Leaf expansion and leaf turnover of perennial C4 grasses growing at moderately low temperatures

Expansión y recambio foliar de gramíneas perennes C4 creciendo a temperaturas moderadamente bajas

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Abstract

Understanding the mechanisms by which some C4 grasses grow more than others at moderately low temperatures (~12-20°C) is valuable to select materials to lengthen the growing season. In turn, the determination of leaf lifespan for each material to be used is relevant to optimize the balance between herbage production and herbage utilization. The objectives of this study were to analyze the growth capacity and the leaf lifespan in two native materials (*Pappophorum caespitosum* and *Trichloris crinita*) and in four materials introduced (*Cenchrus ciliaris* cv. 'Texas-4464', *Cenchrus ciliaris* cv. 'Bella', *Panicum coloratum* cv. 'Klein' and *Panicum maximum* cv. 'Gatton Panic') commonly used in Argentina. Under non-limiting growth conditions, the rate of leaf appearance and leaf elongation, the number of growing leaves and the leaf lifespan, were measured. The materials showed similar leaf growth capacity through contrasting mechanisms: while three of them (*P. coloratum*, *P. maximum* and *P. caespitosum*) showed higher growth of individual leaves, the rest (*C. ciliaris* cv. 'Texas-4464', *C. ciliaris* cv. 'Bella' and *T. crinita*) showed higher number of growing leaves. The leaf lifespan was not significantly different between materials evaluated. Interestingly, in agreement with previous results obtained in a comparison of C3 grasses, it was observed that materials possessing a greater number of growing leaves had lower values of leaf lifespan.

Keywords
base temperature • C4 grasses • leaf appearance • leaf growth • leaf lifespan

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Resumen

Conocer los mecanismos por los cuales algunas gramíneas C4 crecen más que otras a temperaturas moderadamente bajas (~12-20°C) es valioso para seleccionar materiales que permitan alargar la estación de crecimiento. Por su parte, conocer la vida media foliar de las especies es relevante para optimizar el balance entre producción y utilización de forraje. Los objetivos del trabajo fueron analizar la capacidad de crecimiento y la vida media foliar en dos materiales nativos (*Pappophorum caespitosum* y *Trichloris crinita*) y en cuatro materiales introducidos (*Cenchrus ciliaris* cv. 'Texas-4464', *Cenchrus ciliaris* cv. 'Bella', *Panicum coloratum* cv. 'Klein' y *Panicum maximum* cv. 'Gatton Panic') comúnmente utilizados en Argentina. Bajo condiciones no limitantes al crecimiento se evaluó la tasa de aparición y elongación foliar, el número de hojas en crecimiento y la vida media foliar. Los materiales tuvieron similar capacidad de crecimiento foliar con mecanismos contrastantes: mientras tres materiales (*P. coloratum, P. maximum* y *P. caespitosum*) mostraron mayor crecimiento por hoja, el resto (*C. ciliaris* cv. 'Texas-4464', *C. ciliaris* cv. 'Bella' y *T. crinita*) mostró mayor número de hojas en crecimiento. La vida media foliar no difirió significativamente entre materiales. Interesantemente, y en concordancia con resultados previos de una comparación de gramíneas C3, se observó que los materiales que poseían un mayor número de hojas creciendo simultáneamente tenían menores valores de vida media foliar.

Palabras clave

temperatura base • gramíneas C4 • aparición foliar • crecimiento foliar • vida media foliar

Introduction

The continuous increase in the land area dedicated to agriculture that took place in Argentina in the last decade (21) has displaced livestock production to areas with adverse environmental conditions (*e. g.* saline soils, high temperatures, high radiation, low air humidity, flooding) in which C4 materials usually perform better than C3 materials (30, 33). Under such conditions, the availability of C4 materials with a higher growing capacity at daily mean temperatures in a range of 12-20°C would be highly valuable as it will result, if rainfall in spring or autumn is adequate, in lengthened growing seasons and increased primary productivities. In such sense, earlier reports have evidenced better spring performance of a non-native (*i. e.* introduced) material (cv. 'Bella') of *Cenchrus ciliaris* L. (13) and of a native material (*Pappophorum caespitosum*) from the Argentinean arid Chaco (28) over other C4 materials more frequently used. However, the mechanisms behind this better spring performance often are not known. The scientific understanding of the mechanisms responsible of the expression of this valuable trait will be highly relevant, *e. g.* for breeding programs focused in 'traits' comparison rather than in 'cultivars' comparison and aided by techniques like the monitoring of gene expression at the molecular level (26).

