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Research paper

Survival strategies of *Centrosema molle* and *C. macrocarpum* in response to drought

Estrategias de sobrevivencia de Centrosema molle *y* C. macrocarpum *a la sequía*

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Abstract

The response of a genotype each of *C. molle* and *C. macrocarpum* to drought (low soil moisture availability) was studied in a seasonally dry tropical environment throughout 3 consecutive years. Changes in soil water content, leaf water relations and gas exchange, chlorophyll fluorescence, forage yield and leaf area index (LAI) were compared in well-watered and droughted plots. Soil water depletion during the study occurred mostly at 0–20 cm depth. Minimum values of leaf relative water content, water potential (Ψ_l) and net photosynthesis in unwatered plants were within the ranges: 68 (*C. molle*) to 70% (*C. macrocarpum*); -1.6 (*C. molle*) to -0.9 MPa (*C. macrocarpum*); and 8 (*C. molle*) to 10 µmol/m^2/s (*C. macrocarpum*), respectively. Leaf movements helped to avoid excessive solar radiation incidence, yet efficiency of chloroplast Photosystem II in stressed leaves of *C. molle* was negatively affected. Above-ground biomass and LAI were reduced only in *C. macrocarpum* (45–50% reduction) as a result of moisture stress. Leaves of both species behaved as isohydric, though larger declines in Ψ_l in *C. molle* may suggest a less effective control of water loss; this promoted more leaf senescence. Drought survival in these species depends on a combination of avoidance and tolerance strategies; the relative importance of both mechanisms depends on species and the duration and intensity of water deficit. Further studies with a higher number of accessions/ecotypes of each species are suggested in order to corroborate our findings.

Keywords: Acclimation, physiological response, soil transpirable water, tropical forage legumes, water stress.

Resumen

Entre 2011 y 2013 en un ambiente de trópico estacional seco de Maracay, Venezuela, fueron evaluados por su sobrevivencia a la sequía (baja disponibilidad de agua en el suelo) sendos genotipos de *Centrosema molle* y *C. macrocarpum*. Para el efecto en campo se determinaron los cambios en la humedad del suelo, las relaciones hídricas, el intercambio gaseoso de la hoja, la fluorescencia de la clorofila, el rendimiento del forraje y el índice de área foliar (IAF) en plantas bajo riego y con estrés por sequía. El agotamiento de la humedad en el suelo ocurrió principalmente entre 0 y 20 cm. En las plantas bajo condiciones de sequía, los valores mínimos foliares del contenido relativo de humedad, potencial hídrico (Ψ_l) y fotosíntesis neta variaron, respectivamente, dentro de los rangos siguientes: 68 (*C. molle*) a 70% (*C. macrocarpum*), -1.6 (*C. molle*) a -0.9 MPa (*C. macrocarpum*), y 8 (*C. molle*) a 10 µmol/m^2 por segundo (*C. macrocarpum*). Los movimientos foliares contribuyeron a la reducción de la alta incidencia de la radiación solar, aunque en las hojas estresadas de *C. molle* la eficiencia del Fotosistema II del cloroplasto fue disminuida. La biomasa aérea y el IAF fueron afectados solo en *C. macrocarpum* (45–50% de reducción). Las hojas de ambas especies se comportaron como isohídricas, aunque la caída más pronunciada del Ψ_l en *C. molle* posiblemente es debida a un control menos

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efectivo de la pérdida de agua, induciendo así una mayor senescencia foliar. Los resultados sugieren que la sobrevivencia de estas especies ante la sequía está basada en la combinación de estrategias de evasión y tolerancia, donde la importancia relativa de ambas dependerá de la especie y la intensidad y duración del déficit hídrico. Se requieren más estudios con un número mayor de accesiones/genotipos para corroborar los resultados obtenidos.

Palabras clave: Aclimatación, agua transpirable del suelo, estrés hídrico, leguminosas forrajeras tropicales, respuesta fisiológica.

Introduction

Low soil moisture level is regarded as the main environmental factor limiting plant survival and crop production worldwide (Passioura 2007; Lambers et al. 2008), and is considered the abiotic factor most responsible for variations in dry matter yield among forage plants (Barker and Caradus 2001; Islam and Obour 2014).

Among interrelated effects of water deficit on plant functioning are: a decrease in cell division and extension; reduction of net CO_2 assimilation by either stomatal or non-stomatal limitations; reduced shoot and/or root growth; and alteration of water relations, oxidative defense, mineral nutrition and water use efficiency (Turner and Begg 1981; Ludlow 1989; Farooq et al. 2012).

Among perennial plants (including herbaceous grasses and legumes), 2 basic strategies to survive water deficits have been identified, i.e. high and low tissue sensitivity to dehydration (Fisher and Ludlow 1984; Ludlow 1989). High sensitivity to water deficit involves an “escape” or “evasion” strategy; plants of this functional group close stomata (see below) at the onset of drought to control water loss and maintain higher leaf water potentials during the drought spell. In addition, turgor loss and tissue death can be delayed/avoided by other plant traits associated with efficient use of soil water, e.g. deep and extensive root systems, low or high root-stem hydraulic conductivity, reduced leaf and canopy area and leaf movements, until air evaporative demand can no longer be balanced by soil water uptake (Blum 2005; Bacelar et al. 2012). Plants with low sensitivity to soil desiccation can “tolerate” progressive drought stress mainly by physiological mechanisms. Here, a significant decrease of leaf water potential may occur, while tissue turgor and hence CO_2 exchange for growth are maintained by osmotic adjustment or changes in cell wall elasticity (Bacelar et al. 2012; Sanders and Arndt 2012). In this case, plant growth may or may not be affected (Bacelar et al. 2012).

Since stomatal closure is the most rapid and effective mechanism to reduce early water loss during drought, “isohydric” plants show a very effective stomatal control during the day, to maintain high leaf water potential values within a narrow range and are thus less exposed to hydraulic failure as drought develops. This plant response is classified as an evasive strategy. On the contrary, “anisohydric” plants can tolerate more negative leaf water potentials and maintain photosynthesis (though with the penalty of continuous water loss) as soil water deficit increases (McDowell et al. 2008; Vilagrosa et al. 2012). Conservative water usage may imply high water use efficiency and probability of survival during relatively short-term events of low soil moisture, as long as avoidance strategies sustain tissue hydration. Anisohydric plants are predictably more resistant to xylem cavitation, thus displaying essentially tolerant mechanisms to cope with soil water deficits. This may sustain C fixation and growth at very low soil water levels during prolonged drought periods, until tissue turgor can no longer be sustained (Bacelar et al. 2012; Vilagrosa et al. 2012).

Despite this broad separation of water and C economy into 2 functional types, species may respond with a combination of structural and physiological traits of either short- or long-term-adaptive value (Baruch and Fisher 1991; Pang et al. 2011; Rao 2014).

Schultze-Kraft and Clements (1990; and references therein), Keller-Grein et al. (2000) and Rodríguez et al. (2003) noted the importance of *Centrosema* as a forage source for improving pasture productivity in the Neotropics. Nevertheless, to our knowledge, there is little published work linking potential for dry matter production in species of this forage legume and plant strategies to cope with water stress under drought conditions. Pot and field studies on several *Centrosema* species and related forage legumes (Ludlow et al. 1983; Fisher and Ludlow 1984; Sheriff et al. 1986) confirmed the above contrasting responses to drought: a) high tissue sensitivity to reduction in soil water potential, with complete stomatal closure at relatively high leaf water potentials (i.e. ± -1.9 MPa), and other common plant traits typical of drought avoider plants (e.g. *Macroptilium atropurpureum* cv. Siratro) such as deep rooting and early leaf senescence; and b) low tissue sensitivity to reduction in soil water potential, with water potentials at zero-leaf conductance within the range: -4.2 to -8.0 MPa, and variable levels of osmotic adjustment associated with drought-tolerant plants, e.g. *C. brasilianum*, *C. molle* and *C. pascuorum*. Meanwhile, several combinations of avoidance (escape) and tolerance characters within *Centrosema* species have been reported (Clements 1990; Guenni et al. 2007). They can vary from an annual life cycle, rapid growth rate, narrow leaflets, high seed production and tolerance of very low water potentials (*C. pascuorum*), to perennial behavior, slow growth, thickened leaves and the presence of underground storage organs (*C. venosum*).

In this field experiment over 3 consecutive dry seasons, we studied rate of moisture depletion from the initial stored soil water, and the relationship between soil water content and leaf water relations and photosynthesis of 2 herbaceous perennial tropical forage legumes: *C. molle* (formerly known as *C. pubescens*) and *C. macrocarpum*. These legumes were selected because they represent two of the most promising forages for animal feeding in savanna ecosystems. The aim of the study was to identify those plant characters involved in dry matter production and acclimation to progressive drought conditions, and to assess the role of particular plant strategies in enabling these forage legumes to persist in seasonally dry tropical environments.

Materials and Methods

Site, weather conditions and soil measurements

The field study was performed at the Animal Production Institute, Agronomy Faculty of Universidad Central de Venezuela (UCV), Maracay (10º16´ N, 67º36´ W; 459 masl), during 3 consecutive years (2011–2013). According to Köppen-Geiger classification, the local climate is regarded as tropical dry to sub-humid (Aw), with a mean annual rainfall (1995–2015) of 1,074 mm (USICLIMA 2016). Around 95% of the annual precipitation falls between April and November (wet period), with a dry period extending from December to March. Average minimum and maximum temperatures (1995–2015) are 19.5 ºC and 32.1 ºC, respectively (USICLIMA 2016). Daily temperatures during the experiment were recorded at the meteorological station of the Institute of Agricultural Engineering (UCV), located about 1 km from the experimental site. Rainfall data were collected from an automatic station installed nearby (ca. 300 m from the field plots). Plant and soil measurements were restricted to the dry period of each year.

The soil is classified as Mollisol (Fluventic Haplustoll, Soil Survey Staff 2014) that presented no mechanical or chemical restrictions to rooting depth. Soil samples (0–20 cm depth) were collected and analyzed to give mean values (n = 3) of the following properties: loam to loamy-sand texture; pH (1:2): 5.5; EC (dS/m): 0.12; OM (%): 1.8; P (Mehlich I extraction), K, Ca and Mg: 36, 23, 847 and 327 mg/kg, respectively. From 5 different soil-pits located across the experimental site, non-disturbed soil samples were taken at the following depths: 10, 20, 30, 40, 60 and 100 cm, and bulk density determined in the laboratory. The tension plate technique was used to develop the water retention curve at each of the previous soil depths. Bulk density values were used to transform gravimetric water content to volumetric soil water content (θ, v/v). Total transpirable soil water (TSW) in each soil layer was calculated as: θ at field capacity (FC, Ψ_p^m = -0.01 MPa) minus θ at permanent wilting point (PWP, Ψ_p^m = -1.5 MPa).

Species, plant establishment and plot management

Seed of 2 *Centrosema* accessions [*C. molle* Mart. ex Benth. CIAT 15160 (cv. Barinas), and *C. macrocarpum* Benth. CIAT 5713] was scarified with sand paper and sown on plastic trays containing a commercial organic substrate; then seedlings were maintained in a greenhouse with daily irrigation. In July 2010, 45-day-old seedlings were transplanted into the field. Field plots (4.4 x 3.6 m) were planted at a density of about 60 plants/m^2. After 30 days of growth, plants within a plot were supplied with a mixed fertilizer (12:11:18:3:9) of N:P:K:Mg:S at 200 kg/ha. Weeds were controlled by hand. By December 2010 plots were considered established and the first uniformity cut (±20 cm height) was performed by using a mower, with fertilizer applied at the same rate as before. At the beginning of 2011, a second uniformity cut was applied 30–40 days before day 0 (when measurements started). During this regrowth period, all plots were watered weekly with an irrigation hose (≈ 6–8 mm of water). Additionally, the whole experimental area was flooded a week before day 0 in order to bring the entire soil profile to field capacity. The same plot management was performed during 2012 and 2013 before measurements started, but weekly irrigations were increased to 10 mm/plot. The measurement periods were: 10 March–4 May 2011 (47 days); 15 February–12 April 2012 (64 days); and 5 February–11 April 2013 (57 days). During the wet periods of those years, plots were maintained by an annual fertilizer application and regular cuts every 90 days.

Treatments consisted of: 1) half of all plots for each species were maintained at about field capacity down to 20 cm depth, by weekly irrigations (watering/irrigated treatment: WT); and 2) the remaining half of the plots received no watering (drought/unwatered treatment: DT), except for occasional rainfall events.

An access tube was installed at the center of each plot, so soil water contents at 10, 20, 30, 40, 60 and 100 cm depth were monitored once or twice a week, by the use of a PR2/6 sensor probe connected to a data logger (HH2, Delta-T Devices Ltd, Cambridge, UK). For calibration purposes and in order to have some estimate of soil evaporation, in 2011 2 access tubes were installed outside the plots, and bare soil maintained around the tubes. In 2013, an additional control tube was set up near the plots. For each plot and bare soil, soil water content at depth was estimated by converting the sensor probe reading (mv) into θ. In this case, the required bulk density value at depth was obtained from the nearest soil-pit. To compare patterns of change in soil water with depth, the fraction of transpirable soil water [FTSW (mm/mm of TSW) x 100] was plotted over time.

Plant measurements and harvesting

As soon as water treatments started, the central leaflet of the trifoliate leaf immediately below the most recently developed one on a selected stem or stolon, was chosen weekly to record: a) leaf water potential (Ψ_l) at dawn (6.00–7.00 h) during 2011 and 2013, and at midday (11.00–13.00 h) during 2012, by using a pressure pump (3005, Soil Moisture Equipment Corporation, Goleta, CA, USA); b) leaf relative water content (RWC) (Guenni et al. 2004); and c) leaf gas exchange (9.00–11.00 h), with a portable IRGA (CI-340, CID Bio-Science Inc., Camas, WA, USA), during 2011 and Lci-ADC, coupled to a leaf chamber LCA2 (ADC BioScientific Ltd., Hoddesdon, UK) during 2012–2013. Derived measurements from the IRGA included net CO_2 exchange (A, µmol/m^2/s) and stomatal conductance (g_s, mol/m^2/s). At each sampling date, 3–4 leaves per plot were selected for water relations and gas exchange measurements. In addition, by the end of the 2013 drought period, 3 consecutive daily courses of 'chlorophyll a' fluorescence were recorded. After selecting 2–3 leaves per plot, the central leaflets were darkened during the day with special clips and the ratio of variable to maximum fluorescence (F_v/F_m) of Photosystem II (PSII) was then measured from 8.00 to 16.00 h with a portable chlorophyll fluorometer (OS-30p, OPTI Sciences, Hudson, NH, USA).

Field plots were harvested at the end of the dry period in 2011 (± 50 days after the start of DT), and more frequently in 2013: 1, 15, 30 and 45 days after watering ceased in the DT plots. At each harvest, aerial biomass (green leaves, stems and standing dead) was collected from a randomly selected area (0.5 x 0.5 m) within the plots. Plant material was oven-dried (60 °C) for dry matter (DM) determination. A subsample of green leaves was taken to calculate specific leaf area (SLA, cm^2/g), and afterwards the leaf area index (LAI) was derived as leaf DM (g/m^2) x SLA (m^2/g).

Experimental design and data analysis

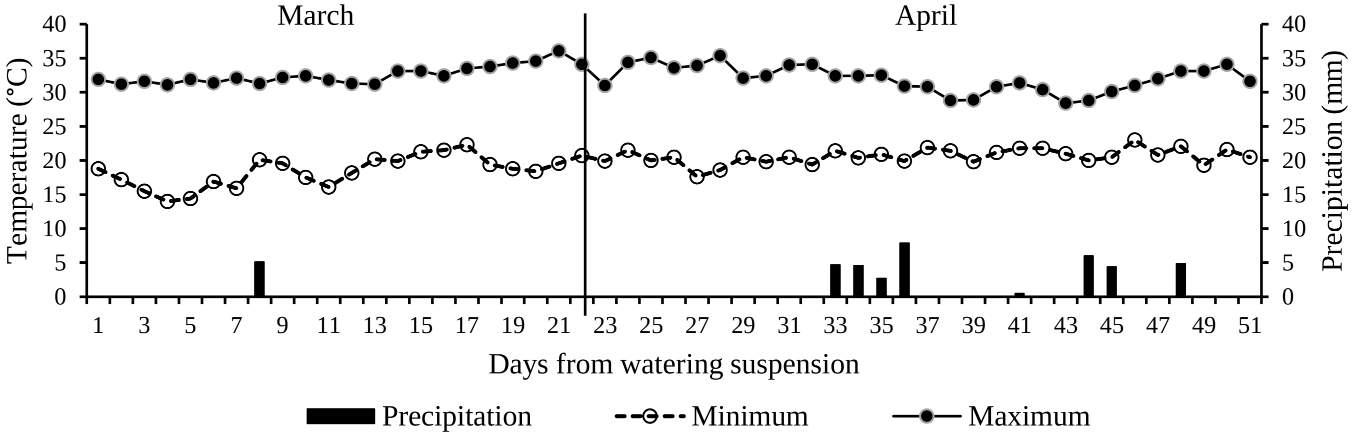
A complete randomized block design was used with 4 repetitions. Each repetition (block) consisted of 4 plots representing the combinations of 2 accessions x 2 irrigation treatments. Since sampling dates differed among years, comparisons between sampling dates, species and treatments were analyzed separately by year, using analysis of variance in accordance with the experimental design. Normality condition of the field data was tested with the UNIVARIATE procedure (Shapiro-Wilk test, SAS Institute 2002). Thus, data were transformed to the $\log_{10}(x+1)$ or arcsine when necessary; otherwise, data were analyzed with non-parametric statistic through the RANK procedure (Friedman test, SAS Institute 2002). The ANOVA procedure was used for balanced data, whereas the GLM procedure (SAS Institute 2002) was applied for unequal number of replicates within treatments. To detect differences between measurement dates, species and treatments, means of all recorded variables were compared with the Tukey's HSD test ($P=0.05$).

Results

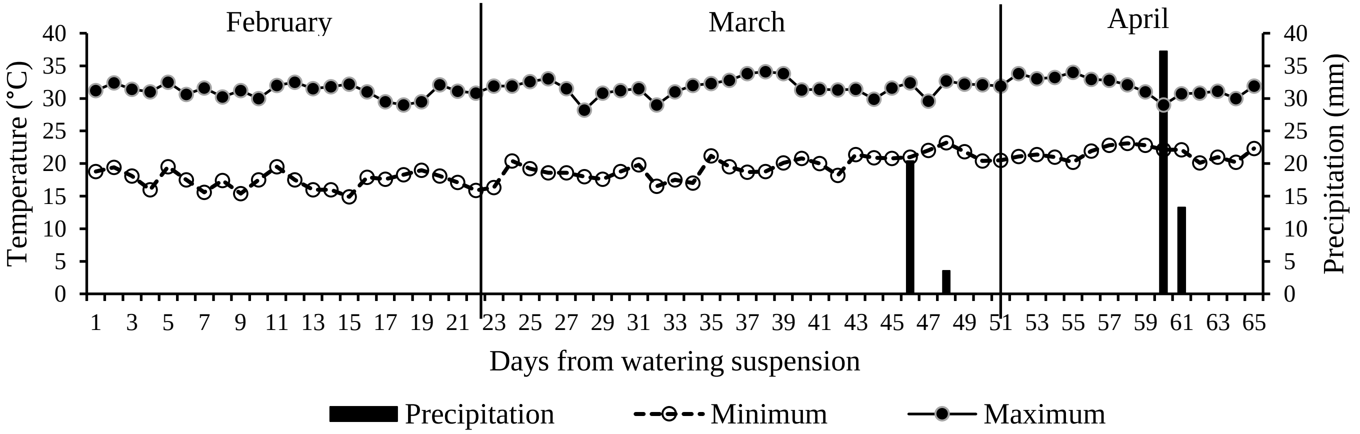
Environmental conditions during the field study

Figure 1 shows the daily maximum and minimum temperatures and precipitation during each evaluation period. Minimum temperatures varied within the range 15–20 °C, whereas maximum temperatures were always above 30 °C, and around 35 °C by 2013 (Figure 1). Isolated rains occurred mostly during the second half of each dry period, totaling 41, 75 and 31 mm for 2011, 2012 and 2013, respectively. Atmospheric evaporative demand estimated as vapor pressure deficit, resulted in mean ± SD daily values of 1.30 ± 0.34, 1.41 ± 0.27 and 1.94 ± 0.25 kPa for the dry periods of 2011, 2012 and 2013, respectively. Therefore, the 2013 measuring period was considered the driest of the 3 evaluated.

(a) 2011



(b) 2012



(c) 2013

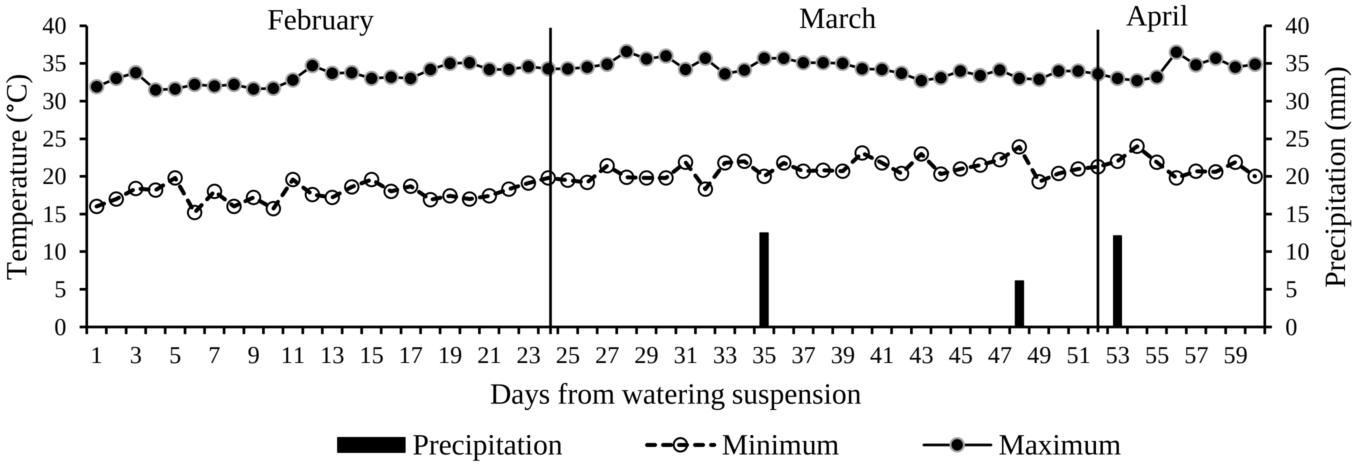


Figure 1. Daily rainfall (mm) and maximum and minimum air temperatures (˚C) during the dry periods of: (a) 2011; (b) 2012; and (c) 2013.

Pattern of water depletion down the soil profile

Total transpirable soil water (TSW) for the entire profile (0–100 cm, n = 5) was 252.3 ± 55.5 mm. The corresponding values for 0–20, 20–60 and 60–100 cm segments of the profile represented 19 (46.8 ± 8.3 mm), 38 (97 ± 30.1 mm) and 43% (108.5 ± 24.9 mm) of the total TSW, respectively.

