206.

DURU M. and DUCROCQ H. (2000) Growth and senescence of the successive grass leaves on a tiller. Ontogenic development and effect of temperature. *Annals of Botany*, **85**, 635–643.

FERNÁNDEZ GRECCO R.C., MAZZANTI A. and ECHEVERRÍA H.E. (1995) Efecto de la fertilización nitrogenada sobre el crecimiento de forraje de un pastizal natural de la Pampa Deprimida bonaerense (Argentina). [Effect of nitrogen fertilization on the growth of forage in a natural grassland of the Flooding Pampas of Buenos Aires (Argentina)]. Memorias XIV Reunión ALPA- 19th Congreso. AAPA, 2, Mar del Plata, Argentina, pp. 173–179.

FOWLER N. (1982) Competition and coexistence in a North Carolina Grassland. III: mixtures of component species. *Journal of Ecology*, **70**, 77–92.

FUSTEC J., GUILLEUX J., LE CORFF J. and MAITRE J.P. (2005) Comparison of early development of three grasses: *Lolium perenne*, *Agrostis stolonifera* and *Poa pratensis*. *Annals of Botany*, **96**, 269–278.

GARDEN D.L. and BOLGER T.P. (2001) Interaction of competition and management in regulating composition and sustainability of native pasture. In: Tow P.G. and Lazenby A. (eds) *Competition and succession in pastures*, pp. 213–232. Wallingford, UK: CAB International.

GASTAL F. and DURAND J.L. (2000) Effects of nitrogen and water supply on N and C fluxes and partitioning in defoliated swards. In: Lemaire G., Hodgson J., de Moraes A., Nabinger C., de F. and Carvalho P.C. (eds) *Grassland ecophysiology and grazing ecology*, pp. 15–39. Wallingford, UK: CAB International.

GASTAL F. and LEMAIRE G. (2002) N uptake and distribution in crops: an agronomical and ecophysiological perspective. *Journal of Experimental Botany*, **53**, 789–799. Inorganic Nitrogen Assimilation Special Issue.

GASTAL F., BELANGER G. and LEMAIRE G. (1992) A model of the leaf extension rate of tall fescue in response to nitrogen and temperature. *Annals of Botany*, **70**, 437–442.

GATTI M.L., AYALA TORALES A.T., CIPRIOTTI P.A. and GOLLUSCIO R.A. (2011) Establishment and implantation efficiencies of temperate pastures. *Revista Argentina de Producción Animal*, **31**, 441.

GAUTIER H., VARLET- GRANCHER C. and HAZARD L. (1999) Tillering responses to the light environment and to defoliation in populations of perennial ryegrass (*Lolium perenne*) selected for contrasting leaf length. *Annals of Botany*, **83**, 423–429.

HARRIS W. (2001) Formulation of pasture seed mixtures with reference to competition and succession in pastures. In: Tow P.G. and Lazenby A. (eds) *Competition and succession in pastures*, pp. 149–174. Wallingford, UK: CAB International.

HIRATA M. and PAKIDING W. (2002) Dynamics in tiller weight and its association with herbage mass and tiller density in a bahia grass (*Paspalum notatum*) pasture under cattle grazing. *Tropical Grasslands*, **36**, 24–32.

HUBER H., LUKÁCS S. and WATSON M.S. (1999) Spatial structure of stoloniferous herbs: an interplay between structural blueprint, ontogeny and phenotypic plasticity. *Plant Ecology*, **141**, 107–115.

HUME D.E. (1991) Leaf and tiller production of prairie grass (*Bromus willdenowii* Kunth) and two ryegrass (*Lolium*) species. *Annals of Botany*, **67**, 111–121.

KEMP D.R. and KING W.McG. (2001) Plant competition in pastures – implications for management. In: Tow P.G. and Lazenby A. (eds) *Competition and succession in pastures*, pp. 85–102. Wallingford, UK: CAB International.

KEMP D.R., MICHALK D.L. and VIRGONA J.M. (2000) Towards more sustainable pastures: lessons learnt. *Australian Journal of Experimental Agriculture*, **40**, 343–356.

LAIDLAW A.S. and TEUBER N. (2001) Temperate forage grass- legume mixtures: advances and perspectives. Proceedings of the XIX International Grassland Congress, Brazil, pp. 85–92.

LATTANZI F., MARINO M.A. and MAZZANTI A. (1996) Efecto de la fertilización nitrogenada sobre la morfogénesis de raigrás anual cv. Grasslands Tama. [Effect of nitrogen fertilization on the morphogenesis of annual ryegrass cv. Grasslands Tama.] *Revista Argentina de Producción Animal*, **16**, 240–241.