In absence of water and nutritional constraints, differences between grasses in growth per hectare should be explained by the differences in growth per tiller and/or the differences in tiller density (*i.e.* tillers per hectare). Tiller growth depends of the inter-relationship between the activity of individual intercalary meristems (*i. e.* individual leaf growth) and the number of active meristems (*i. e.* number of growing leaves) (6). Since leaf growth in the *Gramineae* is predominantly unidirectional, parallel to the longitudinal axis of the leaf (37) the leaf elongation rate (LER) is the variable generally used to analyze leaf growth (10). In turn, since each appeared leaf implies a new potential tiller, materials with faster leaf appearance rate per tiller (LAR_T) have the potential to increase faster their tiller population (17). It is important to note that for temperate grasses LER and LAR variables were found to be useful tools to evaluate forage materials likely to be introduced in a region (3, 5, 11, 14, 23).

Another way to increase the productivity of pasture-based livestock systems is an efficient grazing management (18), which involves a compromise between the aim of maximizing light interception by forage leaf area and the aim of maximizing the harvest of leaf tissue before senescence occurs (25).

Consequently, to gain knowledge about leaf lifespan of materials used as forage is a major aim to control and optimize the balance between herbage production and utilization (18). In addition, leaf lifespan is a key plant trait since it links leaf ecophysiology, whole-plant growth and ecosystem processes (31). In fact, differences in leaf biomass turnover rate can lead to different nutrient cycling rates in the ecosystem (7, 35).

Therefore, knowledge about leaf lifespan of native and non-native materials is highly relevant to design efficient and sustainable livestock production systems.

Objectives

The first objective of this study was to compare the leaf growth capacity at relatively low temperatures of *P. caespitosum* and *C. ciliaris* cv. 'Bella' with other native [*Trichloris* crinita (Lag.) Parodi] and introduced materials (*C. ciliaris* cv. 'Texas-4464', *Panicum coloratum* L cv. 'Klein', *Panicum maximum* J cv. 'Gatton Panic') commonly used in Argentina. The second objective was to quantify the leaf lifespan of these materials. It is important to note that, unlike the case for C3 grasses, such kind of comparison among C4 grasses is scarce (23).

Material and methods

Site and experimental conditions

The experiment was carried out at the Estación Experimental Balcarce of the Instituto Nacional de Tecnología Agropecuaria, Argentina (37°45' S, 58°18' W). On 15 September 2003 (early spring), seeds of *C. ciliaris* cv. 'Texas-4464', *C. ciliaris* cv. 'Bella', *P. maximum* cv. 'Gatton', *P. coloratum* cv. 'Klein', *P. caespitosum* and *T. crinita* were sown equally spaced (50 mm between rows and 30 mm among seeds within a row) in twelve 0.25 m depth · 0.75 m length · 0.35 m width wooden boxes (two boxes per material). Therefore, dense swards were generated. Boxes ("mini-swards") were filled with a 1 : 1 sand : soil mixture. Soil was the A horizon of a Typical Argiudol (organic matter content of 62 g kg^{-1}, pH 6.2). Mini-swards were maintained in a greenhouse until December 1, 2003, when they were transferred outdoors. Mini-swards were fertilized once (December 2003) with superphosphate (3 g P m^{-2}) and weekly with urea (5 g N m^{-2}), and irrigated twice a week up to soil saturation. Weeds were hand controlled.

Mini-swards were defoliated at a height of 5-7 cm once a month (November 1 and December 1, 2003, January 2, 2004). An appropriate defoliation frequency for each material, based in the leaf lifespan of each material (12, 24) was not possible because values of such parameter were not available and actually, to determine them was one of the objectives of this work.

The measurement period started 28 days after the last defoliation (January 30) at a time when all materials had recovered a substantial amount of leaf green area to reduce the effect of 'potential' differences in defoliation tolerance among materials (8).

The measurement period finished when most of the materials showed an uninterrupted elongation of the pseudostem which was a clear sign of the true stem growth. Thus, the measurement period extended from January 30 to February 24, 2004.