Across the entire soil profile, moisture reduction in all irrigated (WT) plots was around 20% (Figure 2), with slight increases after isolated rains occurring from day 30 onwards in each dry period (see Figure 1). For *C. molle*, the fraction of available water in unwatered (DT) plots during 2012 and 2013 was almost always significantly lower ($P<0.05$) than in irrigated (WT) plots, and followed more closely the trend over time observed with bare soil (Figures 2b and 2c). Minimum FTSW values in DT plots (Figures 2a–2c) were within the range: 48 (Year 2013) – 58% (Year 2011). This corresponds with maximum losses from initial available moisture of 23 (Year 2011) to 56% (Year 2013). For *C. macrocarpum*, FTSW in DT plots was significantly lower ($P<0.05$) only in 2012, at day 43 after watering ceased and before rain replenished soil water to levels comparable with those in WT plots (Figure 2e). Over all dry periods, minimum FTSWs in DT plots of C. *macrocarpum* were always above 60%, with a 36% maximum reduction of initial available moisture (Figures 2d–2f).

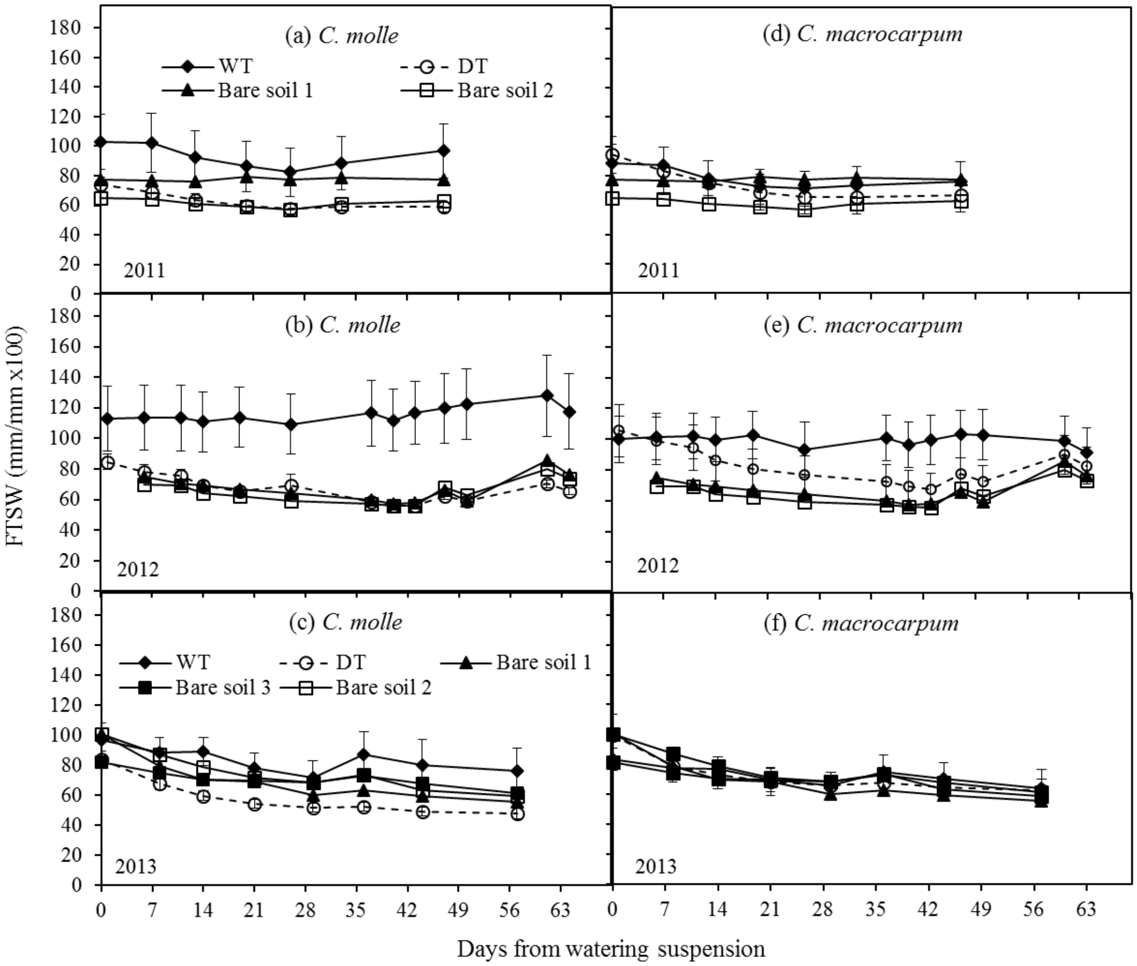


Figure 2. Changes in the fraction of transpirable soil water (FTSW) for the entire profile (0–100 cm), in plots (n = 4) of *C. molle* and *C. macrocarpum* during the dry periods of 2011, 2012 and 2013. WT: irrigated treatment, DT: drought treatment. Recorded values for bare soils (1–2 for years 2011 and 2012; 1–3 for year 2013) are included. Vertical bars denote standard errors.

Major decreases in the fraction of transpirable soil water (FTSW) were recorded at 0–20 cm depth (Figure 3). For both legumes, the pattern of soil moisture loss in 2011 was similar between treatments (Figures 3a and 3d), with minimum FTSW values for DT plots, which were in the range: 39 (*C. macrocarpum*) to 56% (*C. molle*). Accordingly, maximum percentages of moisture loss were 70 and 53% of initial stored soil water, respectively. For the drier periods of 2012 and 2013, reductions in the initial available soil moisture were more evident. Regardless of species, significant differences ($P<0.05$) were found between WT and DT plots within a week of watering suspension in the DT plots (Figures 3b–3f). Lowest values of FTSW were recorded in 2013 (13–20%), followed by 2012 (25–30%). By 2013, this implied a near complete depletion of soil moisture at 20 cm depth (85–90% reduction of initial TSW). The sudden and large increases in FTSW at this depth in both years were the result of isolated showers (see Figure 1). The unexpected and significant decreases of available water in the WT plots were probably related to insufficient irrigation to counter evapotranspiration. However, these drops of FTSW did not affect water relations in either legume (as discussed below). The relatively higher differences in the time trend of FTSW among bare soils at 0–20 cm (Figure 3) when compared with 0–100 cm (Figure 2) may reflect a much higher soil variability in water holding capacity in the upper soil layers.

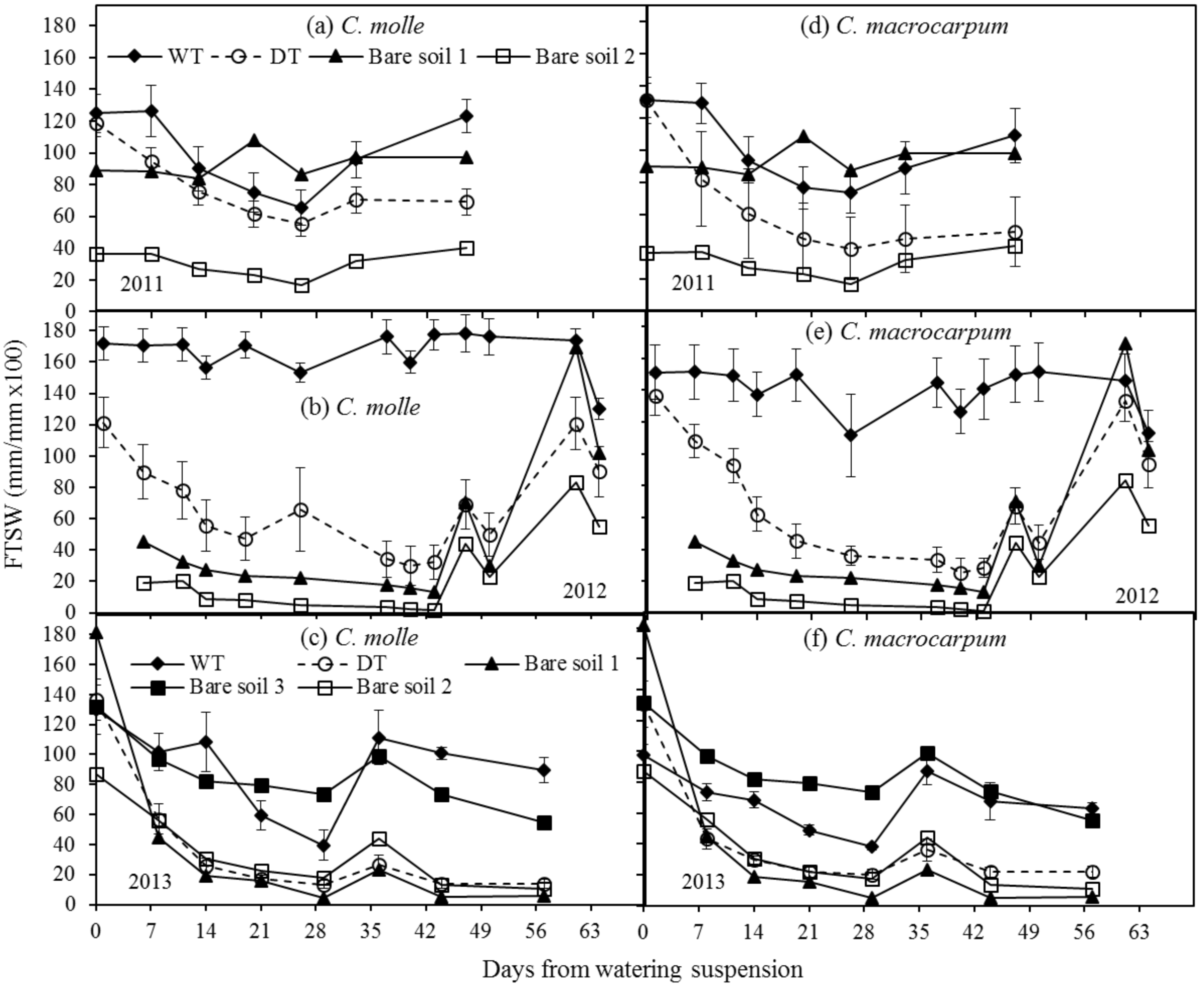


Figure 3. Changes in the fraction of transpirable soil water (FTSW) for the topsoil (0–20 cm), in plots (n = 4) of *C. molle* and *C. macrocarpum* during the dry periods of 2011, 2012 and 2013. WT: irrigated treatment, DT: drought treatment. Recorded values for bare soils (1–2 for years 2011 and 2012; 1–3 for year 2013) are included. Vertical bars denote standard errors.

At 20–60 cm depth, changes in FTSW over time were similar for both legumes, with no significant differences (P>0.05) between WT and DT plots and relatively small losses of stored moisture (15–30% reduction across years) (data not shown). Changes in FTSW for WT and DT plots were very similar to those in bare soils, and changes in soil water content at 60–100 cm depth were negligible (data not shown). Smaller differences in FTSW among bare soils indicated a rather lower soil variation in texture of these soil layers.

Dry matter yield and biomass partitioning, SLA and LAI

In general, total forage yields were 37–207% greater in 2013 than 2011. By the end of the 2011 dry period, aerial biomass of both species and treatments was relatively similar among plots, varying from 93 ± 35 g/m^2 (*C. molle*, WT) to 120 ± 14 g/m^2 (*C. macrocarpum*, DT) (Table 1). Similarly, partitioning of shoot biomass was not affected by drought (P>0.05, Table 1). Dry matter yields of green leaf, stem and standing dead varied, respectively, within the ranges: 53 ± 24 (*C. molle*, DT) to 74 ± 16 g/m^2 (*C. macrocarpum*, DT); 27 ± 10 (*C. molle*, WT) to 45 ± 8 g/m^2 (*C. macrocarpum*, DT); and 2 ± 2 (*C. macrocarpum*, DT) to 10 ± 5 g/m^2 (*C. molle*, WT) (Table 1), while leaf:stem ratio remained relatively constant (1.7–2.3). In each species, SLA and LAI did not differ significantly between watering treatments (P>0.05). Overall, SLA varied from 220 ± 26 (*C. macrocarpum*, DT) to 310 ± 33 cm^2/g (*C. molle*, WT), whereas the corresponding range for LAI was 1.3 ± 0.6 (*C. molle*, DT) to 1.7 ± 0.6 (*C. molle*, WT; *C. macrocarpum*, WT and DT) (Table 1).

For the 2013 harvest, differences in forage biomass were more evident, with WT plots of *C. macrocarpum* having a higher LAI, and yielding more than *C. molle* (P<0.05, Table 1). Differences between WT and DT were present only in *C. macrocarpum*, especially after 30 days of regrowth (data not shown), with nearly 50% reduction of LAI and above-ground biomass (P<0.05) by the end of the experiment (day 45, Table 1). Lower forage yields in DT plots were associated with proportional reductions in green leaf and stem DM; thus the leaf:stem ratio (1.2 ± 0.1) remained unaffected (P>0.05) by low soil moisture (Table 1). Likewise, leaf morphology (expressed by SLA) in both legumes was not altered by drought, with mean values within the range 202–262 cm^2/g (Table 1). However, DT plants of *C. macrocarpum* produced lower (P<0.05) leaf area per unit dry weight than *C. molle* (Table 1). The highest production of dead biomass (25 g/m^2) was recorded in DT plots of *C. molle*, in comparison with only 4–6 g/m^2 in *C. macrocarpum* (Table 1).

Leaves of both legumes showed paraheliotropic movements during the day: the 3 leaflets of each trifoliate leaf folded parallel to the direction of incoming solar radiation (Plate 1). Daily leaf movements in both species started earlier in DT plots.

Table 1. Leaf, stem, standing dead and total aerial biomass (g/m^2), leaf:stem ratio (L:S), specific leaf area (SLA, cm^2/g) and leaf area index (LAI) of 2 *Centrosema* species. Data were recorded from plots harvested at 47 (2011) and 45 days (2013) after watering of the drought treatment ceased. WT: irrigated treatment, DT: drought treatment.

Year	Species/ treatment	Leaf	Stem	Dead	Total	L:S	SLA	LAI
2011	*C. molle*							
	WT	56.5aA[1]	26.8aA	9.9aA	93.1aA	2.2aA	310.2a	1.7a
	DT	53.3aA	29.7aA	3.8aA	104.2aA	1.9aA	250.3a	1.3a
	C. macrocarpum							
	WT	70.8aA	36.3aA	2.0aB	109.1aA	2.3aA	235.5a	1.7a
	DT	74.0aA	44.8aA	1.5aA	120.2aA	1.7aA	220.0a	1.7a
2013	*C. molle*							
	WT	84.7aB	83.4aB	5.9aA	174.1aB	1.0aB	250.8aA	2.2aB
	DT	54.2aA	63.5aA	24.9aA	142.6aA	0.9aB	261.7aA	1.4aA
	C. macrocarpum							
	WT	177.7aA	143.5aA	12.8aA	334.0aA	1.3aA	208.6aA	3.8aA
	DT	83.4bA	76.3bA	3.9aB	163.5bA	1.1aA	201.8aB	1.7bA

[1] For each year and plant trait, different lower- and upper-case letters denote significant differences (P<0.05) between treatments within species, and between species within treatments, respectively.

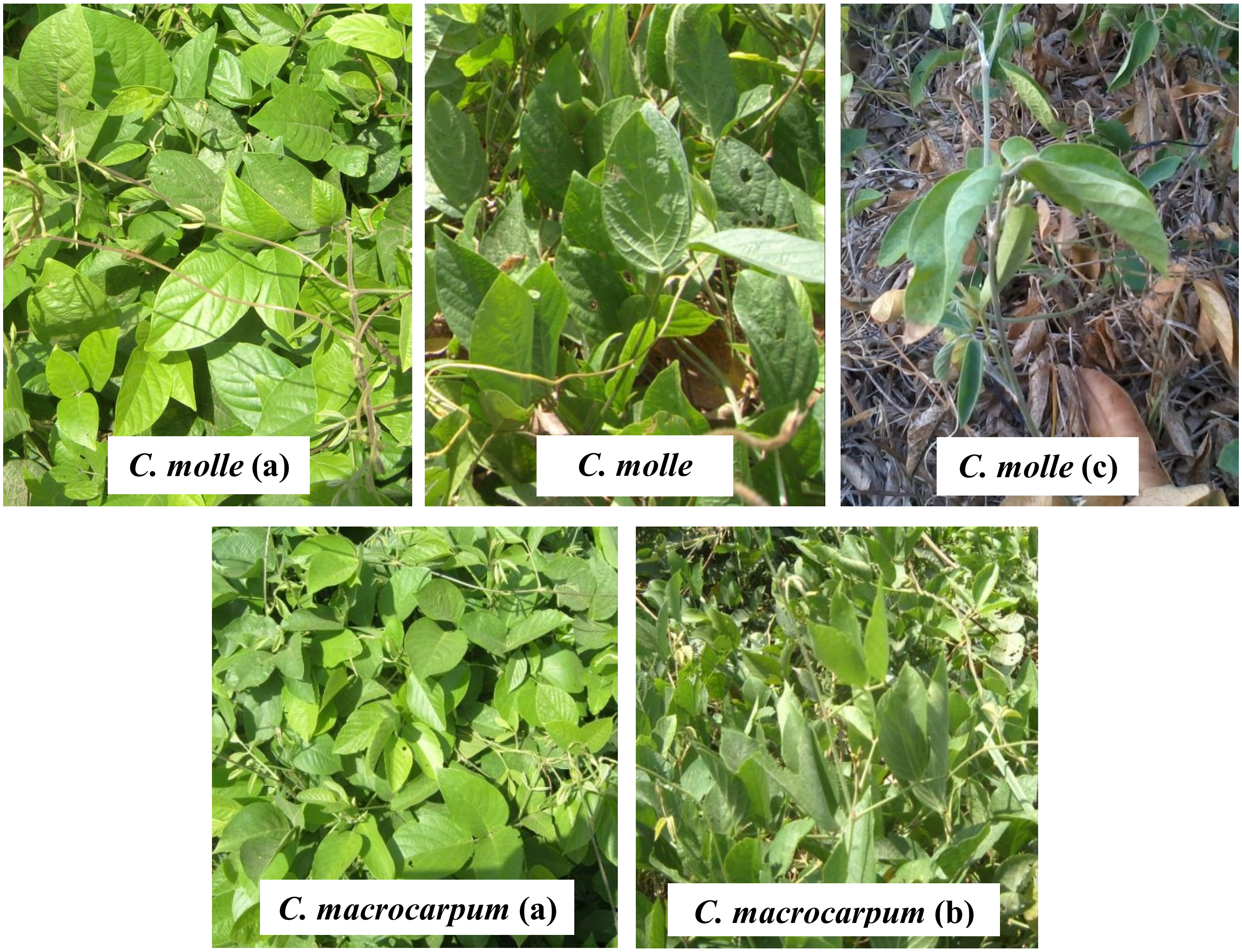


Plate 1. Leaf movements in *C. molle* and *C. macrocarpum*. The corresponding leaf water potentials (Ψ_l) are: (a) -0.2 to -0.1 MPa (well-watered plants); (b) -0.6 to -0.4 MPa (mild water stress); and (c) <-0.7 MPa (severe leaf wilting).

Plant water relations and gas exchange

During 2011 (data not shown), time trends in leaf relative water content (RWC), Ψ_l and net photosynthesis (*A*) were similar between WT and DT plots of both species. However, in DT plots of *C. molle* there was a significant ($P<0.05$) decline in dawn Ψ_l, RWC and *A* a week after watering ceased, when FTSW at 0–20 cm was still above 55–60% (see Figure 3a). Lower values in water relations and *A* persisted for 2 more weeks, when they started recovering in comparison with WT plots due to associated rains at the end of the dry period (Figure 1b). Average leaf RWC, Ψ_l and *A* for the 2 species were within the ranges: 69–90%, -0.8 to -0.3 MPa and 11–25 μmol/m^2/s, with no differences between species.

In 2012, variations in leaf water relations and net photosynthesis within both species followed similar patterns, responding again to changes in soil water content in the upper soil layer. Here, a rather progressive drop in water relations was observed as surface soil dried during the first 4–6 weeks after watering ceased (Figure 4). *Centrosema molle* was more affected by dry soil conditions, DT leaves at day 40 showing significantly ($P<0.05$) lower RWC (75.5 vs. 84.0 %), and *A* (11.1 vs. 15.0 μmol/m^2/s) when compared with leaves on irrigated plants; this resulted in net declines from WT plot values of 10 and 26% in RWC and *A*, respectively (Figures 4a and 4c). In irrigated treatments, minimum midday leaf water potentials varied from -1.25 MPa (*C. molle*) to -0.91 MPa (*C. macrocarpum*). Subsequently, and with the exception of leaf RWC, an increase in midday Ψ_l and *A* in both WT and DT leaves was recorded (Figure 4), in response to the incidence of isolated rains at the end of that dry period (Figure 1b) that recharged surface soil water levels (Figures 3b and 3e).

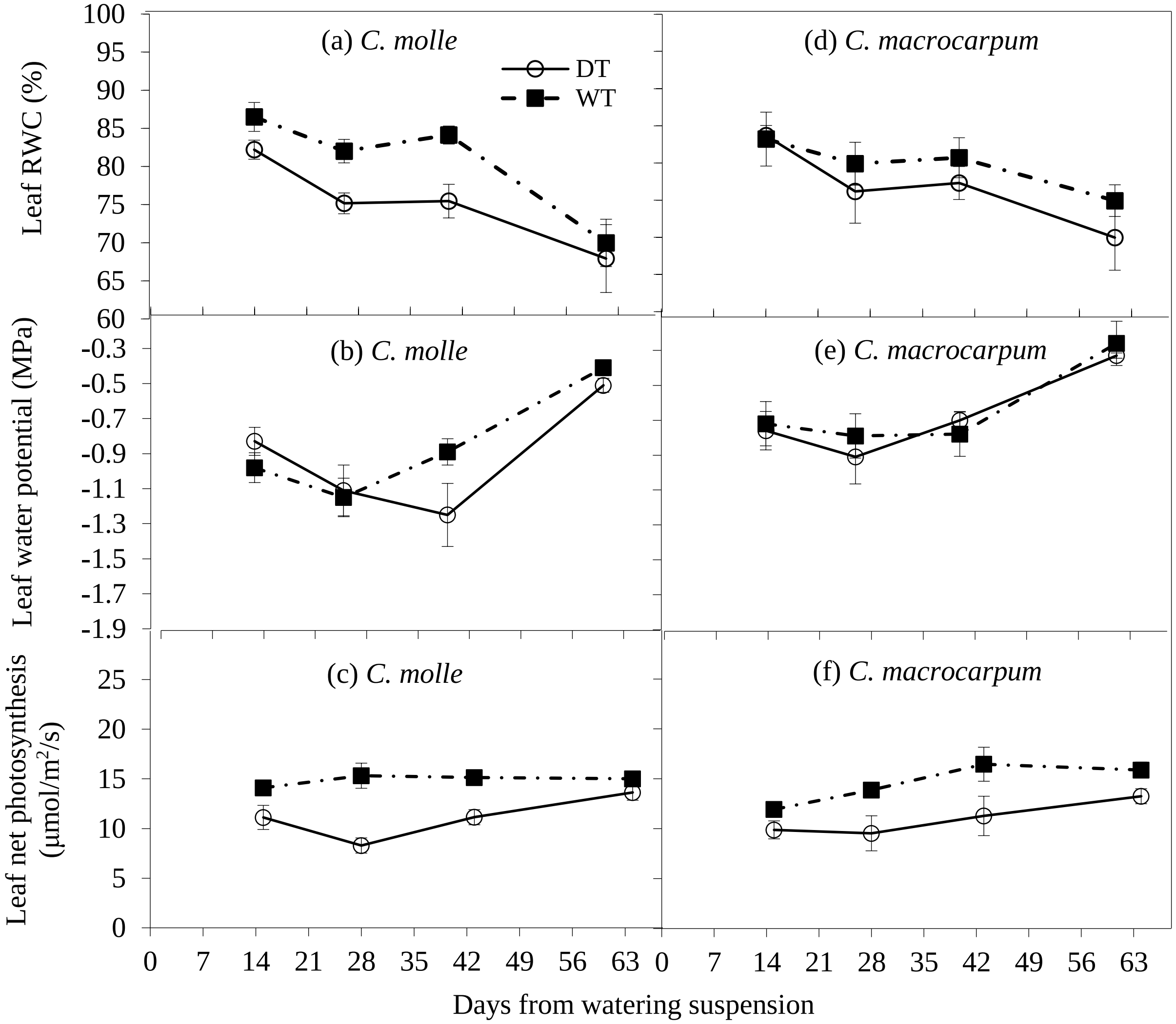


Figure 4. Changes in leaf relative water content (RWC), water potential (Ψ_l) and net photosynthesis (*A*) in *C. molle* and *C. macrocarpum* (n = 12–16) during the 2012 dry period. WT: irrigated treatment, DT: drought treatment. Vertical bars denote standard errors.