LEMAIRE G. (2001) Ecophysiology of grasslands: dynamics aspects of forage plant populations in grazed swards. Proceedings of the International XIX Grassland Congress, Brazil, pp. 29–37.

LEMAIRE G. and AGNUSDEI M. (2000) Leaf tissue turnover and efficiency of herbage utilization. In: Lemaire G., Hodgson J., de Moraes A., Nabinger C., de F. and Carvalho P.C. (eds) *Grassland ecophysiology and grazing ecology*, pp. 265–287. Wallingford, UK: CAB International.

LEMAIRE G. and CHAPMAN D. (1996) Tissue flows in grazed plant communities. In: Hodgson J. and Illius A.W. (eds) *The ecology and management of grazing systems*, pp. 3–36. Wallingford, UK: CAB International.

LEMAIRE G. and MILLARD P. (1999) An ecophysiological approach to modelling resource fluxes in competing plants. *Journal of Experimental Botany*, **50**, 15–28.

MADDALONI J. and FERRARI L. (2001) Cebadilla criolla. In: Maddaloni J. and Ferrari L. (eds) *Forrajeras y pasturas del ecosistema templado húmedo de la Argentina [Fodder and pastures of the temperate humid ecosystem of Argentina]*, pp. 165–182. Buenos Aires: Universidad Nacional de Lomas de Zamora, Facultad de Ciencias Agrarias.

MATTHEW C., ASSUERO S.G., BLACK C.K. and SACKVILLE HAMILTON N.R. (2000) Tiller dynamics of grazed swards. In: Lemaire G., Hodgson J., de Moraes A., Nabinger C., de F. and Carvalho P.C. (eds) *Grassland ecophysiology and grazing ecology*, pp. 127–150. Wallingford, UK: CAB International.

MAZZANTI A., LEMAIRE G. and GASTAL F. (1994) The effect of nitrogen fertilization upon the herbage production of tall fescue swards continuously grazed with sheep. 1. Herbage growth dynamics. *Grass and Forage Science*, **49**, 111–120.

MEDIAVILLA S. and ESCUDERO A. (2003) Photosynthetic capacity, integrated over the lifetime of a leaf, is predicted to be independent of leaf longevity in some tree species. *New Phytologist*, **159**, 203–211.

MÓNACO T.A. and BRISKE D.D. (2000) Does resource availability modulate shade avoidance responses to the ratio of red to far-red irradiation? An assessment of radiation quantity and soil volume. *New Phytologist*, **146**, 37–46.

NAVAS M.L., DUCOUT B., ROUMET C., RICHARTE J., GARNIER J. and GARNIER E. (2003) Leaf life span, dynamics and construction cost of species from Mediterranean old-fields differing in succesional status. *New Phytologist*, **159**, 213–228.

NELSON C.J. (2000) Shoot morphological plasticity of grasses: leaf growth vs. tillering. In: Lemaire G., Hodgson J., de Moraes A., Nabinger C., de F. and Carvalho P.C. (eds) *Grassland ecophysiology and grazing ecology*, pp. 101–126. Wallingford, UK: CAB International.

NEUTEBOOM J.H. and LANTINGA E.A. (1989) Tillering potential and relationship between leaf and tiller production in perennial ryegrass. *Annals of Botany*, **63**, 265–270.

NURJAYA I.G.M.O. and TOW P.G. (2001) Genotype and environmental adaptation as regulators of competitiveness. In: Tow P.G. and Lazenby A. (eds) *Competition and succession in pastures*, pp. 43–62. Wallingford, UK: CAB International.

RAWNSLEY R.P., DONAGHY D.J., FULKERSON W.J. and LANE P.A. (2002) Changes in the physiology and feed quality of cooksfoot (*Dactylis glomerata*) during regrowth. *Grass and Forage Science*, **57**, 203–207.

REICH P.B., ELLSWORTH D.S., WALTERS M.B., VOSE J.M., GRESHAM C.H., VOLIN J.C. and BOWMAN W.D. (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology*, **80**, 1955–1969.

RIES R.E. and SVEJCAR T.J. (1991) The grass seedling: when is it established? *Journal of Range Management*, **44**, 574–576.

ROBSON M.J., RYLE G.J.A. and WOLEDGE J. (1988) The grass plant – its form and function. In: Jones M.B. and Lazenby A. (eds) *The grass crop. The physiological basis for production*, pp. 22–83. London, UK: Chapman and Hall.