Measurements and calculations

Each material was replicated twice (two mini-swards per material) in a completely randomized design. Eight vegetative tillers per mini-sward, with a similar total blade length (an estimator of tiller size) (27, 29), and located in the middle of the canopy, were randomly marked with plastic rings at the beginning of the measurement period. On each tiller, every 3-4 days the green blade length was measured from the tip to its own ligule in fully expanded leaves and from the tip to the ligule of the previous fully expanded leaf in growing leaves. From these measurements, leaf elongation rate per tiller (LER_T; mm tiller^{-1} d^{-1}) and per growing leaf (LER_{Ln}, where *n* is the leaf number with n = 1 for the youngest leaf; mm leaf^{-1} d^{-1}) were calculated, as the positive differences in blade length between successive measurements.

The number of visible growing leaves (N_G), total green leaves per tiller (N_L) and new leaves appeared per tiller were counted on each date. Leaf appearance rate (LAR_T, leaves tiller^{-1} d^{-1}) was calculated as the quotient between appeared leaves per tiller and the duration of the measurement period. The phyllochron (*i. e.* interval time between the appearance of successive leaves on a tiller) was estimated as the inverse of LAR_T. The leaf lifespan (LLS) was quantified as the interval of time comprised between the leaf blade appearance (when its tip surpassed the ligule of the subtending leaf) and the senescence of the blade tip.

Simple linear regression between mean air temperature (independent variable) and leaf growth variables (dependent variable: LER_T, LER_{Ln}, LAR_T, N_L, N_G) were obtained per material and per replicate using ordinary least square regression (38). Base temperature (T_b) for LER_T, LER_{Ln} and LAR_T was estimated, for each material and replicate, by extrapolation (*i. e.* calculating the value of the independent variable when the dependent variable equals zero). However, grass leaves undergo ontological changes in their elongation rate (10), and therefore elongation rates of individual leaves should be compared at the same developmental stage (*e. g.* 3). For this reason, for each measurement period, a subset of growing leaves which lengths were lower than two-thirds of their final length were selected (*e. g.* 3). At this developmental stage, LER is close to maximal (10, 34), and therefore it was termed LER_{max}. Phyllochron and LLS values were expressed in thermal time units (accumulated growing degree-days, GDD).

The GDD were calculated as the sum of daily mean temperatures above a base temperature (T_b). For phyllochron, the T_b used for each material was the value obtained by the regression between LAR_T and mean air temperature. Irrespectively of the material, a T_b of 0°C was used for LLS. Maximum and minimum temperatures were measured daily at 1.5 m height with a portable meteorological station (LI-1200S, Li-Cor Inc., Lincoln, NE).

Statistical analysis

All data were checked for normality and homogeneity of variances. Analyses of variance (ANOVA) were performed for total blade length per tiller (LLT_T) at the beginning of the measurement period, LAR_T, LER_T, LER_{Ln}, N_G, N_L ,LLS and T_b using the SAS GLM procedure (SAS Institute, Cary, NC, USA). Means were separated using LSD ($p = 0.05$).

Slopes and intercepts of the linear functions were compared using dummy variables (18). The Pearson correlation coefficient was used to evaluate the strength of the association between variables of interest.

Three contrasts were made. Contrast 1 compares growth capacity of *C. ciliaris* cv. 'Bella' against the rest of materials. Contrast 2 compares growth capacity of *P. caespitosum* against the rest of materials. Contrast 3, was made to test native *versus* introduced materials.

Results

General

Mean daily temperature and mean daily solar radiation during the experimental period were 19°C (figure 1) and 18 MJ, respectively.

Materials did not differ in total blade length per tiller (LLT_T) at the beginning of the measurement period (table 1, page 74). Likewise, materials did not differ in LER_T, LAR_T, phyllochron and N_L but differences among materials were observed in LER_{Ln}, N_G and LLS (table 1, page 74).

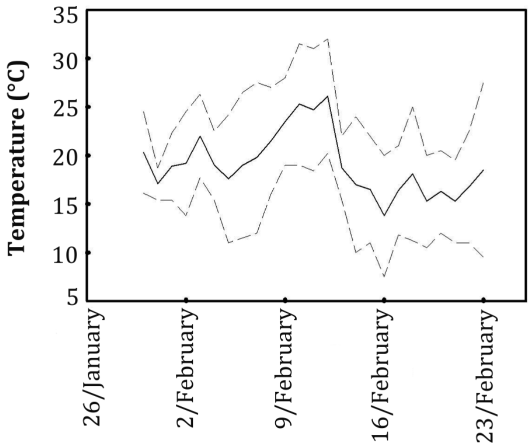


Figure 1. Daily mean (solid line), minimum and maximum air temperatures (dotted line) during the measurement period.