During the driest months of 2013, significant declines ($P<0.05$) in water relations and *A* in DT plots occurred as soon as 2–3 weeks after drought conditions were imposed (Figure 5), as surface soil moisture was rapidly depleted following suspension of watering (Figures 3c and 3f). As expected, net photosynthesis began declining earlier when Ψ_l at dawn was still not affected by dry conditions, though FTSW values were already low (i.e. 13%, see Figure 3c). Drought effects were more evident in *C. molle*, for which the greatest differences between WT and DT leaves were observed. Indeed, droughted leaves of *C. molle* showed final percentage reductions (compared with respective values for WT leaves) of 430% in Ψ_l (-0.3 to -1.6 MPa), 18% in RWC (85 to 70%) and 68% in *A* (25 to 8 µmol/m^2/s), in contrast with 60% (-0.25 to -0.4 MPa), 7% (81 to 75%) and 32% (22 to 15 µmol/m^2/s) for *C. macrocarpum*. In addition, RWC, dawn Ψ_l and *A* in droughted leaves of *C. molle* did not recover (Figures 5a–5c) following the isolated rains received by the end (day 45 onwards) of the dry period (Figure 1c). Lower Ψ_l values in this species were also associated with strong leaf wilting (see Plate 1).

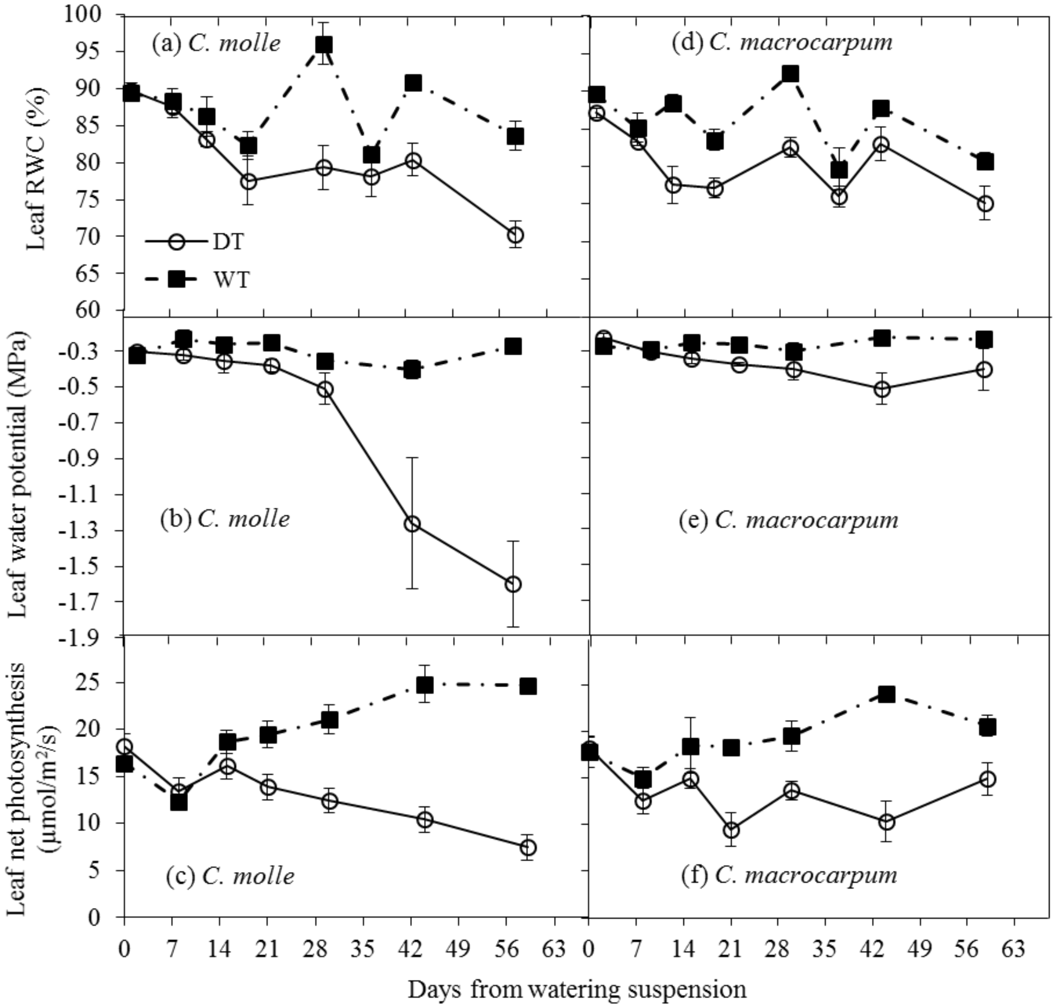


Figure 5. Changes in leaf relative water content (RWC), water potential (Ψ_l) and net photosynthesis (*A*) in *C. molle* and *C. macrocarpum* (n = 12–16) during the 2013 dry period. WT: irrigated treatment, DT: drought treatment. Vertical bars denote standard errors.

The F_v/F_m ratio showed a common trend of decreasing by midday, with a slight recovery late in the afternoon. However, the accumulated effects of drought on chlorophyll a fluorescence appeared to be more detrimental for *C. molle* than *C. macrocarpum*. By day 55 of drought (year 2013), the lowest value of the F_v/F_m ratio in stressed plants of *C. molle* was 0.53 compared with 0.70–0.75 in irrigated plants ($P<0.05$), with no apparent recovery of PSII by the end of the day. By comparison the respective daily values for *C. macrocarpum* were always above 0.70, with no significant differences ($P>0.05$) between watering treatments (Figure 6).

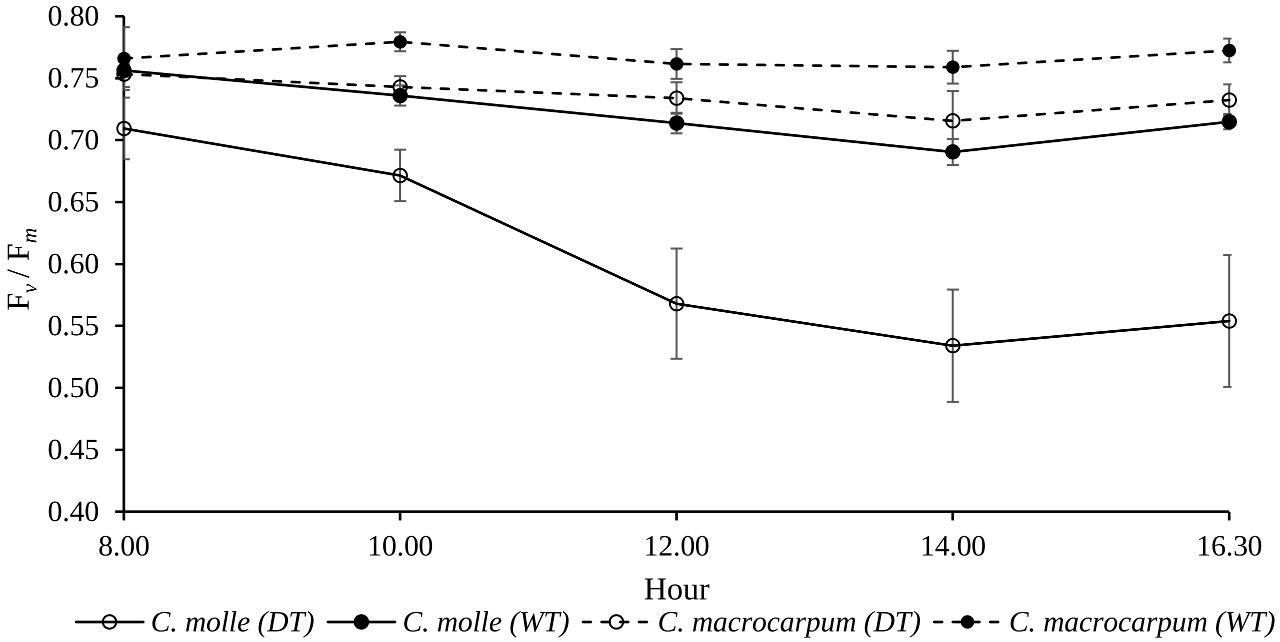


Figure 6. Daily courses of chlorophyll a fluorescence (measured as F_v/F_m ratio) at day 55 of drought (year 2013), in leaves (n = 8–12) of *C. molle* and *C. macrocarpum*. WT: irrigated treatment, DT: drought treatment. Vertical bars denote standard errors.

Data from both species during the 2013 dry period showed a common and rapid decline of stomatal conductance (g_s) within a rather narrow range of leaf water potentials (-0.4 to -0.2 MPa), which was close to that maintained by well-watered plants (Figure 7).

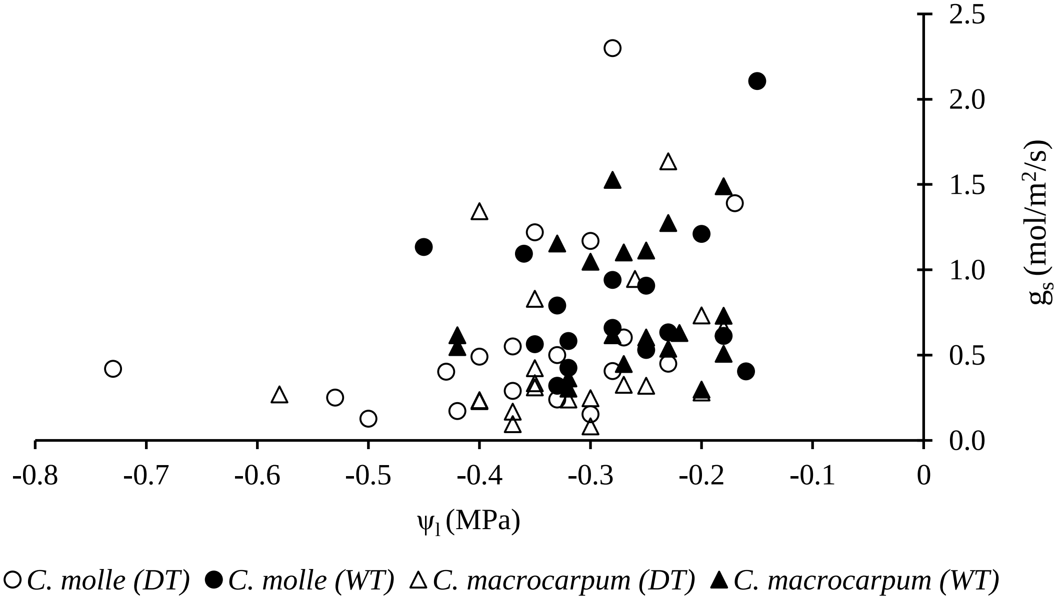


Figure 7. Changes in stomatal conductance (g_s) vs. water potential (Ψ_l), in leaves of *C. molle* and *C. macrocarpum* during the dry period of 2013. Data from irrigated (WT) and unwatered plots (DT) are included. Each value represents a mean from 3–4 leaves per plot.

Discussion

Effects of drought conditions on plants depend on duration, frequency and intensity of water deficits. The 3 consecutive dry periods evaluated in this study simulated this variability as water stress conditions were of variable duration and intensity, because of differences in air evaporative demand and water supply by sporadic rains. Overall, our study showed that *C. molle* and *C. macrocarpum* had a common response to progressive soil water depletion, while some contrasting acclimation traits to cope with long water stress conditions were evident. Some differences between treatments failed to reach significance because of variability in measurements. A larger number of replications may have shown more of the differences as significant.

Growth and dry matter production under water-limited conditions

To optimize C assimilation and usage during drought, DM accumulation is reduced in all plant components, though with different magnitudes (Farooq et al. 2012). In forage plants, a decrease in growth and development of leaf area is the first sign, resulting in smaller leaves and reduced LAI (Sanderson et al. 1997). In addition, rates of leaf and stem production are affected and depend on species and water stress conditions (Likoswe and Lawn 2008). Turgor loss seems the primary factor limiting growth by blocking cell elongation and division (Farooq et al. 2012). Declines in plant growth and DM production in herbaceous legumes can vary between and within species (Likoswe and Lawn 2008; Pang et al. 2011) and in this study the magnitude of the reductions in DM production appeared to vary according to plant age. The absence of effects on DM yields in both legumes in 2011 may be a consequence of transpiration demands of the young plants (leaf:stem ratio about 2.0) being fully supplied by the stored soil water. On the contrary, during the drier period of 2013 larger plants, especially those exposed to continuous drying, demanded more water than was available in the upper soil layers. Consequently, for *C. macrocarpum* reductions in growth of leaf and stem on unwatered plots resulted in lower forage yields than on irrigated plots. We expected similar results in *C. molle*, since field observations showed an even higher level of wilting and leaf senescence in unwatered plots of this legume, which was associated with a larger decline in water relations and gas exchange after 40 days of soil water stress (see below). As mentioned earlier, high variability in forage biomass (with a variation coefficient in shoot DM near 70% among replicates) may explain the lack of significant differences in DM production between WT and DT plots of this species.

Morphological traits contributing to drought acclimation in the field

Drought evasion is related to the ability to sustain initial plant water status for a longer time by increasing access to deep soil water or minimizing water losses through transpiration (Blum 2005), and plasticity in leaf expansion, transpiration control and root proliferation are essential for drought resistance (Turner and Begg 1981).

During the first dry period, small variations in LAI within legumes were the result of very small variations in SLA and leaf biomass among treatments. In this case, comparable patterns of soil water extraction with time may suggest similar plant responses between watering treatments in response to evapotranspiration demand. On the contrary, during the driest evaluation period, the smaller accumulation of LAI in unwatered plots of *C. macrocarpum* when compared with irrigated plots, was due to reduced C investment in assimilatory (leaves) and supporting organs (stems), resulting in reduced leaf area production per unit dry weight (SLA). Therefore, it appears that *C. macrocarpum* controlled transpiration primarily by a reduction in leaf area, since leaf senescence appeared to be delayed by the relatively higher level of leaf stiffness (De Micco and Aronne 2012) present in this species. Conversely, field observations indicated a shorter leaf lifespan and a higher level of leaf turnover in *C. molle*. These species appeared to use different strategies to cope with water deficit, i.e. increased leaf wilting and senescence in *C. molle*, and a decline in leaf biomass production in *C. macrocarpum*. Under water-limited conditions, SLA decreases due to active accumulation of cell wall components (Barker and Caradus 2001), which aids in reducing transpiration (De Micco and Aronne 2012; Farooq et al. 2012). However, this study showed the low plasticity of SLA (or alternatively, LMA: leaf mass area) to reduced water availability. Indeed, in forage grasses and legumes, opposite effects of water stress on cell wall contents have been reported (Wilson 1983; Sanderson et al. 1997; Guenni et al. 2002). Hence, in these *Centrosema* species, parallel modifications of equal magnitude in leaf area and cell wall content may result in SLA (or LMA) having low potential for acclimation to drought. Under water stress, reduction in relative growth rate (RGR) has been positively related to decrease in SLA, leaf mass ratio (LMR) and leaf area ratio (LAR), though there is a high variability of responses among species and growth habits (Lambers et al. 2008; Pang et al. 2011).

An alternative approach to diminish transpiration without compromising surface area for gas exchange is through a daily change in leaf orientation angle (Turner and Begg 1981; De Micco and Aronne 2012). Leaflets of both *Centrosema* species turned to almost vertical orientation by midday (Plate 1). As soil water depletion advanced during the dry period, this condition became more obvious (starting earlier during the day) in unwatered plants. This active leaf movement allows a reduction in heat load on the leaf, and is common in arid environments (De Micco and Aronne 2012), as well as in many cultivated plants, including forage legumes (Fisher and Ludlow 1984; Bell et al. 2007; Pang et al. 2011).

Another complementary strategy to delay negative effects of soil drying is to increase access to deep water in the soil profile, especially when combined with higher stomatal control (Sheriff and Ludlow 1984; Wang and Yamauchi 2006). In this study, though we did not measure root growth and distribution, the pattern of water depletion down the soil profile provided an insight of particular strategies for water use during drought. In both *Centrosema* spp. water needs were rather low. Indeed, most of the extracted soil water (driven mainly by evaporative demand) came apparently from the upper (20 cm) layer, which represented only about 20% of the initial moisture available in the soil profile. This was followed by a much smaller usage from the 20–60 cm stratum, with no apparent utilization of water deeper in the soil profile. This pattern of water usage may not represent a typical evasion strategy for these species with a tap root system, as deep root systems or increased root biomass/lengths at depth in herbaceous legumes (Pang et al. 2011) and grasses (Guenni et al 2004; Cardoso et al. 2015) are traits which potentially improve water extraction. Certainly, this pattern of water usage is opposite from what has been reported for these and other tropical forage legumes growing on deep sandy soils (Guenni et al. 2007). A predominantly sandy soil with lower water retention capacity may induce more root growth to extract water from deeper soil horizons (Guenni et al. 2007). For the loamy soil characteristic of this study, water utilization from only the upper layers appeared to be sufficient to maintain minimum growth and compensate for transpiration demands, but with a possible metabolic cost associated with root nodule activity, which have shown to be adversely affected by water stress (Wery et al. 1986; Silveira et al. 2001). Overall, conservative use of stored water, rather than water extraction from deeper in the profile, was likely to be more advantageous to maintain forage production under the soil conditions of the experimental site. Ultimately, plant survival will depend upon access to deep soil moisture.

Physiological acclimation to drought conditions in the field

Both *Centrosema* species showed a drop in water relations and leaf gas exchange with prolonged drying of soil in the absence of irrigation, similar to other forage and grain legumes (Ludlow et al. 1983; Collinson et al. 1997; Hamidou et al. 2007; Bell et al. 2007; Pang et al. 2011). Without an apparent loss of turgor in leaf tissue during the first 4–5 weeks of the drying cycles, stomatal conductance was very sensitive to a given change in leaf water potential, though net CO_2 exchange was affected only when a significant drop in leaf water content occurred. These effects were again, both time- and species-dependent.

Overall, detrimental effects of soil water deficit were more obvious when the dry period was more severe, with surface FTSW being depleted below a threshold value (ca. ± 25%). Under such conditions, stomatal conductance and photosynthesis in *C. molle* had the highest reductions (about 70%), with predawn leaf water potentials <-0.7 MPa and decreases in relative water contents to 70% at the end of the drying cycle. In contrast, the lower reduction (± 40%) in gas exchange in *C. macrocarpum* was always associated with leaf water potentials and RWCs greater than -0.5 MPa and 70%, respectively. Similar fluctuations in water relations and photosynthesis for these and other herbaceous perennial legumes have been reported elsewhere (Guenni et al. 2007; Pang et al. 2011). Interestingly, Guenni et al. (2007) reported lower reductions in these physiological traits in *C. macrocarpum* when grown in a deep sandy soil during the dry season. This suggests an effective control of water loss as found in *Macroptilium atropurpureum* cv. Siratro (Fisher and Ludlow 1984; Pang et al. 2011), though at the expense of reduced DM production (Turner and Begg 1981; Farooq et al. 2009; Pang et al. 2011).

Therefore, the proposed less effective control of water loss in *C. molle* resulted in a rapid drop in C assimilation as soil continued drying. Subsequently, as stated for C3 plants by Flexas and Medrano (2002), Medrano et al. (2002) and Farooq et al. (2009), a damage to the biochemistry of photosynthesis occurred later as drought became more intense, when water reserves at the soil surface reached minimum values and leaf water potentials were much lower than in *C. macrocarpum*. This situation may trigger considerable leaf senescence to prevent further water losses, thus affecting production of green forage for the remaining dry period. Nevertheless, biochemical damage to the photosynthetic quantum efficiency caused by photo-inhibition under water stress (Pastenes et al. 2005; Lauriano et al. 2006), and possibly increased by high radiation and/or temperature conditions (Lambers et al. 2008), seemed to be easily reversible, due to the rapid regrowth of *C. molle* after the onset of the rainy season.

As no visual wilting was observed in *C. macrocarpum* even after 40 days of low soil moisture levels, it is proposed that leaf turgor and higher leaf water potentials and photosynthetic rates at harvest were sustained mostly by a strong leaf isohydric behavior (Lambers et al. 2008; Limpus 2009). On the other hand, osmotic adjustment in *C. molle* was likely to be more responsible for the maintenance of minimum photosynthesis after the sharp drop in leaf water potential and RWC, which occurred nearly 4–5 weeks after the imposition of water stress. Furthermore, leaves of this species may have been slowly preconditioned for osmotic adjustment during previous milder water stress conditions. Evidence of dehydration tolerance through osmotic adjustment exists in a range of *Centrosema* species (Ludlow et al. 1983; Fisher and Ludlow 1984; Guenni et al. 2007). This physiological strategy may be important as the intensity of water deficits increases, allowing the maintenance of cell turgor and hence soil moisture extraction for minimum photosynthetic activity and biomass production during the last part of the drying cycle (Ludlow et al. 1983; Pang et al. 2011; Farooq et al. 2012). Furthermore, as many species respond at a rather lower range of critical water potentials and a single species can even shift between isohydric and anisohydric behavior (Maseda and Fernández 2006; Limpus 2009), leaves of *C. molle* might have then behaved as partially isohydric (or anisohydric) at more negative soil matrix potentials. This acclimation to water stress should be accompanied by a reduction in leaf area and narrower xylem conduits to avoid embolism and sustain a balance between growth and water loss by transpiration at very low water potentials (Maseda and Fernández 2006; Bresta et al. 2011). The possible link between osmotic adjustment and induced structural changes in xylem hydraulics to drive survival of both *Centrosema* species under prolonged dry conditions deserves more investigation.

Conclusions

This study has confirmed that, to survive in seasonally dry tropical environments, both species of *Centrosema* exploited a combination of water stress postponement strategies and some degree of tolerance of progressive soil drying. In our study, both legumes, in spite of having a taproot system, extracted water mainly from the topsoil, while regulating further water usage from deep storage in the soil profile by a primary control of stomatal opening and the size of leaf area exposed to solar radiation. Subsequently, though not explored in this study, tolerance mechanisms aided by osmotic adjustment and perhaps a reduced vulnerability to embolism, complement the set of plant strategies for survival under seasonal water stress conditions. However, the present results suggest that the expression of such combinations of adaptive traits depends on species plasticity, and the duration and intensity (magnitude) of seasonal low soil moisture conditions. Therefore, the observed expression of morpho-physiological characters and forage production responses to seasonal drought results in enhanced *C. molle* performance in tropical pastoral areas with relatively short dry periods, and *C. macrocarpum* having an advantage in environments with longer dry periods as indicated by Cook et al. (2005). Further studies with a range of accessions/genotypes drawn from different geographical sites within the natural distributions of both species are warranted to corroborate and complement the present findings.