ROSS M.A. and HARPER J.L. (1972) Occupation of biological space during seedling establishment. *Journal of Ecology*, **60**, 77–88.

ROUSSEAUX M.C., HALL A.J. and SÁNCHEZ R.A. (2000) Basal leaf senescence in sunflower *(Helianthus annus)* canopy: responses to increased R:FR ratio. *Physiologia Plantarum*, **110**, 477–482.

RYLE G.J.A. (1970) Partition of assimilates in an annual and a perennial grass. *Journal of Applied Ecology*, **7**, 217–227.

SANDERSON M.A. and ELWINGER G.F. (2002) Plant density and environmental effects on orchardgrass-white clover mixtures. *Crop Science*, **42**, 2055–2063.

SANDERSON M.A., SKINNER R.H. and ELWINGER G.F. (2002) Seedling development and field performance of prairiegrass, grazing bromegrass and orchardgrass. *Crop Science*, **42**, 224–230.

SATO K. (1980) Growth responses of some gramineous forage crops to daylength and temperature. *Journal of Japanese Society of Grassland Science*, **25**, 311–318.

SCHENEITER O. and RIMIERI P. (2001) Herbage accumulation, tiller population density, and sward components of prairie grass under different nitrogen levels. *New Zealand Journal of Agricultural Research*, **44**, 13–22.

SCHENEITER O. and ROSSO B. (2005) Acumulación de forraje y dinámica del macollaje de germonplasma de Cebadilla criolla (*Bromus catharticus* Vhal) en mezcla con alfalfa (*Medicago sativa* L.). [Forage accumulation and tiller dynamics of germoplasm of prairie grass (*Bromus catharticus* Vhal) in a mixture with alfalfa]. *Revista de Invetigaciones Agropecuarias*, **34**, 109–121.

SILVERTOWN J. and WILSON J.B. (2000) Spatial interactions among grassland plant populations. In:

Dieckmann U., Law R. and Metz J.A.J. (eds) *The geometry of ecological interactions. Simplifying spatial complexity*, pp. 28–47. Cambridge, UK: Cambridge University Press.

Skinner R.H. (2005) Emergence and survival of pasture species sown in monocultures or mixtures. *Agronomy Journal*, **97**, 799–805.

Skinner R.H. and Nelson C.J. (1992) Estimation of potential tiller production and site usage during tall fescue canopy development. *Annals of Botany*, **70**, 493–499.

Stoll P. and Weiner J. (2000) A neighborhood view of interactions among individual plants. In: Dieckmann U., Law R. and Metz J.A.J. (eds) *The geometry of ecological interactions. Simplifying spatial complexity*, pp. 11–27. Cambridge, UK: Cambridge University Press.

Suding K.N., Collins S.L., Gough L., Clark C., Cleland E.E., Gross K.L., Milchunas D.G. and Pennings S. (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 4387–4392.

Sugiyama S. (1995) The relationship between growth and development of vegetative shoots in genotypes of tall fescue (*Festuca arundinacea* Schreb.). *Annals of Botany*, **76**, 553–558.

Tallowin J.R.B., Brookman S.K.E. and Santos G.L. (1995) Leaf growth and utilization in four grass species under steady state continuous grazing. *Journal of Agricultural Science, Cambridge*, **124**, 403–417.

Torres M.S., Colabelli M.N. and Peretti A. (2000) Evaluación agronómica de *Lolium perenne* L. libre e infectado por *Neotyphodium lolii* en screen-house. [Agronomic evaluation of *Lolium perenne* L. with and without *Neotyphodium lolii* infection in a screen-house]. *Revista Argentina de Producción Animal*, **20** (Suppl.1), 164–165.

Tremmel D.C. and Bazzaz F.A. (1993) How neighbor canopy architecture affects target plant performance. *Ecology*, **74**, 2114–2124.

Virkajärvi P. and Järvenranta K. (2001) Leaf dynamics of timothy and meadow fescue under Nordic conditions. *Grass and Forage Science*, **56**, 294–304.

Volontec J.J. and Nelson C.J. (1983) Responses of tall fescue leaf meristems to N fertilization and harvest frequency. *Crop Science*, **23**, 720–724.

Wilhelm W.W. and McMaster G.S. (1995) Importance of the phyllochron in studying development and growth in grasses. *Crop Science*, **35**, 1–3.

Wingler A., Purdy S., MacLean J.A. and Pourtau N. (2006) The role of sugars in integrating environmental signals during the regulation of leaf senescence. *Journal of Experimental Botany*, **57**, 391–399.