Figura 1. Temperaturas del aire: media diaria (línea continua), mínima y máxima (línea punteada) registradas durante el período de mediciones.

Table 1. Means of total blade length per tiller at the beginning of the measurement period (LLT_T), leaf elongation rate per tiller (LER_T), leaf elongation rate per leaf category (LER_{Ln}, were n = 1, 2 and 3 indicates the last, penultimate and antepenultimate appearing leaf respectively), maximal leaf elongation rate of individual leaves (LER_{max}), blade length (BL), number of green leaves per tiller (N_L), number of growing leaves (N_G), phyllochron (PHY) and leaf lifespan (LLS) for the C4 materials evaluated.

Tabla 1. Valores medios de largo de lámina total por macollo al inicio del período de mediciones (LLT_T), tasa de elongación foliar por macollo (LER_T), tasa de elongación foliar por categoría de hoja (LER_{Ln}, donde n = 1, 2 y 3 denota la última, penúltima y antepenúltima hoja aparecida, respectivamente), máxima tasa de elongación foliar de hojas individuales (LER_{max}), longitud de lámina (BL), número de hojas verdes por macollo (N_L), número de hojas en crecimiento (N_G), filocrono (PHY) y vida media foliar (LLS) para los materiales C4 evaluados.

Material	LLT_T (mm per tiller)	LER_T (mm d^{-1})	LER_{L1} (mm d^{-1})	LER_{L2} (mm d^{-1})	LER_{L3} (mm d^{-1})	LER_{max} (mm d^{-1})	BL (mm)	N_L	N_G	PHY (GDD†)	LLS (GDD‡)
C. ciliaris cv. 'Bella'	176 [a]	13.8 [a]	6.5 [a]	5.9 [a]	1.4 [a]	6.6 [b]	105 [b]	3.1 [a]	2.0 [a]	38 [a]	306 [dc]
C. ciliaris cv. 'Texas-4464'	270 [a]	13.2 [a]	6.4 [a]	5.3 [a]	1.5 [a]	6.9 [b]	91 [b]	3.6 [a]	2.1 [a]	40 [a]	297 [d]
T. crinita	286 [a]	15.1 [a]	8.3 [a]	6.3 [a]	0.5 [a]	8.7 [b]	103 [b]	3.3 [a]	2.0 [a]	30 [a]	324 [bdc]
P. maximum	210 [a]	17.9 [a]	14.0 [a]	3.9 [a]	0.0 [a]	14.6 [a]	205 [a]	2.4 [a]	1.4 [b]	56 [a]	370 [ab]
P. coloratum	232 [a]	12.9 [a]	9.2 [a]	3.7 [a]	0.0 [a]	10.1 [b]	112 [b]	2.9 [a]	1.6 [b]	57 [a]	344 [abc]
P. caespitosum	200 [a]	12.8 [a]	9.2 [a]	3.6 [a]	0.0 [a]	9.7 [b]	141 [b]	2.7 [a]	1.5 [b]	48 [a]	387 [a]

† Growing degree-days calculated using the base temperature obtained for each material by regression between the leaf appearance rate (LAR_T) and mean air temperature (see Table 2 for more details).

‡ Growing degree-days calculated using a base temperature of 0°C.

Different letters indicate differences between materials at $P < 0.05$.

† Grados día de crecimiento calculados utilizando la temperatura base obtenida para cada material por regresión entre la tasa de aparición foliar (LAR_T) y la temperatura media del aire (ver Tabla 2 para más detalles).

‡ Grados días de crecimiento calculados utilizando una temperatura base de 0°C.

Letras diferentes denotan diferencias entre materiales a $P < 0,05$.

Interestingly, the materials achieved a similar LER_T combining different elongation rates of their individual leaves (LER_{Ln}, table 1, page 74). For example, while in *P. maximum* $LER_{L1,}$ and LER_{L2} contributed 78% and 22% to LER_T, respectively, in *T. crinita* the $LER_{L1,}$ $LER_{L2,}$ and LER_{L3} explained 54%, 40% and 6% of LER_T (table 1, page 74).

Such differences imply that materials differed in both, the capacity of individual leaf growth and the mechanisms to achieve a similar LER_T. As it was expected, the materials showed differences in LER_{max} (table 1, page 74) and, consequently, in leaf blade length (table 1, page 74).