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References

Bacelar ELVA; Mountinho-Pereira JM; Gonçalves BMC; Brito CVQ; Gomes-Laranjo J; Ferreira HMF; Correia CM. 2012. Water use strategies of plants under drought conditions. In: Aroca R, ed. Plant responses to drought stress: From morphological to molecular features. Springer-Verlag, Berlin, Germany. p. 145–170. DOI: 10.1007/978-3-642-32653-0_6

Barker DJ; Caradus JR. 2001. Adaptation of forage plants to drought. In: Proceedings of the XIX International Grassland Congress, São Pedro, SP, Brazil. p. 241–245. (Available at: https://goo.gl/53tGDo).

Baruch Z; Fisher MJ. 1991. Factores climáticos y de competencia que afectan el desarrollo de la planta en el establecimiento de una pastura. In: Lascano CE; Spain JM, eds. Establecimiento y renovación de pasturas: Conceptos, experiencias y enfoque de la investigación. Sexta reunión del comité asesor de la Red Internacional de Evaluación de Pastos Tropicales (RIEPT). CIAT, Cali, Colombia. p. 103–142. (Available at: https://goo.gl/FMT54U).

Bell LW; Williams AH; Ryan MH; Ewing MA. 2007. Water relations and adaptations to increasing water deficit in three perennial legumes, *Medicago sativa*, *Dorycnium hirsutum* and *Dorycnium rectum*. Plant and Soil 290:231–243. DOI: 10.1007/s11104-006-9155-7

Blum A. 2005. Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research 56:1159–1168. DOI: 10.1071/AR05069

Bresta P; Nikolopoulos D; Economou G; Vahamidis P; Lyra D; Karamanos A; Karabourniotis G. 2011. Modification of water entry (xylem vessels) and water exit (stomata) orchestrates long term drought acclimation of wheat leaves. Plant and Soil 347:179–193. DOI: 10.1007/s11104-011-0837-4

Cardoso JA; Pineda M; Jiménez JC; Vergara MF; Rao IM. 2015. Contrasting strategies to cope with drought conditions by two tropical forage C_4 grasses. AoB Plants 7:plv107. DOI: 10.1093/aobpla/plv107

Clements RJ. 1990. *Centrosema* species for semiarid and subtropical regions. In: Schultze-Kraft R; Clements RJ, eds. *Centrosema*: Biology, agronomy, and utilization. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 77–97. (Available at: https://goo.gl/bqg3OS).

Collinson ST; Clawson EJ; Azam-Ali SN; Black CR. 1997. Effects of soil moisture deficits on the water relations of bambara groundnut (*Vigna subterranea* L. Verdc.). Journal of Experimental Botany 48(309):877–884. DOI: 10.1093/jxb/48.4.877

Cook BG; Pengelly BC; Brown SD; Donnelly JL; Eagles DA; Franco MA; Hanson J; Mullen BF; Partridge IJ; Peters M; Schultze-Kraft R. 2005. Tropical forages: An interactive selection tool. [CD-ROM], CSIRO, DPI&F (Qld.), CIAT and ILRI, Brisbane, Australia. (Available at: www.tropicalforages.info).

De Micco V; Aronne G. 2012. Morpho-anatomical traits for plant adaptation to drought. In: Aroca R, ed. Plant responses to drought stress: From morphological to molecular features. Springer-Verlag, Berlin, Germany. p. 37–61. DOI: 10.1007/978-3-642-32653-0_2

Farooq M; Wahid A; Kobayashi N; Fujita D; Basra SMA. 2009. Plant drought stress: Effects, mechanisms and management. Agronomy for Sustainable Development 29:185–212. DOI: 10.1051/agro:2008021

Farooq M; Hussain M; Wahid A; Siddique KHM. 2012. Drought stress in plants: An overview. In: Aroca R, ed. Plant responses to drought stress: From morphological to molecular features. Springer-Verlag, Berlin, Germany. p. 1–33. DOI: 10.1007/978-3-642-32653-0_1

Fisher MJ; Ludlow MM. 1984. Adaptation to water deficits in *Stylosanthes*. In: Stace HM; Edye LA, eds. The biology and agronomy of *Stylosanthes*. Academic Press, Sydney, Australia. p. 163–179. DOI: 10.1016/B978-0-12-661680-4.50013-5

Flexas J; Medrano H. 2002. Drought-inhibition of photosynthesis in C_3 plants: Stomatal and non-stomatal limitations revisited. Annals of Botany 89:183–189. DOI: 10.1093/aob/mcf027

Guenni O; Marín D; Baruch Z. 2002. Responses to drought of five *Brachiaria* species. I. Biomass production, leaf growth, root distribution, water use and forage quality. Plant and Soil 243:229–241. DOI: 10.1023/A:1019956719475

Guenni O; Baruch Z; Marín D. 2004. Responses to drought of five *Brachiaria* species. II. Water relations and leaf gas exchange. Plant and Soil 258:249–260. DOI: 10.1023/B:PLSO.0000016555.53797.58

Guenni O; Gil JL; Baruch Z; Rodríguez I; Tam J. 2007. Relaciones hídricas e intercambio gaseoso en una colección de leguminosas forrajeras cultivadas en las sabanas orientales de Venezuela. Revista de la Facultad de Agronomía (LUZ) 24:348–354. (Available at: https://goo.gl/sbH45f).

Hamidou F; Zombre G; Braconnier S. 2007. Physiological and biochemical responses of cowpea genotypes to water stress under glasshouse and field conditions. Journal of Agronomy and Crop Science 193:229–237. DOI: 10.1111/j.1439-037X.2007.00253.x

Huang B. 2000. Role of root morphological and physiological characteristics in drought resistance of plants. In: Wilkinson RE, ed. Plant–environment interactions. 2nd Edn. Marcel Dekker, New York, USA. p. 39–64. DOI: 10.1201/9780824746568.ch2

Islam MA; Obour AK. 2014. Drought physiology of forage crops. In: Pessarakli M, ed. Handbook of plant and crop physiology. 3rd Edn. CRC Press, Boca Raton, FL, USA. p. 427–440.

Keller-Grein G; Schultze-Kraft R; Franco LH; Ramírez G. 2000. Multilocational agronomic evaluation of selected *Centrosema pubescens* germplasm on acid soils. Tropical Grasslands 34:65–77. (Available at: https://goo.gl/YOtVsj).

Lambers H; Chapin III FS; Pons TL. 2008. Plant physiological ecology. 2nd Edn. Springer, New York, USA. DOI: 10.1007/978-0-387-78341-3

Lauriano JA; Ramalho JC; Lidon FC; Céu Matos M do. 2006. Mechanisms of energy dissipation in peanut under water stress. Photosynthetica 44:404–410. DOI: 10.1007/s11099-006-0043-4

Likoswe AA; Lawn RJ. 2008. Response to terminal water deficit stress of cowpea, pigeonpea, and soybean in pure stand and in competition. Australian Journal of Agricultural Research 59:27–37. DOI: 10.1071/AR07155

Limpus S. 2009. Isohydric and anisohydric characterisation of vegetable crops: The classification of vegetables by their physiological responses to water stress. Report PR09-4248.

Department of Primary Industries and Fisheries, Qld, Australia. (Available at: https://goo.gl/r1KwJi).

Liu F; Jensen CR; Andersen MN. 2003. Hydraulic and chemical signals in the control of leaf expansion and stomatal conductance in soybean exposed to drought stress. Functional Plant Biology 30:65–73. DOI: 10.1071/FP02170

Ludlow MM. 1989. Strategies in response to water stress. In: Kreeb KH; Richter H; Hinkley TM, eds. Structural and functional responses to environmental stresses: Water shortage. Proceedings, XIV International Botanical Congress, Berlin, Germany, 24 July to 1 August 1987: SPB Academic Publishing, The Hague, The Netherlands. p. 269–281.

Ludlow MM; Chu ACP; Clements RJ; Kerslake RG. 1983. Adaptation of species of *Centrosema* to water stress. Australian Journal of Plant Physiology 10(2):119–130. DOI: 10.1071/PP9830119

Maseda PH; Fernández RJ. 2006. Stay wet or else: Three ways in which plants can adjust hydraulically to their environment. Journal of Experimental Botany 57(15):3963–3977. DOI: 10.1093/jxb/erl127

McDowell N; Pockman WT; Allen CD; Breshears DD; Cobb N; Kolb T; Plaut J; Sperry J; West A; Williams DG; Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? New Phytologist 178:719–739. DOI: 10.1111/j.1469-8137.2008.02436.x

Medrano H; Escalona JM; Bota J; Gulías J; Flexas J. 2002. Regulation of photosynthesis of C_3 plants in response to progressive drought: Stomatal conductance as a reference parameter. Annals of Botany 89:895–905. DOI: 10.1093/aob/mcf079

Pang J; Yang J; Ward P; Siddique KHM; Lambers H; Tibbett M; Ryan M. 2011. Contrasting responses to drought stress in herbaceous perennial legumes. Plant and Soil 348:299–314. DOI: 10.1007/s11104-011-0904-x

Passioura J. 2007. The drought environment: Physical, biological and agricultural perspectives. Journal of Experimental Botany 58(2):113–117. DOI: 10.1093/jxb/erl212

Pastenes C; Pimentel P; Lillo J. 2005. Leaf movements and photoinhibition in relation to water stress in field-grown beans. Journal of Experimental Botany 56(411):425–433. DOI: 10.1093/jxb/eri061

Rao IM. 2014. Advances in improving adaptation of common bean and *Brachiaria* forage grasses to abiotic stresses in the tropics. In: Pessarakli M, ed. Handbook of plant and crop physiology. 3rd Edn. CRC Press, Boca Raton, FL, USA. p. 847–889. DOI: 10.1201/b16675-49

Rodríguez I; Flores A; Schultze-Kraft R. 2003. Potencial agronómico de *Centrosema pubescens* en condiciones de sabana bien drenada del Estado Anzoátegui, Venezuela. Zootecnia Tropical 21(2):197–217.

Sanders GJ; Arndt SK. 2012. Osmotic adjustment under drought conditions. In: Aroca R, ed. Plant responses to drought stress: From morphological to molecular features. Springer-Verlag, Berlin, Germany. p. 199–229. DOI: 10.1007/978-3-642-32653-0_8

Sanderson MA; Stair DW; Hussey MA. 1997. Physiological and morphological responses of perennial forages to stress. Advances in Agronomy 59:171–224. DOI: 10.1016/S0065-2113(08)60055-3

SAS Institute. 2002. SAS/STAT: Guide for personal computers. Version 8.1. SAS Institute Inc., Cary, NC, USA.

Schultze-Kraft R; Clements RJ, eds. 1990. *Centrosema*: Biology, agronomy, and utilization. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. (Available at: https://goo.gl/DuFizc).

Sheriff DW; Ludlow MM. 1984. Physiological reactions to an imposed drought by *Macroptilium atropurpureum* and *Cenchrus ciliaris* in a mixed sward. Australian Journal of Plant Physiology 11(2):23–34. DOI: 10.1071/PP9840023

Sheriff DW; Fisher MJ; Rusitzka G; Ford CW. 1986. Physiological reactions to an imposed drought by two twining pasture legumes: *Macroptilium atropurpureum* (desiccation sensitive) and *Galactia striata* (desiccation insensitive). Australian Journal of Plant Physiology 13:431–445. DOI: 10.1071/PP9860431

Silveira JAG da; Costa RCL da; Oliveira JTA. 2001. Drought-induced effects and recovery of nitrate assimilation and nodule activity in cowpea plants inoculated with *Bradyrhizobium* spp. under moderate nitrate level. Brazilian Journal of Microbiology 32:187–194. DOI: 10.1590/S1517-83822001000300005

Soil Survey Staff. 2014. Keys to soil taxonomy. 12th Edn. United States Department of Agriculture; Natural Resources Conservation Service, Washington, DC, USA. (Available at: https://goo.gl/CWErfI).

Turner NC; Begg JE. 1981. Plant-water relations and adaptations to stress. Plant and Soil 58:97–131. (Available at: www.jstor.org/stable/42933783).

USICLIMA. 2016. Unidad de Servicios Integrados Climatológicos para la Investigación en Agricultura y Ambiente (USICLIMA). Cátedra de Climatología Agrícola, Facultad de Agronomía, UCV, Maracay, Venezuela.

Vilagrosa A; Chirino E; Peguero-Pina JJ; Barigah TS; Cochard H; Gil-Pelegrín E. 2012. Xylem cavitation and embolism in plants living in water-limited ecosystems. In: Aroca R, ed. Plant responses to drought stress: From morphological to molecular features. Springer-Verlag, Berlin, Germany. p. 63–109. DOI: 10.1007/978-3-642-32653-0_3

Wang H; Yamauchi A. 2006. Growth and function of roots under abiotic stresses in soil. In: Huang B, ed. Plant-environment interactions. 3rd Edn. CRC Press, New York, USA. p. 271–320. DOI: 10.1201/9781420019346.ch10

Wery J; Turc O; Salsac L. 1986. Relationship between growth, nitrogen fixation, and assimilation in a legume (*Medicago sativa* L.). Plant and Soil 96:17–29. DOI: 10.1007/BF02374992

Wilson JR. 1983. Effects of water stress on *in vitro* dry matter digestibility and chemical composition of herbage of tropical pasture species. Australian Journal of Agricultural Research 34(4):377–390. DOI: 10.1071/AR9830377

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Research paper

Forage yield and nutritive value of *Arachis* spp. genotypes in the Brazilian savanna

Producción de forraje y valor nutritivo de genotipos de Arachis *spp. en el Cerrado brasileño*

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Abstract

Forage yield, nutritive value, ground cover and mineral concentration of 10 genotypes of *Arachis* spp. were evaluated over 3 years in Planaltina, Federal District, Brazil. Experimental plots were arranged in a randomized complete block design with 4 replications. Treatments were 5 genotypes of *A. pintoi* (accessions 2, 4, 5, 6 and 8), 2 genotypes of *A. repens* (accessions 1 and 7), 1 hybrid *A. pintoi* × *A. repens* (accession 3) and 2 *A. pintoi* cultivars, BRS Mandobi and Belmonte. All genotypes established well and achieved good ground cover in the first year. Cultivar Belmonte and accessions 2 and 4 produced the highest DM yields (means of 8.8, 8.5 and 8.8 t DM/ha/yr, respectively) throughout, while cv. BRS Mandobi and accession 6 were the worst (5.7 and 5.6 t DM/ha/yr). Most genotypes maintained ground cover above 80% throughout the study but cv. BRS Mandobi plus accessions 6 and 8 had declined to 60% or less by the third year. Mean crude protein concentration overall was 166 g/kg with a range of 154–182 g/kg among genotypes. There was no major genotypic variation in mineral concentrations, which in all cases were considered marginal to adequate for tropical forage legumes. In conclusion, accessions 2 and 4 (BRA-039799 and BRA-039187, respectively) of *A. pintoi* are considered the most promising forage peanut options under the edaphoclimatic conditions of the experimental site. More effort is needed to improve seed set in these genotypes to increase adoption by farmers. Their persistence under grazing and impact on production should also be demonstrated.

Keywords: *Arachis pintoi*, *Arachis repens*, crude protein, digestibility, minerals.

Resumen

En Planaltina, Distrito Federal, Brasil, se evaluaron la producción de forraje, el valor nutritivo, la cobertura del suelo y la concentración mineral de 10 genotipos de *Arachis* spp. durante 3 años. Las parcelas experimentales se dispusieron en un diseño de bloques completos al azar con 4 repeticiones. Los tratamientos consistieron en 5 genotipos de *A. pintoi* (accesiones 2, 4, 5, 6 y 8), 2 genotipos de *A. repens* (accesiones 1 y 7), 1 híbrido *A. pintoi* × *A. repens* (accesión 3) y 2 cultivares de *A. pintoi*, BRS Mandobi y Belmonte. Todos los genotipos presentaron buen establecimiento y buena cobertura del suelo en el primer año. Considerando los 3 años del experimento, el cultivar (cv.) Belmonte y las accesiones 2 y 4 presentaron los rendimientos de materia seca (MS) más altos, con promedios de 8.8, 8.5 y 8.8 t MS/ha por año, respectivamente, mientras que cv. BRS Mandobi y la accesión 6 fueron los menos productivos, con promedios de 5.7 y 5.6 t MS/ha por año, respectivamente. La mayoría de los genotipos mostraron una cobertura del suelo superior a 80% durante la duración del estudio; no obstante el cv. BRS Mandobi y las accesiones 6 y 8 presentaron una reducción al 50–60% al finalizar el tercer año. En total, la concentración promedio de proteína cruda fue de 166 g/kg con un rango de 154–182 g/kg entre genotipos. Las concentraciones de minerales no mostraron mayores variaciones entre genotipos y se consideraron marginales a adecuadas para leguminosas forrajeras tropicales. En conclusión, las accesiones 2 y 4 de

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A. pintoi (BRA-039799 y BRA-039187, respectivamente) mostraron ser las opciones más promisorias bajo las condiciones edafoclimáticas del sitio experimental. Se requieren esfuerzos para mejorar la producción de semilla de estos materiales para lograr una mayor adopción por los productores. Además su persistencia bajo pastoreo y su impacto en la producción deben demostrarse.

Palabras clave: *Arachis pintoi*, *Arachis repens*, digestibilidad, minerales, proteína cruda.

Introduction

Cattle farms in the Brazilian savannas (Cerrados) are practically totally dependent on pastures sown with African grasses, which may be exposed to overgrazing, low soil fertility and water deficits (Oliveira et al. 2004). Despite being considered a sustainable way to improve livestock productivity at low cost (Boddey et al. 1997), the sowing of legumes by farmers is not a regular practice in Brazil. The use of tropical forage legumes is restricted to particular areas, like the northwestern humid tropics in Acre State, where *Pueraria phaseoloides* and *Arachis pintoi* are sown in mixed grass-legume pastures (Assis et al. 2013). Limited numbers of suitable legume cultivars is one of the barriers to the use of legumes in forage-based production systems. Furthermore, low persistence of legumes, cost of seed, the need for vegetative propagation in some cases and undefined grazing management recommendations also contribute to their limited usage (Wünscher et al. 2004), as opposed to the wide-spread use of more resilient and accessible grasses like *Brachiaria* spp.

Several *Arachis* species with forage potential ("forage peanut") are endemic in Brazil (Valls and Simpson 1994), presenting satisfactory performance, especially when sown in humid areas (>1,500 mm rainfall per year). Within the *Arachis* genus, *A. pintoi* is the most commonly sown for forage in the world and the only one with registered cultivars in Brazil. Approximately 0.14 M ha are cultivated with cv. Belmonte (*A. pintoi*) in the State of Acre, but expansion of this area is slow owing to the predominant use of vegetative propagation (Assis et al. 2013). The Australian cv. Amarillo (*A. pintoi*) is the only cultivar for which seed is available in the Brazilian market, and it is usually imported from Bolivia and Peru. Under savanna conditions, annual dry matter (DM) yields from *A. pintoi* of up to 13 t/ha have been reported, and for *A. repens* yields up to 6 t DM/ha have been obtained in a lowland area (Pizarro and Rincón 1994). In addition to tolerance to heavy grazing and adaptation to infertile soils (Rao and Kerridge 1994), *Arachis* presents high nutritive value and acceptability (Argel and Pizarro 1992) and supports better cattle performance than grass monocultures (Lascano 1994). However, because of irregular rainfall distribution in savannas, yield and persistence of *A. pintoi* are not always satisfactory, especially when it is grown in highland areas. The objective of this study was to evaluate forage yield, ground cover, nutritive value and macronutrient concentrations of genotypes, cultivars and a hybrid of *Arachis* spp. in a Brazilian savanna ("Cerrado") environment.

Materials and Methods

Experimental field and environmental conditions

The experiment was carried out in Planaltina, Federal District, Brazil (15º35' S, 47º42' W; 993 masl) during 3 consecutive years. The climate at the site is Aw according to Köppen-Geiger classification (Peel et al. 2007). Monthly rainfall and mean air temperature were recorded at 600 m from the experimental site and are presented along with the historical averages for annual rainfall and temperature (Table 1). The soil in the area is classified as an Oxisol, with very clayey texture, pH (in H_2O) 5.2, organic matter content of 0.22 g/kg, phosphorus (P) 6.6 mg/dm^3 (Mehlich-I), potassium (K) 38.7 mg/dm^3, calcium (Ca) 2.6 mg/dm^3, magnesium (Mg) 0.64 mg/dm^3 and aluminum (Al) 0.14 mg/dm^3 in the 0–0.2 m soil layer. On 31 March 2008, after the first harvest of the experimental period, 25 kg K/ha as potassium chloride and 4 kg P/ha as single superphosphate were applied; this was the only fertilization during the entire evaluation period.

Arachis *spp. germplasm sources*

The treatments were 10 genotypes of *Arachis* spp. (Table 2), consisting of 5 genotypes of *Arachis pintoi* Krapov. & W.C. Greg. (accessions 2, 4, 5, 6 and 8), 2 genotypes of *Arachis repens* Handro (accessions 1 and 7), 1 inter-specific hybrid (accession 3) from a cross between *A. pintoi* cv. Amarillo and *A. repens* BRA-029220 and 2 cultivars of *A. pintoi* (cvv. Belmonte and BRS Mandobi). The genotypes and hybrid were drawn from the *Arachis* spp. germplasm collection of Embrapa Acre, located in Rio Branco, Acre State, Brazil. Cultivar Belmonte was collected in Bahia State, Brazil, and released in 1999 (Paganella and Valls 2002). Cultivar BRS Mandobi was obtained from a network of mass selection promoted by Embrapa in Brazil including the states Acre, Bahia and the Federal District (Assis et al. 2013).

Table 1. Monthly rainfall and mean temperature during the experimental period and historical means (1974–2006) for Planaltina, Federal District, Brazil.

Month	Rainfall (mm)					Temperature (°C)				
	2007	2008	2009	2010	Avg.[1]	2007	2008	2009	2010	Avg.[1]
Jan	152	227	150	113	250	22.0	21.7	22.1	22.6	22.3
Feb	161	135	161	122	193	21.5	21.2	22.2	23.1	22.4
Mar	11	107	64	271	219	22.6	21.1	22.2	22.0	22.6
Apr	7	92	220	68	93	22.2	21.6	21.1	22.2	22.2
May	1	0	77	3	25	21.3	20.1	20.3	21.6	21.0
Jun	0	0	4	0	5	20.1	19.5	19.3	20.4	19.9
Jul	0	0	0	0	5	20.5	19.1	20.2	20.0	19.8
Aug	0	0	52	0	16	20.8	21.4	20.9	21.3	21.6
Sep	0	44	65	0	41	23.6	23.4	23.0	23.3	23.1
Oct	57	17	126	122	129	24.2	24.5	21.9	23.3	23.2
Nov	79	155	113	252	187	22.5	22.0	22.5	21.1	22.5
Dec	298	152	158	309	228	21.8	21.3	21.4	21.7	22.3
Total	766	929	1,190	1,260	1,391					

[1] Historical means (1974–2006).

Table 2. Identification, register and species of 10 *Arachis* spp. genotypes.

Identification	BRA germplasm register[1]	Scientific name
Belmonte	031828 (cv. Belmonte)	*Arachis pintoi*
Mandobi	040550 (cv. BRS Mandobi)	*Arachis pintoi*
Accession 1	033260	*Arachis repens*
Accession 2	039799	*Arachis pintoi*
Accession 3	013251(cv. Amarillo) × 029220	hybrid *A. pintoi* × *A. repens*
Accession 4	039187	*Arachis pintoi*
Accession 5	014991	*Arachis pintoi*
Accession 6	035114	*Arachis pintoi*
Accession 7	034436	*Arachis repens*
Accession 8	034142	*Arachis pintoi*

[1] Official germplasm accession number at Embrapa Recursos Genéticos e Biotecnologia.

Experimental design and sampling

The experimental design was a randomized complete block with 4 replications. Plots consisted of 4 rows of 2 m length with 0.5 m spacing between rows and 0.25 m between plants within rows, totaling an area of 4.0 m^2. Vegetative material was struck in a greenhouse, using 200-mL plastic cups, and rooted plants were transplanted to plots on 4 December 2007. Plots were harvested on 31 March and 30 May 2008 to estimate dry matter yield (DMY) in the establishment year. Plants were cut at 0.05 m from the soil surface and forage mass was quantified in a 1-m^2 quadrat placed in the center of the plot. In subsequent years, plots were harvested every 42 days during the rainy season (November–April). The evaluation extended from Dec 2007 to Apr 2010 and harvests were categorized in 3 periods as described earlier and referred to herein as Year 1, Year 2 and Year 3. Harvests were made as follows: Year 1 - 31 March (1) and 30 May 2008 (2); Year 2 - 4 Dec 2008 (1), 15 Jan (2), 26 Feb (3), 9 Apr (4) and 21 May 2009 (5); and Year 3 - 20 Oct (1), 1 Dec 2009 (2), 12 Jan (3), 23 Feb (4) and 6 Apr 2010 (5).

A few days before each harvest (except for harvest 4 of Year 3), ground cover (GC) percentage of the *Arachis* spp. genotypes was estimated. This visual evaluation was performed by 2 observers who attributed scores to the plots in a range of 0–100%, and these scores were meaned.

Forage samples were dried in a forced-air oven at 55 °C for 72 h and then ground in a Wiley mill fitted with a sieve of 1 mm mesh for subsequent chemical analysis. Dry matter content, and crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were evaluated as described by Silva & Queiroz (2002). In vitro dry matter digestibility (DMD) was determined by the 2-stage method of Tilley & Terry (1963). All samples were analyzed, except those for harvest 1 of Year 3. The concentrations of macronutrients (Ca, Mg, P, K and S) of aerial plant parts were also determined according to Adler and Wilcox (1985). Forage samples from harvest 5 of Year 2 and from harvests 1 and 2 of Year 3 were not analyzed for macronutrient concentrations.

Statistical analyses

Dry matter yield data were analyzed in terms of annual forage accumulation considering all harvests during 3 years of evaluation. Nutritive value variables like DMD and CP, NDF, ADF and macronutrient concentrations were evaluated as annual mean values of forage samples analyzed. Agronomic and chemical analysis data were analyzed using Proc Mixed of SAS (Statistical Analysis System, version 9.2) with genotype, year and genotype × year as fixed effects, while block was considered a random effect. Year was tested as a fixed effect and a repeated measure because an effect on forage yield in a perennial experiment was expected. However, since genotype × year interactions were significant for most data ($P<0.05$), these data were analyzed by year using Proc GLM of SAS. Means for the effect of genotype were assessed by Least Significant Difference (LSD) by t test ($P<0.05$). Ground cover (GC) percentage for each harvest was described graphically with standard deviation bars.

Results

Dry matter yield (DMY) was affected by a genotype × year interaction (Table 3; $P<0.0001$). In the establishment year, DMY was lower than in Years 2 and 3 (mean yields of 4.2 vs. 8.4 and 8.7 t/ha, respectively). *Arachis pintoi* accessions 2 and 4 were quite productive throughout the evaluation (mean yields of 8.5 and 8.8 t DM/ha, respectively) along with cv. Belmonte (8.8 t DM/ha). Cultivar BRS Mandobi (5.7 t DM/ha) and *A. pintoi* accession 6 (5.6 t DM/ha) were the least productive genotypes throughout the study.

Ground cover was affected by a genotype × year interaction (Table 3; $P<0.0001$). In the establishment year GC of the various genotypes ranged from 83 to 96%. In the second and third years, larger differences emerged with ranges of 62–100% in Year 2 and 54–99% in Year 3. Throughout the evaluation, GC was greatest for *A. pintoi* accessions 2 and 4 and cv. Belmonte (Figure 1). Ground cover was maintained above 86% throughout by all genotypes, except for Mandobi and *A. pintoi* accessions 6 and 8, where cover had declined to about 60% or less by the end of the third year.

Table 3. Dry matter yield (DMY) and ground cover (GC) of 10 *Arachis* spp. genotypes in 3 years of evaluation (2008–2010) in Planaltina, Federal District, Brazil.

Genotype[1]	DMY (t/ha)			GC[2] (%)		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Belmonte	4.6	10.5	11.3	96.0	99.7	95.7
Mandobi	3.8	6.4	6.8	88.6	62.1	60.7
Accession 1	2.6	8.8	7.4	92.8	90.1	88.8
Accession 2	5.5	9.6	10.3	87.5	99.8	96.6
Accession 3	3.2	7.2	9.1	95.0	87.5	86.6
Accession 4	5.0	10.4	11.1	92.4	99.5	99.4
Accession 5	4.6	7.8	9.1	91.5	86.0	86.6
Accession 6	3.6	7.0	6.2	82.5	65.1	53.8
Accession 7	4.4	8.4	8.8	89.8	89.8	86.6
Accession 8	4.9	8.2	6.7	91.8	80.0	62.5
LSD ($P<0.05$)	0.6	2.0	1.5	12.8	13.0	13.1

[1] Values presented are averages across 4 replications, with 2 harvests in Year 1 (2008), 5 harvests in Year 2 (2008/09) and 5 harvests in Year 3 (2009/10).

[2] Ground cover (%) was transformed to (GC/100) arcsin to perform statistical analyses.

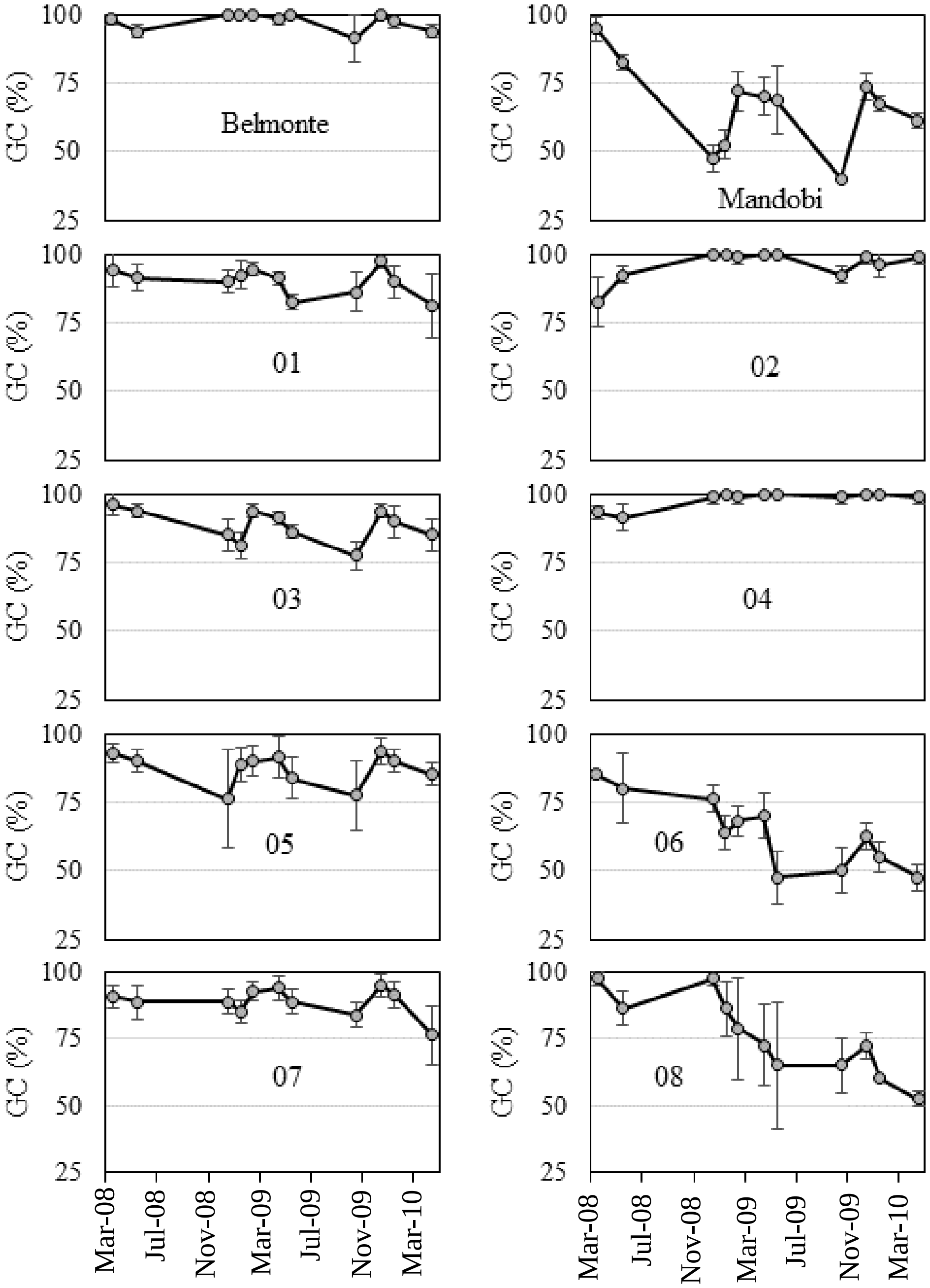

Figure 1. Ground cover (GC) percentage for 10 *Arachis* spp. genotypes in 3 years of evaluation (2008–2010) in Planaltina, Federal District, Brazil. Numbers 1 and 7 represent accessions of *A. repens,* numbers 2, 4, 5, 6 and 8 represent accessions of *A. pintoi* and number 3 represents an *A. pintoi* × *A. repens* hybrid. Small vertical bars represent 2 standard deviations. Each point plotted represents the mean of 4 replicates.

Dry matter digestibility (DMD) was affected by a genotype × year interaction (Table 4; P = 0.0118). Mean values were greater for the first 2 years of evaluation (610 and 614 g/kg, respectively) than for the third year (532 g/kg). In the first year, DMD of the hybrid accession 3 (678 g/kg) was 11% superior (P<0.05) to the mean of the remaining genotypes (610 g/kg), but there were no differences between genotypes in the second and third years (P>0.05).

There were significant genotype × year interactions for ADF (Table 4; P<0.0001) and NDF (Table 4; P = 0.0022) concentrations. The only consistency in ADF or NDF concentrations in the various genotypes throughout the study was that the hybrid (accession 3) had the lowest ADF values throughout.

Crude protein concentrations were affected by genotype (P = 0.0004) and year (P<0.0001). *Arachis repens* accession 7 and *A. pintoi* accessions 2 and 4

Table 4. In vitro dry matter digestibility (DMD), and acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude protein (CP) concentrations of 10 *Arachis* spp. genotypes in 3 years of evaluation (2008–2010) in Planaltina, Federal District, Brazil.

Genotype[1], year	DMD (g/kg)			ADF (g/kg)			NDF (g/kg)			CP (g/kg)
	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	3 years
Belmonte	596	624	530	255	234	259	442	396	408	174
Mandobi	602	615	520	221	233	272	409	400	423	158
Accession 1	602	583	559	241	234	246	462	423	432	162
Accession 2	583	621	524	252	242	258	456	392	403	177
Accession 3	678	618	556	197	210	228	409	386	398	154
Accession 4	602	631	541	232	229	248	425	391	394	176
Accession 5	618	608	501	239	248	280	434	410	432	160
Accession 6	608	599	515	217	233	278	407	403	422	154
Accession 7	597	610	518	252	250	268	445	420	440	182
Accession 8	610	632	556	208	228	249	394	391	400	163
LSD (P<0.05)	38.2	33.9	43.3	10.7	15.7	22.6	26.2	21.5	24.3	13.1
Year 1										142
Year 2										197
Year 3										159
LSD (P<0.05)										5.9

[1] Values presented are averages across 4 replications, 2 harvests in Year 1 (2008), 5 harvests in Year 2 (2008/09) and 4 harvests in Year 3 (2009/10).

as well as cv. Belmonte had higher CP concentrations than all other genotypes (Table 4; P = 0.0004). In the first year of evaluation, mean CP concentration was 142 g/kg, increasing to 197 g/kg in Year 2, before decreasing to 159 g/kg in Year 3 (P<0.0001).

Concentrations of all macronutrients were affected by genotype × year interactions (P<0.05) (Table 5). However, the main difference that occurred in P concentrations was a marked drop from 2.1 and 2.3 g/kg in Years 1 and 2, respectively, to 1.3 g/kg in Year 3. A similar result was found for K concentration, where values dropped from 9.8 and 10.0 g/kg in Years 1 and 2 to 8.3 g/kg in Year 3. Magnesium values also declined from 4.7 and 3.9 g/kg in Years 1 and 2 to 1.5 g/kg in Year 3.

Table 5. Concentrations of macroelements (g/kg DM) in 10 *Arachis* spp. genotypes over 3 years of evaluation (2008–2010) in Planaltina, Federal District, Brazil.

Genotype[1]	P			K			Ca			Mg			S		
	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3
Belmonte	1.8	2.2	1.3	9.0	9.4	5.7	17.5	11.6	22.1	5.0	4.8	2.0	1.6	1.2	1.7
Mandobi	2.1	2.3	1.3	10.9	10.4	10.8	14.9	10.7	19.5	3.5	3.7	1.2	1.8	1.4	1.8
Accession 1	2.7	2.3	1.4	11.0	9.6	7.8	12.0	9.5	18.2	2.8	3.4	1.2	1.5	1.3	1.6
Accession 2	2.0	2.1	1.3	11.1	9.0	6.2	19.1	11.6	23.3	5.1	4.9	2.2	1.8	1.2	1.7
Accession 3	2.1	2.4	1.2	10.3	11.7	8.1	14.8	10.0	16.9	3.8	3.0	0.8	1.5	1.4	1.5
Accession 4	2.1	2.2	1.3	9.4	9.1	6.8	21.5	11.7	24.7	5.3	4.8	2.2	1.7	1.3	1.8
Accession 5	1.9	2.3	1.2	9.4	10.8	8.3	16.1	9.9	14.9	4.7	4.0	1.3	1.5	1.3	1.4
Accession 6	2.1	2.3	1.3	8.8	10.7	12.0	14.8	8.8	14.5	5.0	3.0	1.0	1.6	1.5	1.9
Accession 7	1.8	2.4	1.5	8.4	9.8	7.9	18.5	10.3	18.2	5.5	4.0	1.8	1.5	1.4	1.7
Accession 8	2.2	2.2	1.3	9.2	9.0	9.5	18.2	10.5	15.8	5.9	3.6	1.2	1.8	1.3	1.5
LSD (P<0.05)	0.5	0.2	0.1	2.3	1.3	1.5	3.0	1.1	1.6	1.0	0.7	0.3	0.2	0.2	0.1

[1] Values presented are averages across 4 replications, 2 harvests in Year 1 (2008), 4 harvests in Year 2 (2008/09) and 3 harvests in Year 3 (2009/10).

Discussion

Although *Arachis* spp. yields were lower than those of introduced African C4 grasses in Brazil, they were similar to those of native perennial legumes from Brazilian savannas like *Stylosanthes* spp. (Ramos et al. 2010). In Acre State, cv. BRS Mandobi frequently reaches yields of 9–15 t DM/ha in the establishment year (Assis et al. 2013), much higher than the yields obtained in this study (Table 3). Even in Year 3, Mandobi failed to reach yields of that magnitude. On the other hand, cv. Belmonte performed at a higher level and appears better adapted to this environment than Mandobi (Ramos et al. 2010). Assis et al. (2008), in comparing *Arachis* spp. selected for the Amazonian region, observed that *A. pintoi* accessions 2 and 4 presented higher DMYs than other genotypes, including *A. repens*. In this same study, in the establishment year, accessions 2 and 4 yielded about 4 t DM/ha of forage while *A. repens* accessions 1 and 7 yielded about 3 t DM/ha. In Florida, USA, a collection of *A. pintoi* genotypes yielded 4.4 t DM/ha of forage in the first year of evaluation (Carvalho and Quesenberry 2012), similar to the DMYs reported in the first year of the current study. Genotypes of *A. repens* (accessions 1 and 7) showed lower DMYs than *A. pintoi*, which was to be expected for a Brazilian savanna environment (Pizarro and Rincón 1994). Considering the low productivity of most unfertilized grass pastures in Brazilian savannas, the mean DMYs displayed by the *Arachis* spp. could be considered satisfactory, particularly in the second and third years of evaluation.

Persistence of forage peanut in pastures is related to its capacity to cover the ground and thereby prevent the ingress of weeds that leads to pasture degradation. As a strategy to colonize an area, species from the botanical *Arachis* section Caulorrizhae, such as *A. pintoi* and *A. repens*, spread by stolons. This clonal reproduction is one of the most efficient mechanisms to ensure the persistence of forage legumes. In addition, the persistence of forage peanut is due to its high tolerance to trampling and defoliation, since it has a prostrate habit, with abundant and well protected growth points. However, the persistence and vigor of *Arachis* spp. in savannas can be short-lived due to irregular distribution of rainfall throughout the year, resulting in severe moisture stress. In 2008, rainfall during the first dry season (May–October) was very low (Table 1) resulting in decline in ground cover of most genotypes, especially Mandobi and accessions 5 and 6. Better rainfall distribution throughout 2009 failed to maintain maximum ground cover for most *Arachis* spp. genotypes during the dry season (Figure 1), because of low minimum temperatures in this period (<15 ºC). However, the ability of these genotypes to maintain such high ground cover until the end of the study demonstrates their ability to survive in edaphoclimatic conditions of savannas. Nevertheless, their performance under grazing, when extra stresses would be experienced, still needs to be evaluated. The genetic variability existing among the germplasm evaluated would enable selection of more adapted genotypes and further gains through breeding. In Acre State, Valentim et al. (2003) observed that Belmonte reached 96% ground cover just 70 days after planting, higher than cv. Amarillo and other genotypes of *A. pintoi* and *A. repens*. For Amazonian conditions, Assis et al. (2008) observed that cvv. Belmonte and BRS Mandobi and accessions 2 and 4 were the best performing genotypes during the establishment phase in terms of DM yield and ground cover. Except for Mandobi, these results were similar to our findings under savanna conditions in the current study (Figure 1). In general, the most productive genotypes in the rainy season were those which achieved the highest values for ground cover.

As establishment of *Arachis* spp. is considered slow, the lesser productivity is continually associated with stand formation problems, even in humid areas (Valentim et al. 2003). In the current study, the slow initial development limited the number of cuts (2) in the first rainy season. This can require additional resources to assure an adequate initial ground cover and forage production (Carvalho and Quesenberry 2012), e.g. new germplasm and fertilization strategies. Otherwise, the expected benefits of the legume in the short term can be reduced (Pizarro and Rincón 1994). Because of its ability to produce acceptable seed yields, cv. BRS Mandobi was chosen from a selection network including many regions of Brazil. Assis et al. (2013) stated that about 18–21 months after planting, Mandobi produced 3 t/ha of pure seeds. This is an important characteristic, considering the restrictions on farmer adoption of *Arachis* spp. caused by the need for vegetative propagation, as can be observed for cv. Belmonte, which produces little seed. Although DM yield and persistence in savannas of some *Arachis* spp. might not be satisfactory, particularly for cv. BRS Mandobi, high seed production would allow for a lesser seed price, which could compensate for the need to have to replant the legume periodically. However, as a consequence of the geocarpic reproductive growth, lack of relevant seed harvesting technology could increase costs of seed production (Ferguson 1994).

In general, dry matter digestibility (DMD) of *Arachis* spp. is greater than that of other herbaceous tropical legumes, regardless of the season of the year (Gama et al. 2014). As it is strongly associated with forage quality and intake, DMD greatly affects animal performance at pasture. Dry matter digestibility of tropical grasses is usually low (<600 g/kg) compared with that of legumes. Despite differences in thickness of stems, *A. repens* genotypes 1 and 7 had similar DMD to that of *A. pintoi* genotypes, unlike hybrid accession 3 that had the greatest DMD in the first year (678 g/kg). In Florida, mean DMD of *A. pintoi* genotypes was 670 g/kg, ranging from 600 to 730 g/kg, similar to *A. glabrata* cvv. Florigraze and Arbrook (Carvalho and Quesenberry 2012), while in the Amazonian region, cv. BRS Mandobi showed DMD of 660 g/kg (Assis et al. 2013). Both studies showed greater mean DMD than the current study (613 g/kg in Years 1 and 2 and 532 g/kg in Year 3). In Brazilian savanna, Gama et al. (2014) observed that cv. Belmonte in mixed legume-grass pastures achieved DMD of 700 g/kg in the rainy season and 610 g/kg in the dry season, exceeding DMD of woody legumes like *Leucaena leucocephala* cv. Cunningham (590 g/kg) and *Cratylia argentea* (560 g/kg). Although selection and development of genotypes and hybrids are focused on other attributes, since the species has already high values of DMD, there is enough variability to improve the nutritive value of *Arachis* germplasm through hybridization.

Cultivar Belmonte and accessions 2 and 4, as the most productive genotypes in terms of DM yield, also presented greater crude protein (CP) concentrations, together with *A. repens* accession 7. There was, therefore, no evidence for a dilution effect of the N concentration in the most productive genotypes, possibly as a consequence of increased biological-N fixation over time. In Campo Grande, Mato Grosso do Sul State, cv. Belmonte showed CP concentrations of 190 g/kg in mixed grass-legume pastures throughout the year, slightly lower than those of the woody legumes *Cratylia argentea* and *Leucaena* spp., with 200 and 210 g CP/kg, respectively (Gama et al. 2014), but greater than reported in the current study for this same cultivar (174 g/kg). In a similar way, CP concentration for cv. BRS Mandobi in Acre State reached 230 g/kg (Assis et al. 2013), much greater than reported in the current study (158 g/kg). In Florida, mean CP concentration of a collection of *A. pintoi* genotypes was about 180 g/kg (Carvalho and Quesenberry 2012), while a collection of *A. pintoi* genotypes and cultivars (Belmonte and Alqueire 1) had mean CP concentration of 197 g/kg and *A. repens* genotypes showed 193 g CP/kg in Acre State (Valentim et al. 2003). These values are similar to those reported in the second year of the current study (197 g CP/kg). Crude protein concentrations ranging from 142 to 197 g/kg confirm the great potential of this germplasm to provide nutritious forage for livestock.

Nutritive value decay in the last year of the evaluation (Year 3), in terms of both DMD and CP, was also observed through the increased values of ADF, the fiber fraction associated with poor quality forage due to anti-nutritional lignin encrusted in cellulose. This increase in ADF can be related to the higher proportion of stems in the forage harvested and reduction in more nutritional leaves (assessed by visual observations). In Acre State, evaluations of BRS Mandobi presented approximately 276 g ADF/kg and 538 g NDF/kg (Assis et al. 2013), both greater than observed in the current study (242 and 411 g/kg, respectively). In a broad literature review of *A. pintoi*, Ramos et al. (2010) specified mean values of ADF and NDF of 236 and 403 g/kg, respectively, which are similar to the values obtained in the current study (241 and 415 g/kg, respectively).