Changes in LER_T may be explained by changes in the number of leaves elongating at a given time (N_G) and/or the rate at which each individual leaf elongates (*i. e.* the LER_{max}).

The materials evaluated achieved similar LER_T values by different mechanisms and that can be illustrated by a strong negative correlation between N_G and LER_{max} (figure 2a, page 76). Roughly, two contrasting groups can be visualized. A 'low-N_G' group integrated by *P. coloratum, P. maximum* and *P. caespitosum* and a 'high-N_G' group integrated by *C. ciliaris* cv. 'Bella', *C. ciliaris* cv. 'Texas-4464' and *T. crinita* (figure 2a, page 76).

The evaluated materials showed a similar leaf growth-response to temperature. First, the slope of the relationship between leaf growth variables (LER_T, LER_{max} and LAR_T) and mean air temperature was similar among materials (table 2, page 77). Second, T_b for LER_T, LER_{max} and LAR_T was also similar among materials (table 2, page 77).

Contrasts

The leaf growth capacity (LER_T, LER_{max}, LAR_T) of *C. ciliaris* cv. 'Bella' and *P. caespitosum* did not differ from that of the rest of the materials evaluated (table 3, page 79; contrast 1 and 2).

Moreover, no difference was observed for these contrasts when T_b and the temperature-responses (*i. e.* slopes of relationships between leaf growth and mean air temperature) for such variables were analyzed (data not shown). Native species showed similar leaf growth capacity (*i. e.* LER_T, LER_{max}, LAR_T) and similar tissue turnover (*i.e.* LLS) than the introduced species (table 3, page 79; contrast 3).

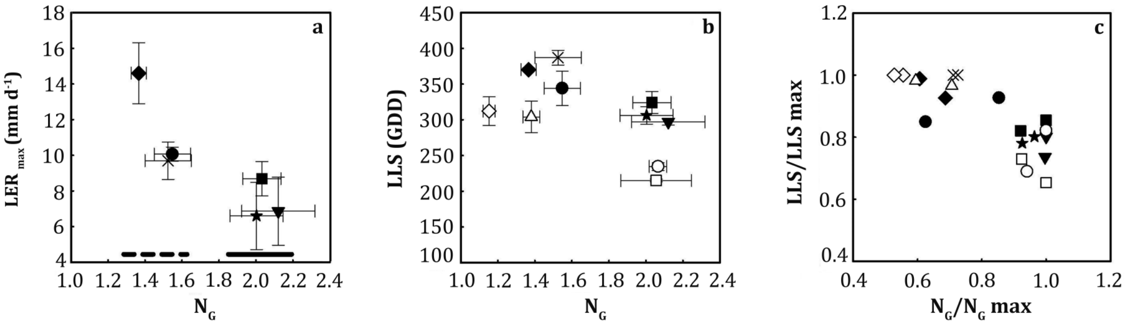
Discussion

Leaf growth at moderately low temperatures

This article shows that, given adequate growing conditions (*i. e.* non-limiting water and nutrients availability) the materials evaluated did not differ in the activity of shoot apical meristem (quantified by phyllochron; 35) and also, did not differ in the leaf elongation rate per tiller (LER_T).

Therefore, the previously reported superior canopy spring growth of *C. ciliaris* cv. 'Bella' (13) and *P. caespitosum* (28) can not be attributed to a higher capacity of leaf tissue production at tiller level, at relatively low temperatures for C4 species to grow (~ 14-20°C).

The absence of differences could be explained by the occurrence of several days (~ 36% of days) with a mean daily temperature ranging around values (~ 14-17°C; figure 1, page 73) closed to the base temperature for leaf growth determined for the materials evaluated (~ 15°C; table 2, page 77). In fact, differences between genotypes in the capacity to grow at moderately low temperature usually diminish as temperature approaches the temperature base (3, 22).



In **(a)**, horizontal lines denote the species integrating the 'high-N_G' (solid line) and the 'low-N_G' (dashed line) groups. In **(a)** and **(b)** each symbol is the mean of two replicates and the bars are the standard error of each mean. In **(c)** each symbol is a replicate and each replicate is a mean of 7–8 tillers for materials evaluated at present research (black symbols) and 18–20 tillers for materials evaluated in Berone 2005 (white symbols). *Pappophorum caespitosum* (black cross), *Panicum maximum* (black diamond), *Panicum coloratum* (black circle), *Cenchrus ciliaris* cv. 'Bella' (black star), *C. ciliaris* cv. 'Texas-4464' (black triangle), *Trichloris crinita* (black square), *Bromus stamineus* cv. 'Experimental' (white circle), *B. stamineus* cv. 'Zamba' (white square), *Lolium perenne* cv. 'Experimental' (white diamond), *L. perenne* cv. 'Horizon' (white triangle).

En **(a)**, las líneas horizontales denotan las especies que integran el grupo 'alto-N_G' (línea continua) y el grupo 'bajo-N_G' (línea discontinua). En **(a)** y **(b)** cada símbolo es el valor medio de dos repeticiones y las barras son el error estándar de cada valor medio. En **(c)** cada símbolo es una repitición y cada réplica es el valor medio de 7-8 macollos para los materiales evaluados en el presente trabajo (símbolos negros) y de 18-20 macollos en los materiales evaluados en Berone 2005 (símbolos blancos). *Pappophorum caespitosum* (cruces negras), *Panicum maximum* (diamantes negros), *Panicum coloratum* (círculos negros), *Cenchrus ciliaris* cv. 'Bella' (estrellas negras), *C. ciliaris* cv. 'Texas-4464' (triángulos negros), *Trichloris crinita* (cuadrados negros), *Bromus stamineus* cv. 'Experimental' (círculos blancos), *B. stamineus* cv. 'Zamba' (cuadrados blancos), *Lolium perenne* cv. 'Experimental' (diamantes blancos), *L. perenne* cv. 'Horizon' (triángulos blancos).

Figure 2. (a) Relationship between the maximal leaf elongation rate of individual leaves (LER_{max}), and the number of growing leaves (N_G) for materials evaluated at present research. Correlation analysis: $r = -0.86$; $p < 0.05$. **(b)** Relationship between absolute values of N_G and leaf lifespan (LLS) for materials evaluated at present research (black symbols) and in Berone 2005 (white symbols). Correlation analysis for data of present research: $r = -0.90$, $p < 0.05$. Correlation analysis for data of Berone 2005: $r = -0.98$; $p < 0.05$. **(c)** Relationship between the relative maximum N_G and the relative maximum LLS for materials evaluated at present research (black symbols) and in Berone 2005 (white symbols). Correlation analysis: $r = -0{,}91$; $p < 0{,}01$.

Figura 2. (a) Relación entre la máxima tasa de elongación foliar de hojas individuales (LER_{max}), y el número de hojas en crecimiento. Análisis de correlación: $r = -0{,}86$; $p < 0{,}05$. (N_G) para los materiales evaluados en el presente trabajo. **(b)** Relación entre los valores absolutos de N_G y de vida media foliar (LLS) para los materiales evaluados en el presente trabajo (símbolos negros) y en Berone 2005 (símbolos blancos). Análisis de correlación para los datos del presente trabajo: $r = -0{,}90$, $p < 0{,}05$. Análisis de correlación para los datos de Berone 2005: $r = -0{,}98$; $p < 0{,}05$. **(c)** Relación entre el máximo valor relativo de N_G y el máximo valor relativo de LLS para los materiales evaluados en el presente trabajo (símbolos negros) y en Berone 2005 (símbolos blancos). Análisis de correlación: $r = -0{,}91$; $p < 0{,}01$.

Table 2. Parameters (slope and intercept) of the equations relating leaf elongation rate per tiller (LER_T-temp), maximal leaf elongation rate of individual leaves (LER_{max}-temp) and leaf appearance rate (LAR_T-temp) with mean air temperature, and the corresponding base temperature ($^{\dagger}T_b$, °C) for the C4 materials evaluated.

Tabla 2. Parámetros (pendiente e intercepta) de las ecuaciones que relacionan la tasa de elongación foliar por macollo (LER_T-temp), la máxima tasa de elongación foliar de hojas individuales (LER_{max}-temp) y la tasa de aparición foliar (LAR_T-temp) con la temperatura media del aire y, la correspondiente temperatura base ($^{\dagger}T_b$, °C) para los materiales C4 evaluados.