According to Rao and Kerridge (1994), critical concentrations of P, K and S for *A. pintoi* (whole aerial biomass) are 2.3, 5.0 and 1.1 g/kg, respectively. The same authors considered a value of 18 g Ca/kg as adequate. Concentrations of K and S we measured are similar to the critical values, regardless of year, while concentrations of P and Ca were marginal at best. In a sandy soil in the humid region (~1,200 mm of annual rainfall) of Benin, West Africa, *A. pintoi* genotypes over 3 years achieved concentrations of 1.7, 5.3, 14.8, 10.1 and 1.2 g/kg of P, K, Ca, Mg and S, respectively (Adjolohoun et al. 2013). The occurrence of mineral deficiency in tropical pastures is very common and depends on not only forage species but also soil fertility, which is mostly poor in Brazilian savannas. In this study with limited fertilizer application, concentrations of macro-minerals were generally marginal to adequate, although the soil fertility of the experimental area was better than that of typical soils of Brazilian savannas. High values of K in whole plant tissue observed in this study indicate a significant amount of this nutrient being extracted from soil. Considering mean values of 10 g K/kg DM and 8.0 t DM/ha of yield, the extraction per year would be approximately 80 kg K/ha (equivalent to 96 kg K_2O/ha). Irrespective of absolute values, the same genotypes that presented the highest forage yield also presented the highest values of Ca and Mg, particularly *A. pintoi* accessions 2 and 4.

Considering the benefits of *Arachis* spp. in grazing systems, it is important that breeding programs are continued to select genotypes for different purposes and localities. For Brazilian savannas it is also important to comprehend the potential role of *Arachis* spp. in the cattle production systems for this region. As opposed to the role of other legumes that complement the poor nutritive grass pastures during the dry season (e.g. *Stylosanthes* spp.), *Arachis* spp. have potential to intensify production systems during the rainy season, through a direct contribution to animal diets and also indirectly through promoting substantial recycling of nitrogen in the soil in mixed legume-grass pastures and increasing their longevity. While introduction, management and persistence of *Arachis* spp. are still relevant issues, seed production technology is one of the most important aspects that should be addressed in order to provide affordable and readily accessible seed for farmers. Under savanna conditions, in terms of forage production, nutritive value, ground cover and tissue macronutrient concentration, the new *A. pintoi* accessions 2 and 4 (accessions BRA-039799 and BRA-039187, respectively) were considered the most promising in this study. Their performance under grazing needs to be evaluated and steps made to improve seed production of these new materials.

References

Adjolohoun S; Bindelle J; Adandedjan C; Toleba SS; Houinato M; Sinsin B. 2013. Variety and environmental effects on crude protein concentration and mineral composition of *Arachis pintoi* (Krapovickas & Gregory) in Benin (West Africa). Journal of Applied Biology and Biotechnology 1:24–28. DOI: 10.7324/JABB.2013.1304

Adler PR; Wilcox GE. 1985. Rapid perchloric acid digest methods for analysis of major elements in plant tissue. Communications in Soil Science and Plant Analysis 16:1153–1163. DOI: 10.1080/00103628509367673

Argel PJ; Pizzaro EA. 1992. Germplasm case study: *Arachis pintoi*. In: Hardy B, ed. Pastures for the tropical lowlands - CIAT's contribution. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 57–76. (Available at: https://goo.gl/mx85wP).

Assis GML; Valentim JF; Carneiro Jr. JMC; Azevedo JMA; Ferreira AS. 2008. Seleção de genótipos de amendoim forrageiro para cobertura do solo e produção de biomassa aérea no período de estabelecimento utilizando-se metodologia de modelos mistos. Revista Brasileira de Zootecnia 11:1905–1911. DOI: 10.1590/S1516-35982008001100001

Assis GML; Valentim JF; Andrade CMS. 2013. BRS Mandobi: A new forage peanut cultivar propagated by seeds for the tropics. Tropical Grasslands-Forrajes Tropicales 1:39–41. DOI: 10.17138/TGFT(1)39-41

Boddey RM; Sá JCM; Alves BJR; Urquiaga S.1997. The contribution of biological nitrogen fixation for sustainable agricultural systems in the tropics. Soil Biology and Biochemistry 29:787–799. DOI: 10.1016/S0038-0717(96)00221-0

Carvalho MA; Quesenberry KH. 2012. Agronomic evaluation of *Arachis pintoi* (Krap. and Greg.) germplasm in Florida. Archivos de Zootecnia 61:19–29. DOI: 10.4321/s0004-05922012000100003

Ferguson JE. 1994. Seed biology and seed systems for *Arachis pintoi*. In: Kerridge PC; Hardy B, eds. Biology and agronomy of forage *Arachis*. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 122–133. (Available at: https://goo.gl/UlyiEq).

Gama TCM; Volpe E; Lempp B. 2014. Biomass accumulation and chemical composition of Massai grass intercropped with forage legumes on an integrated crop-livestock-forest system. Revista Brasileira de Zootecnia 43:279–288. DOI: 10.1590/S1516-35982014000600001

Lascano CE. 1994. Nutritive value and animal production of *Arachis*. In: Kerridge PC; Hardy B, eds. Biology and agronomy of forage *Arachis*. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 109–121. (Available at: https://goo.gl/UlyiEq).

Oliveira OC; Oliveira IP; Alves BJR; Urquiaga S; Boddey RM. 2004. Chemical and biological indicators of decline/degradation of *Brachiaria* pastures in the Brazilian Cerrado. Agriculture, Ecosystems and Environment 103: 289–300. DOI: 10.1016/j.agee.2003.12.004

Paganella MB; Valls JFM. 2002. Caracterização morfológica de cultivares a acessos selecionados de *Arachis pintoi* Krapov. & Gregory. Pasturas Tropicales 24(2):23–30. (Available at: https://goo.gl/spLKRh).

Peel MC; Finlayson BL; McMahon TA. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrology Earth System Sciences 11:1633–1644. DOI: 10.5194/hess-11-1633-2007

Pizarro EA; Rincón A. 1994. Regional experience with forage *Arachis* in South America. In: Kerridge PC; Hardy B, eds. Biology and agronomy of forage *Arachis*. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 144–157. (Available at: https://goo.gl/UlyiEq).

Rao IM; Kerridge PC. 1994. Mineral nutrition of forage *Arachis*. In: Kerridge PC; Hardy B, eds. Biology and agronomy of forage *Arachis*. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 71–83. (Available at: https://goo.gl/UlyiEq).

Ramos AKB; Barcellos AO; Fernandes FD. 2010. Gênero Arachis. In: Fonseca DM; Martuscello JA, eds. Plantas forrageiras. Editora da UFV, Universidade Federal de Viçosa, Viçosa, MG, Brazil. p. 249–293.

Silva DJ; Queiroz AC. 2002. Análise de alimentos: Métodos químicos e biológicos. 3rd Edn. Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil.

Tilley JMA; Terry RA. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science 18:104–111. DOI: 10.1111/j.1365-2494.1963.tb00335.x

Valentim JF; Andrade CMS; Mendonça HA; Sales MFL. 2003. Velocidade de estabelecimento de acessos de amendoim forrageiro na Amazônia Ocidental. Revista Brasileira de Zootecnia 32:1569–1577. DOI: 10.1590/S1516-35982003000700005

Valls JFM; Simpson CE. 1994. Taxonomy, natural distribution, and attributes of *Arachis*. In: Kerridge PC; Hardy B, eds. Biology and agronomy of forage *Arachis*. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 1–18. (Available at: https://goo.gl/UlyiEq).

Wünscher T; Schultze-Kraft R; Peters M; Rivas L. 2004. Early adoption of the tropical forage legume *Arachis pintoi* in Huetar Norte, Costa Rica. Experimental Agriculture 40:257–268. DOI: 10.1017/S0014479703001583

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Research paper

Effects of deferred forage as winter cover on spring growth of the tropical grasses *Chloris gayana* and *Panicum coloratum*

Efectos del forraje diferido como cobertura de invierno en el crecimiento primaveral de las gramíneas tropicales Chloris gayana *y* Panicum coloratum

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Abstract

This pot study assessed the effects of deferring forage during autumn and leaving as winter cover on reducing cold damage to plants of 2 tropical (C4) grasses (*Chloris gayana* and *Panicum coloratum*) in a temperate environment in La Plata, Buenos Aires, Argentina. Mature plants were subjected to the following treatments: (i) autumn-deferred forage retained (DF) as control; and (ii) autumn-deferred forage removed (DFR) cutting at 15 cm from soil level at beginning of winter. This experiment had 10 replicates per treatment and 1 plant per pot (experimental unit). Plants of both species were extracted from a commercial beef farm and transplanted into an experimental garden in pots where they grew outdoors from 2 February to 23 May (111 days) when treatments were applied. After winter, both grasses were cut to 15 cm in early spring (27 September) and spring growth was measured in November. Shoot biomass was harvested at ground level and separated into lower and upper layers (above and below 15 cm), leaf blades, sheaths and stolons. Daily air temperature, relative humidity and frost events were registered. Allometric analysis of shoot biomass was performed to determine the stress incidence by cold. DF plants achieved 55–80% higher shoot biomass than DFR plants during spring in both species. The allometric analysis revealed for *P. coloratum* significant relationships between shoot biomass from plant compartments (lower and upper layers, leaf blade and sheath) and total shoot biomass in both treatments, indicating good cold tolerance. However, for *C. gayana*, unlike DF plants, DFR plants were strongly stressed, showing a lack of shoot biomass fit. These results suggest that deferring autumn forage growth and retaining as winter cover may improve survival during winter and productivity during spring of these two tropical grasses in temperate pastoral systems. However, the study needs to be repeated under field conditions and under grazing or cutting over a number of years in different situations to verify these preliminary results.

Keywords: Winter cover, cold damage, frost protection, pasture management.

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Resumen

En La Plata, Buenos Aires, Argentina, en un experimento en macetas a aire libre (condiciones de luz y temperatura naturales) se evaluó el efecto del forraje diferido como cobertura invernal en 2 gramíneas tropicales C4 (*Chloris gayana* y *Panicum coloratum*). Plantas adultas fueron extraídas de un establecimiento ganadero, trasplantadas a macetas en un jardín experimental y, después de crecer durante 111 días, sometidas el 23 de Mayo a los tratamientos: (1) control [sin remoción del forraje diferido de otoño (DF)]; y (2) remoción del forraje diferido de otoño (DFR) después de un corte a 15 cm del suelo. Se utilizaron 10 repeticiones por tratamiento y una planta por maceta (unidad experimental). Las plantas fueron cosechadas el 27 de Septiembre (después del invierno) y nuevamente en Noviembre, mediante corte a ras del suelo, para medir la biomasa del rebrote primaveral en los estratos superior (>15 cm) e inferior (<15 cm sobre el suelo), láminas, vainas y estolones. Se realizó un análisis alométrico para dilucidar la incidencia del estrés por frio y se registraron la temperatura del aire, la humedad relativa y la ocurrencia de heladas. Las plantas con forraje diferido alcanzaron una mayor biomasa (55–80%) que aquellas con remoción del forraje. *Panicum coloratum* mostró un ajuste significativo entre la biomasa total y la biomasa de los diferentes estratos para ambos tratamientos, mostrando buena tolerancia al estrés por frio. Por otro lado, *C. gayana* mostró falta de ajuste de la biomasa y un mayor estrés por la remoción del forraje, mientras que las plantas sin remoción presentaron menor daño por frío y un mayor ajuste. La cobertura invernal del forraje diferido podría mejorar la productividad y supervivencia de estas especies forrajeras cuando son utilizadas en sistemas pastoriles templados. Estos resultados preliminares deben ser evaluados en condiciones de campo durante un mayor número de años, considerando diferentes estrategias de pastoreo.

Palabras clave: Cobertura invernal, daño por frío, manejo de pasturas, protección de heladas.

Introduction

The successful introduction of new forage species into grassland ecosystems or cultivated pasture systems depends on successful establishment, persistence and forage productivity (Baron and Bélanger 2007). In temperate and subtropical areas, tropical (C4) grasses have the potential to increase forage production during summer, when growing conditions are not ideal for C3 temperate grasses (Davies and McNaughton 1980; Johnston 1996; Crush and Rowarth 2007). As well as having better growth potential, some tropical grasses have shown good adaptation to saline soils (Loch et al. 2004), drought conditions (Pitman 2001), soils with low or high pH (Robinson et al. 1993), infertile soils (Loch 1980), seasonal flooding (Baruch 1994; Imaz et al. 2015a) and other environmental stresses, whereas temperate grasses showed poor persistence (Crush and Rowarth 2007). However, most tropical grasses are seriously adversely affected by winter frost, as a result of sub-zero temperatures (freezing stress). Even at temperatures in the range 0–15 ºC (chilling stress) (Ivory 1975; Ludlow 1980; Anderson and Wu 2011), these species show little or no growth (Sage and Pearcy 2000). When suboptimal temperatures are recorded for extended periods (i.e. 2–6 months), significant tissue damage and subsequent plant death can occur (Ludlow 1980; Márquez et al. 2006).

Given the constraints to growth of warm-season grasses imposed by temperate climatic conditions, only those tropical grasses able to tolerate and to survive under winter temperatures, especially minimum temperatures, are likely to become useful. *Chloris gayana* (Rhodes grass) and *P. coloratum* (Klein grass) are C4 grasses of African tropical and subtropical origin (Cook et al. 2005), that have been incorporated in pastures in lowland areas of humid grasslands and cultivated pastures in temperate livestock systems (Loch et al. 2004; Tischler and Ocumpaugh 2004; Crush and Rowarth 2007; Imaz et al. 2012, 2015a). Over recent years this introduction has been facilitated by the extended warm summers and less restrictive winters (i.e. higher minimal temperatures and less risk of frost) resulting from global warming (Long 1999; IPCC 2006; Chapman et al. 2012). Both grasses are cultivated in the United States (Texas), Africa, Australia, Japan, South America and under irrigation in the Middle East (Boschma et al. 2008). They are regarded as tolerant of soil salinity and drought (Dear et al. 2008) and grow satisfactorily in areas experiencing flooding (Boschma et al. 2008). *Chloris gayana* and *P. coloratum* have a lower critical daily mean temperature threshold for growth (8 ºC) than other tropical grasses like *Pennisetum ciliare* (syn. *Cenchrus ciliaris*) and *Megathyrsus maximus* (syn. *Panicum maximum*).

While *C. gayana* demonstrates high frost tolerance at plant level and medium tolerance at leaf level, *P. coloratum* has shown a higher ability to maintain its leaves active (tolerance at leaf level) during winter than other tropical grasses (Ludlow 1980). However, leaves of both grasses are damaged at temperatures below -2 ºC to -3 ºC, mainly with high air humidity, and plants are killed by temperatures about -10 ºC. In this sense, while cold tolerance has a major impact on where the species can grow, grazing management designed to protect plants from the direct physical damage of frost and low temperatures could enhance their utilization. We hypothesize that, while making adequate use of the pasture during the growing season, allowing accumulation of forage in late summer and autumn to provide herbage cover in winter (deferred forage) could help to improve pasture survival in winter and productivity in the following spring.

Thus, the aim of this study was to test this hypothesis by evaluating the effects of autumn-deferred forage as winter cover on growth of *P. coloratum* and *C. gayana* in spring under temperate environmental conditions in a pot study. A positive outcome could allow grazing management strategies to be devised to minimize the impact of cold winter temperatures on spring growth. To the best of our knowledge, this is the first attempt to assess the effects of autumn-deferred forage on spring growth of different plant components of these grass species.

Materials and Methods

Experimental details

Chloris gayana (cv. Finecut; Rhodes grass) and *Panicum coloratum* (cv. Klein) plants were extracted from a commercial beef farm located in Chascomús (35°34'42.9" S, 58°0'49.9" W), Buenos Aires province, Argentina, and transplanted into 15 L plastic pots (1 mature plant per pot) filled with a mixture of sand with top soil (1:2) from a lowland grassland of the Flooding Pampa of Argentina (organic carbon 3.3%; further details in Soriano 1991). There were 10 replicates. Pots were transferred to the experimental garden of INFIVE, the Plant Physiology Institute of La Plata National University, Buenos Aires (34°55'7" S, 57°57'17" W; 45 masl). In order to avoid nutrient limitation, plants were fertilized with di-ammonium phosphate (dose equivalent to 150 kg/ha) 10 days after transplanting. Randomly arranged plants grew outdoors for 4 months from late summer (2 February) until late autumn (23 May), when they were subjected to the following treatments: (i) Control, autumn-deferred forage retained (DF); and (ii) autumn-deferred forage removed (DFR) at 15 cm from top soil. This cutting height corresponds with pasture height at high-intensity grazing or cutting for hay, both of which are common farm practices in late autumn (Chaparro et al. 1995; Sollenberger et al. 2004). All pots were kept at field capacity during the study and plants allowed to grow during winter. In order to promote plant regrowth in early spring, plants were cut again at 15 cm height on 27 September and then allowed to grow during spring. The final harvest was performed when 50% of tillers showed evidence of reproductive structures (Flores et al. 1993), which occurred after 53 (20 November) and 35 (2 November) days for *C. gayana* and *P. coloratum*, respectively.

Daily maximum, minimum and mean air temperatures and relative humidity (RH) were registered by a micrometeorological station located in the experimental garden. Daily mean air temperature and RH were used to calculate the air vapor pressure deficit (VPD; Figure 1) in order to characterize the air evaporative demand during the experimental periods, i.e. late autumn, winter and spring.

Biomass responses

Shoot dry matter biomass of initial plants was determined at the beginning of the experiment, when treatments were applied (23 May). At the end of spring growth (20 November and 2 November for Rhodes grass and Klein grass, respectively) plants were harvested by cutting at ground level (final harvest) and biomass determined. Biomass from both early winter (only DFR plants) and spring cuts (both species) was registered and used to calculate the total shoot biomass accumulation, considering both cuts (early winter and spring) and the final harvest. Shoot biomass was separated into upper and lower layers (above and below 15 cm) as recorded in previous grazing studies (Imaz et al. 2015b). Stolons were separated only in *C. gayana*. Subsequently, shoot biomass was divided into leaf blades and sheaths. There was no root accumulation at the bottom of the pots at the final harvest, which suggested no potential constraints on plant growth due to pot size (Poorter et al. 2012).

Statistical analysis

Shoot biomass data were analyzed separately for each harvest by using Student’s T-test (P<0.05).

The relationships between total shoot biomass and: (i) lower layer biomass; (ii) upper layer biomass; (iii) sheath biomass; and (iv) leaf blade biomass, were studied through linear regression using log-transformed data (Poorter and Nagel 2000). Slope tests were carried out to compare these relationships among treatments. Whenever slopes and intercepts among linear adjustments did not differ, data were pooled and a single linear equation was presented. Shoot biomass results are presented as non-transformed means (± standard errors) of 10 replicates. Allometric analysis was used to determine whether shoot biomass differences among plant compartments were due to a size reduction or changes in biomass allocation.

Results

The experimental period extended from late autumn to spring, showing average daily minimum temperature of 7.8 ± 5.6 ºC, average daily maximum temperature of 18.5 ± 5.1 ºC and daily mean temperature of 12.8 ± 4.8 ºC. During the experiment, 13 frost events were recorded, when minimum temperature was lower than 0 ºC (between -0.2 and -3.9 ºC). The atmospheric evapotranspirative demand, estimated through the air vapor pressure deficit, gradually increased till spring, ranging from 0.42 to 2.10 kPa (mean of 1.10 kPa; Figure 1). In addition, average daily temperatures by month from 2 February to 23 May were 21.7, 20.1, 16.8 and 12.4 ºC for February, March, April and May, respectively.

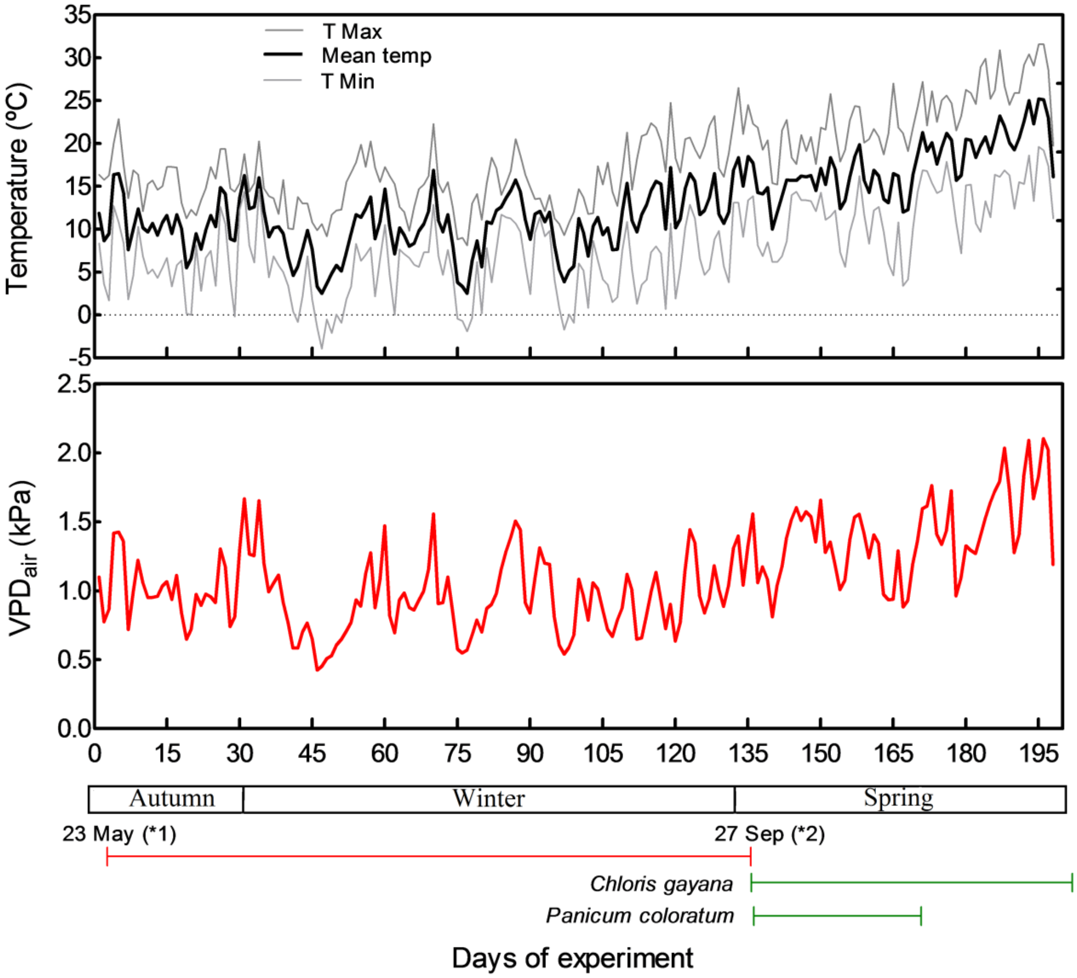

Figure 1. Daily maximum, mean and minimum air temperatures (upper panel) and air vapor pressure deficit (VPD, lower panel) during the study. (*1) Deferred forage cut (autumn); (*2) Early spring cut.