	LER_T-temp				LER_{max}-temp				LAR_T-temp			
	T_b	**slope**	**intercept**	**R^2**	**T_b**	**slope**	**intercept**	**r^2**	**T_b**	**slope**	**intercept**	**r^2**
C. ciliaris cv. 'Bella'	14.9	4.2	-62.6	0.55	14.5	2.1	-30.5	0.60	14.4	0.0328	-0.4798	0.80
C. ciliaris cv. 'Texas-4464'	15.9	5.5	-87.4	0.52	15.2	2.4	-36.9	0.47	14.8	0.0329	-0.4794	0.56
T. crinita	15.4	5.2	-80.5	0.84	15.2	2.9	-43.8	0.88	15.2	0.0427	-0.6482	0.73
Panicum maximum	15.4	6.3	-97.1	0.70	15.4	5.0	-76.0	0.68	15.1	0.0227	-0.3457	0.53
Panicum coloratum	14.2	3.2	-46.2	0.52	13.9	2.3	-32.3	0.58	13.0	0.0215	-0.2820	0.60
Pappophorum Caespitosum	14.8	3.8	-55.8	0.62	14.3	2.4	-34.6	0.70	15.9	0.0279	-0.4422	0.66

$^{\dagger}T_b$ was estimated as the value of the independent variable when the dependent variable equals zero.

Regressions were obtained using replication data. All equations were statistically significant ($P < 0.05$). Slopes and intercepts did not differ ($P > 0.05$) between materials evaluated.

$^{\dagger}T_b$ fue estimada como el valor de la variable independiente cuando la variable dependiente es igual a cero (*i.e.* valor de y cuando x = 0).

Las regresiones fueron obtenidas utilizando los datos de cada repetición. Todas las ecuaciones fueron estadísticamente significativas ($P < 0,05$). Las pendientes e interceptas no difirieron ($P > 0,05$) entre los materiales evaluados.

Interestingly, the present study demonstrates contrasting mechanisms for genotypes to achieve a similar leaf growth per tiller (LER_T). *Panicum coloratum, P.* and *P. caespitosum* showed a lower number of active meristems (*i. e.* number of visible growing leaves, N_G) but a higher activity of individual intercalary meristems (*i. e.* maximal leaf elongation rate of individual leaves, LER_{max}) than *C. ciliaris,* and *T. crinita*. As it was expected (31), these findings imply that genotypic differences observed at one organization level (*i.e.* leaf growth) will not necessarily translate to a higher organization level (*i. e.* tiller growth).

The growth of a grass sward can be explained by the growing capacity of individuals (*i. e.* LER per tiller) and the number of individuals growing at the same time (*i. e.* tiller density). Therefore, potential differences between materials in tiller density and canopy growth can not be discarded. It is generally accepted that each appeared leaf has the potential to form a tiller (23) and then, differences among materials in LAR_T could lead to differences in tiller density (17).

However, the materials evaluated here did not differ in the LAR_T. In other words, under the prevailing conditions of the present study, the materials showed the same capacity to generate sites for tiller appearance and consequently the same capacity to generate canopies with a similar 'potential' tiller density.

Since the referred works with *P. caespitosum* and *C. ciliaris* cv. 'Bella' (13, 28) were performed under natural conditions (*i. e.* without addition of nutrients and water) their superior spring growth should be explained by other factors than the intrinsic temperature response of LER_T and LAR_T. Additional research focusing on other traits than LER_T and LAR_T, carried out under different levels of water and nutrients availability, seem to be necessary to better understand the behaviour of different C4 materials and to allow selecting those with a superior growth capacity at the beginning and at the end of the growing season.

Leaf turnover at moderately low temperatures

Under the environmental conditions of present research (*i. e.* moderately low temperatures and adequately water and nutrients supply) differences in leaf lifespan between materials were observed. Therefore, a specific defoliation interval (*i. e.* material dependent) is needed to optimize the balance between the production and the utilization of herbage (18). For sites/periods with a mean daily temperature similar of present research (~ 19°C) the interval between defoliations will range between 15 days for the material with the higher leaf turnover (*e. g.* leaf lifespan of *C. ciliaris* cv. 'Texas 464' = 297 GDD; 297 GDD/19°C = 15 days) and 20 days for the material with the lower leaf turnover (*e. g.* leaf lifespan of *P. caespitosum* = 387 GDD; 387 GDD/19°C = 20 days). Assuming that, at higher temperatures, differences in leaf lifespan are sustained, the intervals between defoliations become similar between these materials. As an example, at 27°C the optimal interval between defoliations will range between 11 and 14 days for *C. ciliaris* cv. 'Texas 464' and *P. caespitosum*, respectively.