Plants that retained autumn-deferred forage during winter (DF) attained a higher shoot biomass (*C. gayana*, P<0.01; *P. coloratum*, P<0.05) following spring growth than plants whose deferred forage was removed at the beginning of winter (DFR). This effect was expressed in both species with average increases of 55 and 83% in *C. gayana* and *P. coloratum*, respectively (Figure 2). Noticeably, no plants died throughout the course of the experiment. The change in shoot biomass occurred only in the lower biomass layer in *C. gayana* (lower layer, P<0.05; upper layer, P=0.73) and in both layers in *P. coloratum* (lower layer, P<0.01; upper layer, P<0.01) (Figure 2A). The biomass of stolons in *C. gayana* was not affected by the removal of deferred forage (P>0.05). Total shoot biomass accumulated during the experimental period including growth in spring, winter and that removed at the beginning of the experiment (DFR treatment only), was 53 and 80% higher in *C. gayana* and *P. coloratum*, respectively, for the treatment where deferred forage was retained (Figure 2B).

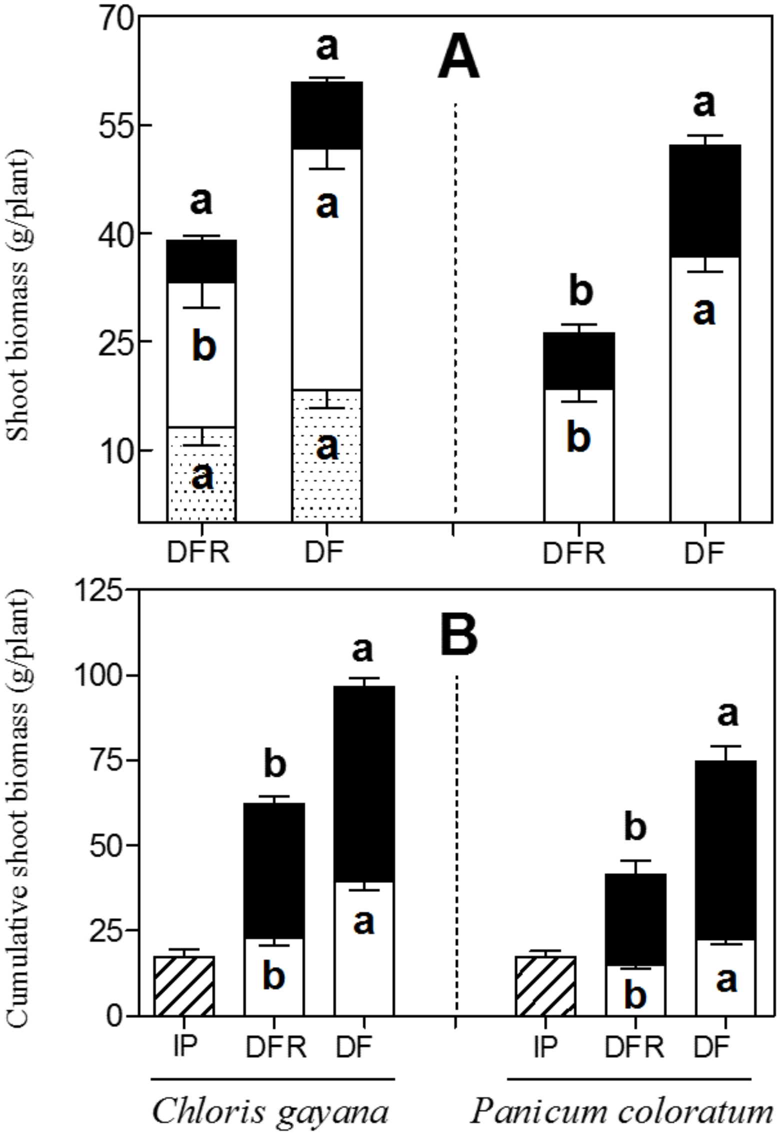

Figure 2. **A)** Shoot dry matter biomass after spring growth of *Chloris gayana* and *Panicum coloratum* plants, allowed to grow during autumn and then subjected to deferred forage removal at beginning of winter (DFR) or left as control plants (DF), then cut in early spring. Forage is divided into upper layer (black bars, >15 cm), lower layer (white bars, <15 cm) and stolons (shaded bars, *C. gayana* only). **B)** Cumulative shoot dry matter biomass during the experiment, including spring growth (black bars), removed autumn forage and early spring cut (DFR plants) and early spring cut (DF plants) (white bars) and initial plant biomass (IP). Within plant species and components, bars with different letters are significantly different based on Student's T-test. Values are means and s.e. based each on 10 replicates at each harvest.

The relationships between the biomass located in different plant layers (upper and lower) and both blade and sheath biomass were studied in correlations with total shoot biomass during spring growth. There were significant linear relationships (Table 1, R values higher than 0.80) between accumulated biomass (log-transformed data) of both layers (above and below 15 cm) and total shoot biomass (Figure 3, left panel) in *C. gayana* exposed to the DF treatment. No linear relationships between measurements of biomass were found in plants in which deferred forage was removed (DFR) in this species (Figure 3, empty points; Table 1, R values lower than 0.17). The situation with *P. coloratum* was different, since there were significant linear relationships for both treatments across all compartments (Figure 3, right panel; Table 1).

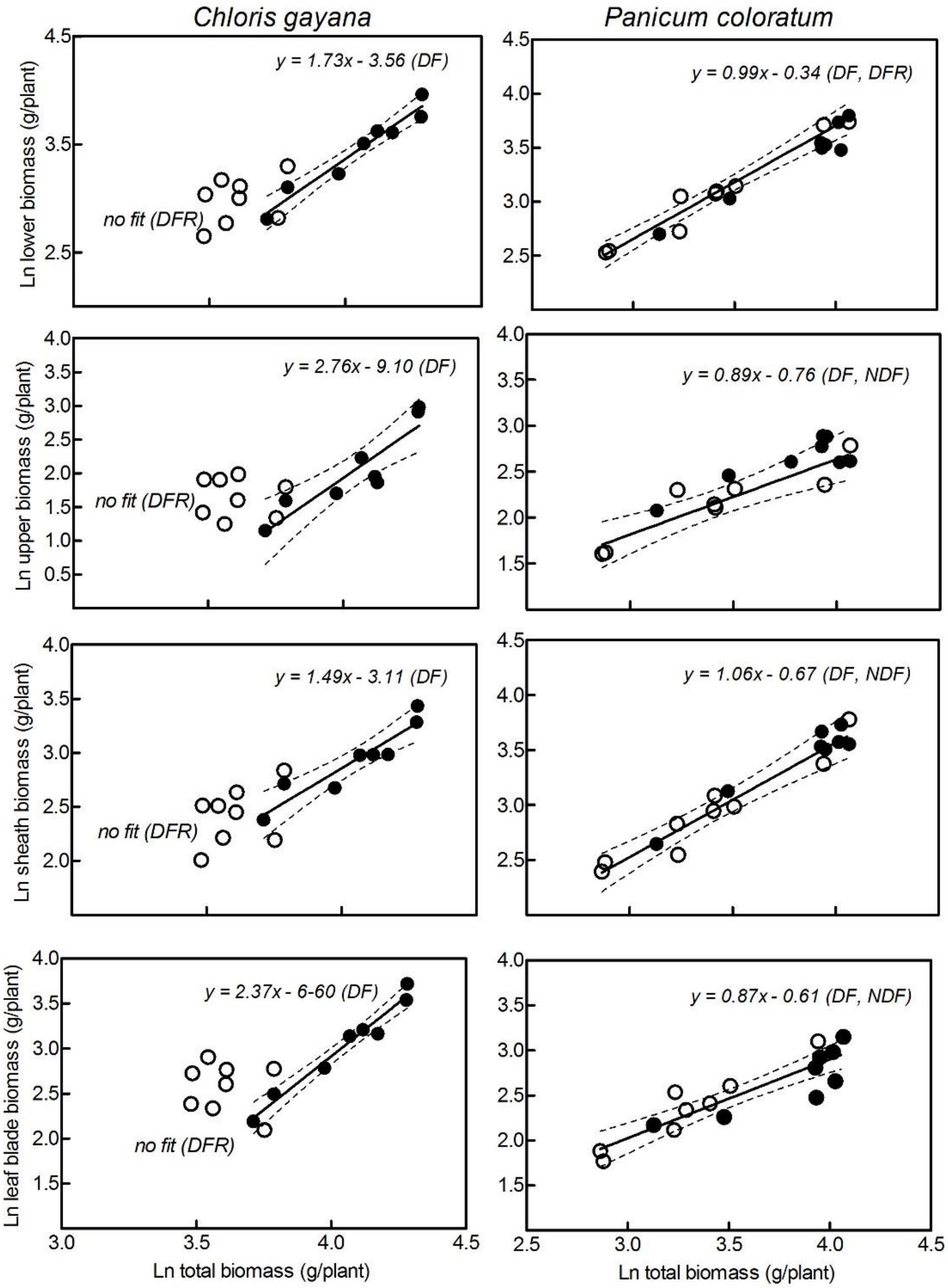

Figure 3. Allometric relationships between Ln of total shoot biomass at the end of spring and: (i) Ln of lower layer biomass, (ii) Ln of upper layer biomass, (iii) Ln of sheath biomass and (iv) Ln of leaf blade biomass of *Chloris gayana* and *Panicum coloratum* plants subjected to deferred forage removal at the beginning of winter (open symbols, DFR) and control plants (closed symbols, DF). Note: when slopes and intercepts among fitted regression lines did not differ, data were pooled and a single regression line for both species is presented for clarity, i.e. for *P. coloratum*.

Table 1. Relationships between Ln of total shoot biomass and: (i) Ln of lower layer biomass, (ii) Ln of upper layer biomass, (iii) Ln of sheath biomass and (iv) Ln of leaf blade biomass of *Chloris gayana* and *Panicum coloratum* plants subjected to deferred forage removal at the beginning of winter (DFR) and control plants (DF) (Slopes ± s.e.). R^2 values and F values are presented for each fitted regression line.

	Slope		F		R^2	
	DFR	DF	DFR	DF	DFR	DF
Chloris gayana						
Lower layer biomass	0.739 ± 0.726	1.732 ± 0.163	1.037 NS	112.60 ***	0.147	0.949
Upper layer biomass	- 0.196 ± 1.027	2.760 ± 0.514	0.036 NS	28.73 **	0.001	0.827
Sheath biomass	0.970 ± 0.873	1.494 ± 0.226	1.234 NS	43.42 ***	0.170	0.878
Leaf blade biomass	- 0.406 ± 0.973	2.379 ± 0.183	0.173 NS	168.90 ***	0.028	0.965
Panicum coloratum						
Lower layer biomass	1.047 ± 0.078	1.075 ± 0.108	175.70 ***	99.04 ***	0.961	0.942
Upper layer biomass	0.820 ± 0.149	0.697 ± 0.176	30.27 **	15.69 **	0.834	0.723
Sheath biomass	1.041 ± 0.112	1.048 ± 0.106	86.14 ***	97.24 ***	0.924	0.941
Leaf blade biomass	1.180 ± 0.144	0.886 ± 0.233	66.79 ***	14.37 ***	0.917	0.705

Discussion

The presence of the standing deferred forage during winter produced a positive effect on plant regrowth of both species during spring that could be a result of maintaining the viability of meristems, which produce new vegetative growth after winter, and protecting stem bases from cold (Ferro et al. 2015). Tropical grasses grow very slowly under cool temperatures, as a result of markedly low rates of leaf formation, leaf expansion and photosynthesis (Moore et al. 2004). Since grass regrowth depends mainly on current carbon assimilation (Schnyder and Visser 1999), stem bases are important as a reserve compartment of non-structural carbohydrates (NSC). After the cool season, when tropical grasses begin to grow, these reserves are the main source of carbon until leaves appear and rates of photosynthesis increase as the warm season progresses (de Visser 1997). Some studies with tropical grasses suggest that seasonal variation in the allocation of photo-assimilates is a mechanism used by plants to ensure their survival during winter. Carvalho et al. (2001) observed that *Cynodon* spp. plants increased the NSC in root bases from mid-spring, while NSC in the base of stems began to decrease as a result of plant growth. In this experiment, removing the deferred forage in early winter possibly promoted vegetative growth at this time, reducing reserves which had accumulated in stem bases during the previous growing season (Ferro et al. 2015). Therefore, DFR plants could have faced winter and early spring with lower stem-base reserves than DF plants.

Regarding the physical effects of herbage cover, we hypothesize that deferred forage may protect plant meristems from the pernicious action of frosts, allowing them to largely remain viable. Thus, the physical barrier provided by deferred grass creates a microenvironment, which safeguards the active meristems from the cold temperatures (Davies and McNaughton 1980). In fact, temperature measurements carried out on stem bases (between 3 and 5 cm above soil surface) support this hypothesis, showing that temperatures recorded in DF plants were 1–3 °C higher than those in DFR plants, where air temperatures were below zero (data not shown). Since active plant meristems are sinks of C and N compounds and more relevant than roots (Wardlaw 1990), DF plants might be able to achieve a rapid transition when temperatures increase in spring, and utilize the stored nutrients to produce early spring growth.

Spring regrowth differences between species could be associated with the better cold tolerance of *P. coloratum*, which suffered less death of tissues during winter. This species achieved faster regrowth than *C. gayana* due to its ability to retain more green leaf area at the commencement of spring (Kobayashi et al. 1978). Despite the temperature increase during spring and resulting response in shoot biomass, temperatures had not reached optimal values for maximum growth. This is one possible reason why statistically significant differences in biomass in the upper layer between treatments in *C. gayana* were not observed. Sustaining plant activity, even if rates of photosynthesis are low, could contribute to preserving NSC reserves located in the roots/crown fraction (top 5 cm of roots and 2.5 cm above-ground stubble) during cold winter conditions. Previous studies have shown that *Brachiaria decumbens* could increase NSC reserves by maintaining plant activity during less restrictive winter conditions in a tropical climate (Andrade and Marques Neto 1989; Soares Filho 1991), while NSC reserves in *Cynodon* spp. declined in order to ensure vital functions of tissues, when reduction in leaf area was severe as a result of a hard winter in mid-west USA (Missouri) (Dunn and Nelson 1974). Additionally, structures like stolons or rhizomes are important for the survival of tropical grasses which lack hardy aerial shoots, because their location is generally near soil level where temperatures are warmer (Davies and McNaughton 1980). Biomass of stolons was not reduced in either DF or DFR *C. gayana* plants; this finding could be important not only in terms of winter plant survival but also for pasture recovery during the warm season.

The practice of retaining deferred forage to reduce cold damage under freezing conditions proved effective in *C. gayana*. While *C. gayana* plants, where deferred forage was removed (DFR treatment), suffered significant death of shoot biomass and were strongly stressed, DF plants showed a considerable proportion of green foliage in the lower layer. On the other hand, *P. coloratum* was able to maintain green foliage on almost whole plants during the cool season in both treatments. Although high cardinal base temperature can be the factor limiting winter growth of tropical grasses to minimal levels, water deficit can be a limiting factor in areas where a marked dry season coincides with the cool season (Jones 1985; Pearson et al. 1985). Accumulation of shoot biomass in late autumn and winter in both species was considerable and was greater under grass deferral (Figure 2B, white bars). However, it is important to mention that the rate of growth in spring was much greater than in winter (see dates in experimental details and Figure 1). The presence of adequate soil moisture during the study probably resulted in pasture growth mainly in late autumn and early spring, when no frost events were recorded (Figure 1, T min line). Other studies (Kobayashi et al. 1978; Ostrowski and Fay 1979; Tischler and Ocumpaugh 2004) under high water availability (rainfall) in southeast Queensland (Australia) found that *P. coloratum* produced forage growth longer into winter than other tropical grasses.

In tropical and subtropical climates with a marked dry winter season, soil moisture conditions are usually inadequate to support pasture growth, but soil moisture is often adequate in temperate climates (Soriano et al. 1991). This can have a positive impact on productivity because grass response to environmental stresses depends not only on the plant’s tolerance of the actual event, but also on the ability to grow after the stress is removed, when water supplies could be adequate for growth (Striker 2008). Studies that analyze growth patterns and water use efficiency in tropical pastures under temperate climates are scarce or even non-existent. The fact that DF plants produced more shoot biomass than DFR plants as well as suffering reduced green leaf death should contribute to identifying management strategies to allow tropical plants to cope better under hard winter conditions. Thus, producing leafy pastures for utilization during the warm season, when forage quality and availability are high, and resting pastures during the cool season, when herbage protects plants from cold and forage quality is low, could be an effective alternative management strategy (see also O´Reagain et al. 2009).

Poorter and Nagel (2000) highlighted the importance of incorporating allometric analysis in order to study biomass allocation under different stress conditions, as many morphological and biomass ratios change based on plant size. Some factors could affect plant growth rate, but not affect biomass allocation to different structures at a given size. This allometric analysis identified that *C. gayana* was severely stressed in winter, when deferred forage was removed (absence of fitted data, Figure 3), while DF plants of this species were not. We associate the lack of fit between total plant biomass accumulation and different plant compartments with a stressful condition for plant growth in DFR plants (Figure 3, left panels; Table 1, R values). On the other hand, *P. coloratum* showed a good fit in both treatments supporting previous statements about the good tolerance of this species to these experimental conditions (Figure 3, right panels; Table 1, R values). An allometric analysis in *P. coloratum* subjected to other environmental stress (complete flooding) showed a strong correlation among shoot biomass reductions, oxidative stress and the absence of fit (Imaz et al. 2012). Similarly, Kollmann et al. (2002) reported that some allometric relationships were radically altered in the ornamental species, *Kochia scoparia*, in response to over-crowding. These findings agree with previous studies, which demonstrated that *P. coloratum* can tolerate both moderate chilling and freezing stresses, when used as cultivated forage in temperate livestock systems.

Conclusion

The results obtained in this experiment appear significant for the ecophysiology and grazing management of these tropical grasses. This pot study showed a positive effect on spring growth of retaining forage accumulated during autumn as winter cover. In this sense, *P. coloratum* was less stressed than *C. gayana* as reflected in reduced foliage damage and increased shoot biomass accumulation during spring growth, as the allometric analysis has shown. We suggest the following grazing management strategies should be tested for these tropical grasses in temperate environments where frosts occur: (i) allow forage to accumulate in autumn and do not graze or cut it during winter (especially between the first and last frost events) in order to reduce the damage caused by cold and to achieve faster spring regrowth; (ii) commence grazing or cutting in late spring or early summer when temperatures increase and shoot biomass is fully recovered. These strategies need to be verified under field conditions with grazing animals or cutting before recommendations are made to farmers. Future work should also examine forage availability and quality at different times of the year, as part of a temperate pastoral system.

References

Anderson JA; Wu YQ. 2011. Freeze tolerance of forage bermudagrasses. Grass and Forage Science 66:449–452. DOI: 10.1111/j.1365-2494.2011.00802.x

Andrade IF; Marques Neto J. 1989. Efeito da carga animal sobre os níveis de carboidratos solúveis e pesos seco da base do colmo, base do caule e sistema radicular de *Brachiaria decumbens*. Revista da Sociedade Brasileira de Zootecnia 18:426–431.

Baruch Z. 1994. Responses to drought and flooding in tropical forage grasses. I. Biomass allocation, leaf growth and mineral nutrients. Plant and Soil 164:87–96. DOI: 10.1007/bf00010114

Baron VS; Bélanger G. 2007. Climate and forage adaptation. In: Barnes RF; Nelson CJ; Moore KJ; Collins M, eds. Forages: The science of grassland agriculture, Vol. II. Sixth Edn. Blackwell Publishing, Oxon, UK. p. 83–104.

Boschma SP; Lodge GM; Harden S. 2008. Herbage mass and persistence of pasture legumes and grasses at two potentially different saline and waterlogging sites in northern New South Wales. Australian Journal of Experimental Agriculture 48:553–567. DOI: 10.1071/EA07115

Carvalho CA; Silva SC da; Sbrissia AF. 2001. Carboidratos não estruturais e acúmulo de forragem em pastagens de *Cynodon* spp. sob lotação contínua. Scientia Agricola 58:667–674. DOI: 10.1590/S0103-90162001000400003

Chaparro CJ; Sollenberger LE; Jones CS. 1995. Defoliation effects on 'Mott' Elephantgrass productivity and leaf percentage. Agronomy Journal 87:981–985. DOI: 10.2134/agronj1995.00021962008700050034x

Chapman SC; Chakraborty S; Dreccer MF; Howden SM. 2012. Plant adaptation to climate change – opportunities and priorities in breeding. Crop & Pasture Science 63:251–268. DOI: 10.1071/cp11303

Cook BG; Pengelly BC; Brown SD; Donnelly JL; Eagles DA; Franco MA; Hanson J; Mullen BF; Partridge IJ; Peters M; Schultze-Kraft R. 2005. Tropical forages: An interactive selection tool. Web tool. CSIRO, DPI&F (Qld), CIAT and ILRI, Brisbane, Australia. (Available at: www.tropicalforages.info).

Crush JR; Rowarth JS. 2007. The role of C_4 grasses in New Zealand pastoral systems. New Zealand Journal of Agricultural Research 50:125–137. DOI: 10.1080/00288230709510287

Davies LJ; McNaughton KG. 1980. Effect of winter herbage cover on survival and spring growth of tropical grasses in a temperate environment. New Zealand Journal of Agricultural Research 23:331–337. DOI: 10.1080/00288233.1980.10425364

Dear BS; Reed KFM; Craig AD. 2008. Outcomes of the search for new perennial and salt tolerant pasture plants for southern Australia. Australian Journal of Experimental Agriculture 48:578–588. DOI: 10.1071/EA07118

de Visser H. 1997. Nutrient fluxes in splanchnic tissue of dairy cows: Influence of grass quality. Journal of Dairy Science 80:1666–1673. DOI: 10.3168/jds.S0022-0302(97)76098-3

Dunn JH; Nelson CJ. 1974. Chemical changes occurring in three Bermudagrass turf cultivars in relation to cold hardiness. Agronomy Journal 66:28–31. DOI: 10.2134/agronj1974.00021962006600010008x

Ferro MM; Zanine AM; Ferreira DJ; Souza AL de; Geron LJV. 2015. Organic reserves in tropical grasses under grazing. American Journal of Plant Sciences 6:2329–2338. DOI: 10.4236/ajps.2015.614236

Flores JA; Moore JE; Sollenberger LE. 1993. Determinants of forage quality in Pensacola bahiagrass and Mott elephantgrass. Journal of Animal Science 71:1606–1614. (Available at: www.ncbi.nlm.nih.gov/pubmed/8392049).

Imaz JA; Giménez DO; Grimoldi AA; Striker GG. 2012. The effects of submergence on anatomical, morphological and biomass allocation responses of tropical grasses *Chloris gayana* and *Panicum coloratum* at seedling stage. Crop & Pasture Science 63:1145–1155. DOI: 10.1071/CP12335

Imaz JA; Giménez DO; Grimoldi AA; Striker GG. 2015a. Ability to recover overrides the negative effects of flooding on growth of tropical grasses *Chloris gayana* and *Panicum coloratum*. Crop & Pasture Science 66:100–106. DOI: 10.1071/CP14172

Imaz JA; Dos Santos D; Hernández O; Radrizzani A; Arroquy JI. 2015b. Effect of supplementation frequency on forage utilization by heifers grazing a tropical pasture during the dry season. Proceedings of the XXIII International Grassland Congress, New Delhi, India, 2015. (Available at: http://goo.gl/hc4sYp).