Differences in leaf biomass turnover rate can lead to different nutrient cycling rates in the ecosystem (7, 35). Therefore, variation in leaf life-span has long been considered of ecological significance (31). Despite this, a quantitative evaluation of the relationships between leaf life-span and other plant characteristics has been rare. Interestingly, the materials showing a significantly higher number of active intercalary meristems (N_G) showed a lower leaf lifespan (figure 2b, page 76). A similar trade-off between N_G and leaf lifespan was observed in a C3 grasses comparison (2, 4), where the species with higher leaf lifespan (*Lolium perenne*) showed a lower N_G than the species showing lower leaf lifespan (*Bromus stamineus*) (figure 2b, page 76). The quite similar relationship (LLS vs. N_G) observed in species belonging to contrasting functional groups (*i. e.* C3 of a previous report and C4 grasses of present research) can be better appreciated when both variables were expressed in relative terms (figure 2c, page 76).

Such inverse correlation between the N_G and leaf lifespan could be due to the higher nitrogen/phosphorus demand for cell production and expansion (1, 15, 16, 20, 38) of tillers with a higher number of leaves growing simultaneously.

It's also important to note that, under environmental conditions of present research the native materials from the Argentinean arid Chaco showed a similar leaf turnover than that of the non native materials (table 3, contrast 3).

Table 3. Means of leaf elongation rate per tiller (LER_T), maximal leaf elongation rate of individual leaves (LER_{max}), blade length (BL), number of growing leaves (N_G), number of green leaves per tiller (N_L), phyllochron and leaf lifespan in the contrasts evaluated.

Tabla 3. Valores medios de tasa de elongación foliar por macollo (LER_T), máxima tasa de elongación foliar de hojas individuales (LER_{max}), longitud de lámina (BL), número de hojas en crecimiento (N_G), número de hojas verde por macollo (N_L), filocrono y vida media foliar en los contrastes evaluados.

	Contrast 1		**Contrast 2**		**Contrast 3**	
	1† vs.	**2,3,4,5**	**6 vs.**	**2,3,4,5**	**3,6 vs.**	**1,2,4,5**
LER_T (mm day^{-1})	14 a	15 a	13 a	15 a	14 a	14 a
LER_{max} (mm day^{-1})	8 a	10 a	9 a	10 a	9 a	10 a
BL (mm)	105 a	128 a	141 a	128 a	122 a	128 a
N_G	2.0 a	1.8 a	1.5 a	1.8 a	1.8 a	1.8 a
N_L	3.1 a	3.1 a	2.7 a	3.1 a	3.0 a	3.0 a
Phyllochron GDD‡)	38 a	57 a	66 a	57 a	39 a	59 a
Leaf lifespan GDD§)	306 a	334 a	387 a	334 b	356 a	329 a

† 1= *C. ciliaris* cv. 'Bella', 2= *C. ciliaris* cv. 'Texas-4464', 3= *T. Crinita*, 4= *Panicum maximum*, 5= *Panicum coloratum,* 6= *Pappophorum caespitosum.*

‡ Growing degree-days calculated using a base temperature obtained for each material by linear regression between the leaf appearance rate (LAR_T) and mean air temperature (see table 2, page 77; for more details)

§ Growing degree-days calculated using a base temperature of 0°C.

Different letters indicate differences between groups, within each contrast, at P < 0.05.

‡ Grados día de crecimiento calculados utilizando la temperatura base obtenida para cada material por regresión entre la tasa de aparición foliar (LAR_T) y la temperatura media del aire (ver tabla 2, pág. 77; para más detalles)

§ Grados días de crecimiento calculados utilizando una temperatura base de 0°C.

Letras diferentes denotan diferencias entre grupos, dentro de cada contraste, a P < 0,05.

This suggests that the replacement of native materials by non-native materials evaluated at present research will not derive in changes in nutrient cycling rates in the ecosystem (7, 35). However, this remains to be tested at an appropriate spatiotemporal scale.

Conclusions

The results of this study indicate that, at moderate low temperatures, the C4 evaluated materials had a similar tiller growth capacity and similar leaf lifespan. Interestingly they could be grouped according to their contrasting strategies to achieve a similar tiller growth; while *P. coloratum, P. maximum* and *P. caespitosum* showed a higher activity of individual intercalary meristems *C. ciliaris* and *T. crinita* showed a higher number of intercalary active meristems. In addition, and in coincidence with previous findings reported for C3 grasses, materials with a higher number of visible growing leaves showed a lower leaf lifespan.

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