IPCC (Intergovernmental Panel on Climate Change). 2006. 2006 guidelines for national greenhouse gas inventories. Vol. 4: Agriculture, forestry and other land use. Prepared by the National Greenhouse Gas Inventories Programme, Eggleston HS; Buendia L; Miwa K; Ngara T; Tanabe K, eds. Institute for Global Environmental Strategies (IGES), Hayama, Japan. (Available at: www.ipcc-nggip.iges.or.jp/public/2006gl/).

Ivory DA. 1975. The effect of temperature on growth of tropical pasture grasses. Ph.D. Thesis. University of Queensland, Brisbane, Australia.

Johnston WH. 1996. The place of C4 grasses in temperate pastures in Australia. New Zealand Journal of Agricultural Research 39:527–540. DOI: 10.1080/00288233.1996.9513213

Jones CA. 1985. C4 grasses and cereals: Growth, development, and stress response. John Wiley & Sons, New York, USA.

Kobayashi T; Nishimura S; Tanaka S. 1978. Growth of tropical and subtropical grasses in the south-western area of Japan as influenced by air temperature. 1. Effect of sowing date, growth stage and cutting frequency on growth and yield. Science Bulletin of the Faculty of Agriculture, Kyushu University 32:169–175.

Loch DS. 1980. Seed assures future of Callide Rhodes. Queensland Agricultural Journal 106:183–187.

Loch DS; Rethman NFG; Van Niekerk WA. 2004. Rhodesgrass. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C4) grasses. Agronomy Monograph 45. American Society of Agronomy, Madison, WI, USA. p. 833–872. DOI: 10.2134/agronmonogr45.c25

Long SP. 1999. Environmental responses. In: Sage RF; Monson R, eds. C4 plant biology. Academic Press, New York, USA. p. 215–249. DOI: 10.1016/b978-012614440-6/50008-2

Ludlow MM. 1980. Stress physiology of tropical pasture plants. Tropical Grasslands 14:136–145. (Available at: https://goo.gl/1U2Vb9).

Márquez EJ; Rada F; Fariñas MR. 2006. Freezing tolerance in grasses along an altitudinal gradient in the Venezuelan Andes. Oecologia 150:393–397. DOI: 10.1007/s00442-006-0556-3

Moore KJ; Boote KJ; Sanderson MA. 2004. Physiology and developmental morphology. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C4) grasses. Agronomy Monograph 45. American Society of Agronomy, Madison, WI, USA. p. 179–216. DOI: 10.2134/agronmonogr45.c6

O´Reagain P; Bushell J; Holloway C; Reid A. 2009. Managing for rainfall variability: Effect of grazing strategy on cattle production in a dry tropical savanna. Animal Production Science 49:85–99. DOI: 10.1071/EA07187

Ostrowski H; Fay MF. 1979. Cool season productivity of five tropical grasses in a high rainfall area of south-east Queensland. Tropical Grasslands 13:149–153. (Available at: https://goo.gl/D09xuF).

Pearson CJ; Kemp H; Kirby AC; Launders TE; Mikked C. 1985. Responsiveness to seasonal temperature and nitrogen among genotypes of kikuyu, paspalum and bermuda grass pasture of coastal New South Wales. Australian Journal of Experimental Agriculture 25:109–116. DOI: 10.1071/ea9850109

Perelman SB; León RJC; Oesterheld M. 2001. Cross-scale vegetation patterns of Flooding Pampa grasslands. Journal of Ecology 89:562–577. DOI: 10.1046/j.0022-0477.2001.00579.x

Pitman WD. 2001. Environmental constraints to tropical forage plant adaptation and productivity. In: Sotomayor-Rios A; Pitman WD, eds. Tropical forage plants: Development and use. CRC Press, Boca Raton, FL, USA. p. 17–23. DOI: 10.1201/9781420038781.ch2

Poorter H; Nagel O. 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: A quantitative review. Australian Journal of Plant Physiology 27:595–607. DOI: 10.1071/pp99173

Poorter H; Bühler J; van Dusschoten D; Climent J; Postma JA. 2012. Pot size matters: A meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology 39:839–850. DOI: 10.1071/FP12049

Robinson DL; Castillo AE; Kong T. 1993. Acid tolerance of forage species. Better Crops 77(4):8–11.

Sage RF; Pearcy RW. 2000. The physiological ecology of C_4 photosynthesis. In: Leegood RC; Sharkey TD; von Caemmerer S, eds. Photosynthesis: Physiology and metabolism. Kluwer Academic Publishers, Dordrecht, The Netherlands. p. 497–532.

Schnyder H; de Visser R. 1999. Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones. Plant Physiologist 119:1423–1436. DOI: 10.1104/pp.119.4.1423

Soares Filho CV. 1991. Variação sazonal de parâmetros bioquímico-fisiológicos em *Brachiaria decumbens* estabelecida em pastagem. Dissertação (Mestrado). Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, Brazil.

Sollenberger LE; Reis RA; Nussio LG; Chambliss CG; Kunkle WE. 2004. Conserved forage. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C4) grasses. Agronomy Monograph 45. American Society of Agronomy, Madison, WI, USA. p. 355–387. DOI: 10.2134/agronmonogr45.c10

Soriano A. 1991. Río de la Plata grasslands. In: Coupland RT, ed. Ecosystems of the world 8A: Natural grasslands. Introduction and Western Hemisphere. Elsevier, Amsterdam, The Netherlands. p. 367–407.

Striker GG. 2008. Visiting the methodological aspects of flooding experiments: Quantitative evidence from agricultural and ecophysiological studies. Journal of Agronomy & Crop Science 194:249–255. DOI: 10.1111/j.1439-037X.2008.00317.x

Tischler CR; Ocumpaugh WR. 2004. Kleingrass, blue panic and vine mesquite. In: Moser LE; Burson BL; Sollenberger

LE, eds. Warm-season (C4) grasses. Agronomy Monograph 45. American Society of Agronomy, Madison, WI, USA. p. 623–649. DOI: 10.2134/agronmonogr45.c18

Wardlaw IF. 1990. Tansley Review No. 27 The control of carbon partitioning in plants. New Phytologist 116:341–381. DOI: 10.1111/j.1469-8137.1990.tb00524.x

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Research paper

Evaluation of yield and forage quality in main and ratoon crops of different sorghum lines

Evaluación del rendimiento y de la calidad forrajera en la primera cosecha y la soca de líneas de sorgo

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Abstract

Improving the yield and quality of sorghum (*Sorghum bicolor*) forage for livestock feeding is a major breeding objective, because of sorghum's inherently high biomass accumulation, high productivity per unit water utilized and its ability to produce a ratoon crop after harvesting of the plant crop. Newly bred sorghum lines, including 36 lines falling in 5 different categories, i.e. 12 experimental dual-purpose lines, 6 germplasm accessions from the ICRISAT collection, 11 commercial varieties and hybrids, 6 forage varieties and 1 *bmr* mutant line, were evaluated in terms of fodder yield, quality and ratooning ability. The main crop produced more dry biomass ($P<0.05$) at 80 days after planting (mean 22.87 t DM/ha; range 17.32–33.82 t DM/ha) than the ratoon crop (mean 8.47 t DM/ha; range 3.2–17.42 t DM/ha) after a further 80 days of growth. Mean nitrogen concentration in forage did not differ greatly between main and ratoon crops (2.56 vs. 2.40%, respectively) but there was wide variation between lines (2.06–2.89%). The line N 610 recorded highest N percentage of 2.89%, followed by SSG 59 3 (2.86%) and SX 17 (2.81%). Highest acid detergent fiber % was recorded by ICSV 12008 (42.1%), closely followed by CO 31 and IS 34638 (40.0%). The least acid detergent lignin % was observed in MLSH-296 Gold (3.59%), ICSV 700 (3.75%) and ICSSH 28 (3.83%). Metabolizable energy concentration was highest in N 610, Phule Yashodha and SX 17 (mean 8.34 MJ/kg DM), while in vitro organic matter digestibility ranged from 52.5 to 62.6%. The main crop contained much higher mean concentrations of the cyanogenic glycoside, *dhurrin*, than the ratoon (639 vs. 233 ppm, respectively) with ranges of 38 to 2,298 ppm and 7 to 767 ppm, respectively. There was no significant correlation between *dhurrin* concentration and dry biomass yield so breeding and selection for low *dhurrin* concentrations should not jeopardize yields. Hence, breeding for sorghum can target simultaneously both quality and biomass improvement.

Keywords: Cyanogenic glycoside, digestibility, dry biomass production, fodder quality, tillering ability.

Resumen

Mejorar el rendimiento y la calidad del forraje para la alimentación del ganado es un objetivo importante de fitomejoramiento en sorgo (*Sorghum bicolor*), debido al alto potencial de la especie para acumular biomasa, su alta productividad por unidad de agua utilizada y su capacidad de rebrotar después de la primera cosecha. En un experimento de campo en Patancheru, India, fueron evaluadas por rendimiento de forraje, calidad nutritiva y capacidad de rebrote 36 líneas nuevas de sorgo de 5 categorías diferentes: 12 líneas experimentales de doble propósito (grano, forraje); 6 accesiones de germoplasma de la colección del ICRISAT; 11 variedades e híbridos comerciales; 6 variedades forrajeras; y 1 línea de mutante *bmr*. En la primera cosecha, realizada 80 días después de la siembra, la producción promedio de

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MS fue de 22.87 t/ha, con un rango de 17.32–33.82 t/ha), mientras que en la segunda, realizada 80 días después de la primera, disminuyó ($P < 0.05$) alcanzando un promedio de 8.47 t/ha (rango 3.2–17.42 t/ha). La concentración promedio de nitrógeno en el forraje no varió entre cortes (2.56 vs. 2.40%, respectivamente), pero sí se observó una alta variación entre las líneas (2.06–2.89%). La línea N 610 presentó la mayor concentración de N (2.89%), seguida por las líneas SSG 59 3 (2.86%) y SX 17 (2.81%). La concentración más alta de fibra detergente ácida se registró para ICSV 12008 (42.1%), seguida por CO 31 e IS 34638 (40.0%). Los porcentajes más bajos de lignina detergente ácida se observaron en las líneas MLSH-296 Gold (3.59%), ICSV 700 (3.75%) e ICSSH 28 (3.83%). La mayor concentración de energía metabolizable (promedio de 8.34 MJ/kg MS) se presentó en las líneas N 610, Phule Yashodha y SX 17, mientras que la digestibilidad in vitro de la materia orgánica varió de 52.5 a 62.6%. En el forraje de la primera cosecha se encontraron concentraciones mucho más altas de dhurrina, un glucósido cianogénico, que en la soca (639 vs. 233 ppm, respectivamente), con rangos de 38 a 2,298 ppm y de 7 a 767 ppm, respectivamente. No se encontró correlación significativa entre la concentración de dhurrina y el rendimiento de materia seca, por lo que programas de fitomejoramiento y selección buscando concentraciones bajas de dhurrina no estarían comprometiendo el rendimiento. Por tanto, proyectos de fitomejoramiento de sorgo podrían enfocar simultáneamente tanto la calidad de la biomasa como su cantidad.

Palabras clave: Calidad forrajera, capacidad de rebrote, digestibilidad in vitro de la materia orgánica, glucósido cianogénico, producción de materia seca.

Introduction

Sorghum (*Sorghum bicolor*) is a dual-purpose crop used for both human food and animal feed in many Asian and African countries (Sarfraz et al. 2012; Bean et al. 2013), with key characteristics being wide adaptability across environments and tolerance to biotic and abiotic stresses (Krishnamurthy et al. 2007; Dahlberg et al. 2011; Gill et al. 2014). The crop residue is used mainly for feeding livestock by small farmers in the Asian and African continents (Hassan et al. 2015). Owing to very high crude fiber and very low crude protein concentrations, sorghum stover left after harvesting grain does not provide quality fodder for milking cattle (Manjunatha et al. 2014).

The contribution of sorghum as a fodder crop has increased the value of production in recent years, so selection criteria in breeding programs could include biomass production and quality as well as grain yield (Hassan et al. 2015). This thinking has been applied in breeding programs with emphasis given to forage quality improvement and selection of nutritious varieties for fodder purposes (Bean and McCollum 2006), as sorghum is the most preferred alternative silage crop after corn (*Zea mays*) (Kurle et al. 1991). However, feed quality data on the newly bred lines are not available, which makes commercialization challenging (Akabari and Parmar 2014). The important feed trait to be considered is potential of the plant to accumulate high dry matter yields of good quality forage. Sorghum displays wide variability for concentrations of protein, fiber, carbohydrates, crude fat and nitrogen free extract as well as in vitro dry matter degradability (Singh and Shukla 2010; Afzal et al. 2012). Assessment for the anti-nutritional factor, hydrocyanic acid (HCN), also known as *dhurrin* (further used in text), is also of vital importance. The permissible/safe threshold for HCN in sorghum fodder is 500 ppm (dry matter basis) or >200 ppm (fresh weight basis) (Smitha Patel et al. 2013). HCN is rapidly absorbed into the blood stream of grazing ruminants and can cause cellular asphyxiation and eventually death (Hoveland and Monson 1980). Hence, it is necessary to develop varieties or hybrids with high fodder yields, acceptable quality and low HCN concentrations. Sorghum has good ratooning ability from stubble of the plant crop, which is a desirable trait, as it reduces overall inputs in terms of seed for planting and labor for field preparation (Willey 1990).

Hence, the current study focused on the evaluation of forage dry biomass yield, feed quality and HCN concentration in both the main and ratoon crops of a range of sorghum lines, to aid farmers in choosing the most appropriate lines for feeding to their livestock in particular circumstances and to provide background data for planning future breeding programs.

Material and Methods

Field experiment

A total of 36 improved sorghum lines (Table 1) were evaluated for feed quality and agronomic performance, at ICRISAT, Patancheru, India. The field studies were performed during the rainy season (commencing mid-July) on a medium-fertility vertisol. The experimental design adopted was an alpha lattice design with 2 replications and 6 entries in 6 blocks, with 4 rows of each entry in 0.2 ha. The field was fertilized with di-ammonium phosphate at 80 kg/ha and 40 kg KCl/ha at the time of sowing and top dressed with 145 kg urea 21 days after planting. Immediately after the initial harvest, nitrogen was applied at the rate of 45 kg N/ha (top dressing) and 1 irrigation was provided to increase nitrogen absorption. Seed treatment against soil-borne pests and diseases was performed with thiram at 3 g/kg seed. Seedlings were thinned to 1 plant per hill 3 weeks after sowing, maintaining about 20 cm distance between seedlings and 60 cm between rows; gross plot area was 9.6 m^2 (4 rows × 0.6 m × 4 m) and net plot area was 4.8 m^2 (2 rows × 0.6 m × 4 m), where the observations were recorded. For each sorghum line the population maintained was about 80–85 plants. The crop was irrigated during sowing and at critical growth stages. The first sampling from the main crop was performed at 15 cm from ground level from the middle 2 rows excluding borders at 80 days after sowing, and the second sampling 80 days later. After the initial harvest, the remaining rows were cut at 15 cm above ground, the forage removed and plants allowed to tiller from the stubble. All agronomic and feed quality parameters were assessed on harvested forage. Agronomic traits recorded during the experiment were: plant height (measured after flowering, from ground level to the tip of the plant); tillering ability (measured by the number of tillers produced by the mainstem in a clump); ratoon scoring (measured by the percentage of plants that produced productive tillers after harvesting; 1: 81–100% stubble tillering, 2: 61–80% stubble tillering, 3: 41–60% stubble tillering, 4: 21–40% stubble tillering and 5: <21% stubble tillering) and dry biomass yield (determined by harvesting all plants in the middle 2 rows and drying in forced-air ovens at 60 °C for 4–5 days).

Feed quality parameters

Quality analysis of forage was performed with 15 plants per line selected at random from each replication, hand-cut into pieces of 4–5 cm length, dried at 60 °C for 4–5 days, later ground in hammer mills to pass through a 1-mm mesh and analyzed at the livestock nutritional laboratory of ILRI in Patancheru. Concentrations of N, acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL) and metabolizable energy (ME) were determined by Near Infrared Spectroscopy (NIRS), calibrated for this experiment against conventional wet laboratory analyses. The NIRS instrument used was a FOSS Forage Analyzer 5000 with software package Win ISI II. Biological fodder quality traits of the forage samples were analyzed for apparent in vitro digestibility using in vitro gas production procedures (Menke and Steingass 1988).

Dhurrin *estimation*

The youngest leaf (1 leaf from 3 plants per plot) at the booting stage was cut from plants and 100 mg of fresh leaf sample was placed in Eppendorf tubes (2 mL) containing 750 μL of 50% methanol and inserted in a hot water bath at 75 °C for 15 min. The tubes were then cooled to room temperature and 750 μL of 50% methanol was added, to make up the volume to 1.5 mL. Later the tissue was lyzed and centrifuged @ 11,000 rpm for 5 min. One mL of the supernatant was transferred to fresh tubes and stored at 4 °C prior to analysis in Acquity UPLC (Waters, Model D13 CHA 708 G). The mobile phase was 10% acetonitrile and column C-18, with detector-PDA. The *dhurrin* was detected by monitoring the absorbance at 232 nm (De Nicola et al. 2011).

Statistical analyses

Analysis of variance was performed in linear model for Lattice Design: $Yijl = \mu + \tau_i + \gamma_i + \tau_{l(j)} + \mu_{ijl}$, where: τ_i = Treatment effect i= 1, 2,…, t; γ_i = Replicate effect j = 1, 2; $\tau_{l(j)}$ = Block within replicate effect l = 1, 2,…, s; and μ_{ijl} = Random error. Statistical package, GENSTAT 17 edition for Windows (VSN International, Hemel Hempstead, UK, 2015), was used to analyze the data generated.

Results

Agronomic results

Average plant height (Table 1) was greater in the ratoon crop (2.59 m; range 1.75–3.62 m) than in the main crop (2.28 m; range 1.9–2.7 m). The line IS 31553 (2.70 m) recorded the greatest plant height in the main crop, while IS 13553 (3.62 m) was the tallest in the ratoon. Mean ratoon score in the ratoon crop (shoot regeneration from the stubble of the first harvest) was 3 with a range of 1–4 between lines ($P<0.05$). Tillering ability (number of additional shoots from main shoot) was higher in the main crop than in the ratoon (5 vs. 3) with ranges of 1–19 and 1–14, respectively. The ratoonability of plants was negatively associated ($P<0.05$) with the number of tillers in the ratoon, reflecting to some extent the scoring system employed. Mean dry biomass yield for the main crop at 80 days after planting was 22.87 t/ha with a range for different lines of 17.32–33.82 t/ha ($P<0.05$) (Table 1), while mean dry biomass yield for the ratoon crop at 80 days after the first harvest was 8.47 t/ha with a range of 3.2–17.42 t/ha ($P<0.05$). Individual lines which performed well for the main crop were ICSSH 28 (33.82 t/ha), IS 31553 (27.54 t/ha),

Table 1. Mean values for agronomic parameters plant height (PH, m), dry biomass yield (DB, t/ha) and *dhurrin* concentration (DH, ppm) of 36 sorghum lines in main and ratoon crops.

Line	Main crop			Ratoon crop		
	PH	DB	DH	PH	DB	DH
Dual purpose experimental lines						
CSV 24 SS	1.9	23.42	420	2.7	9.73	161
GD 65013	2.08	19.65	612	2	5.96	281
ICSSH 28	2.45	33.82	454	2.53	12.09	94
ICSV 12006	2.1	24.55	1,185	3	9.38	289
ICSV 12008	2.3	26.65	101	2.3	9.16	680
ICSV 12012	2.28	23.24	1,177	2.75	11.29	547
ICSV 12015	2.53	19.45	1,038	3.03	6.31	210
ICSV 25275	2.43	22.39	1,055	2.03	5.24	200
ICSV 25333	2.4	24.27	406	3.13	13.22	219
ICSV 700	2.1	23.8	360	2.2	5.69	153
ICSV 93046	2.3	24.91	320	2.45	9.76	125
SSV 84	2.4	17.32	494	2.35	10.84	674
Collection from Genetic Resource Division, ICRISAT (germplasm lines chosen for high biomass)						
IS 13553	1.9	21.34	471	3.63	10.84	401
IS 14212	2.15	27.52	834	2.45	6.24	66
IS 23143	2.55	17.61	38	3.05	8.67	570
IS 31553	2.7	27.55	216	2.43	17.42	167
IS 33871	2.3	25.67	185	2.7	8.71	392
IS 34638	2.28	19.66	406	3.03	11.24	186
Commercial varieties and hybrids						
MLSH-296 Gold	1.95	21.53	1,545	1.75	5.29	178
Phule Anuradha	2.35	20.24	657	2.25	7.42	257
Phule Chitra	2.43	20.55	725	2.15	9.78	160
Phule Moule	2.3	22.92	965	2.2	8.27	53
Phule Yashodha	2.23	22.2	89	2.4	7.18	25
RSSV 9	2.35	22.31	297	2.93	14.13	37
Seredo	2.03	21.25	2,298	2.43	7.82	767
Star	2.18	28.13	1,363	2.95	6.64	145
SX 17	2.5	26.12	352	2.98	7.38	130
BJV 44	2.63	20.75	507	2.6	6.36	208
CSH 16	2.28	23.37	145	2.8	6.31	7
bmr mutant line						
N 610	2.05	22.56	440	2.23	11.35	277
Forage varieties						
CO 30	2.2	20.33	682	2.08	3.2	156
CO 31	2.3	28.4	639	3.23	3.87	51
CO-FS-27	2.3	18	243	2.8	4	166
CO 19	2.55	18.7	513	2.85	4.18	114
COS 28	2.3	24.58	654	2.1	16.53	171
SSG 59 3	2.23	18.6	199	2.9	3.38	68
Mean	2.28	22.87	639	2.59	8.47	233
Maximum	2.7	33.82	2,298	3.63	17.42	767
Minimum	1.9	17.32	38	1.75	3.2	7
Standard deviation	0.26	5.98	38.4	0.17	0.74	24.7
Least significant difference (P<0.05)	0.53	12.14	78.2	0.35	1.49	50.2
Coefficient of variation (%)	11.5	26.1	6	6.6	8.7	10.6

Star (28.13 t/ha) and CO 31 (28.40 t/ha), while highest yields for the ratoon crop were recorded with IS 34638 (17.42 t/ha), RSSV 9 (14.13 t/ha) and COS 28 (16.53 t/ha). In terms of total yield (main + ratoon crop) the highest yields came from ICSSH 28 (45.91 t DM/ha), IS 31553 (44.97 t DM/ha) and COS 28 (41.11 t DM/ha).

Table 2. Means, ranges and statistical differences for nitrogen, fiber (NDF, ADF) and lignin (ADL) concentrations, metabolizable energy (ME), in vitro organic matter digestibility (IVOMD), *dhurrin* concentration, plant height, ratooning ability, tiller numbers and dry biomass yield in 36 sorghum lines in main and ratoon crops.

Parameter	Mean		Range		LSD		$P<0.05$	
	Main	Ratoon	Main	Ratoon	Main	Ratoon	Main	Ratoon
Nitrogen (%)	2.56	2.40	2.23–2.89	2.06–2.72	0.54	0.40	0.4371	0.985
NDF (%)	58.0	55.6	56.1–59.8	52.6–60.7	2.48	3.31	0.1289	0.092
ADF (%)	37.9	34.1	35.2–42.1	31.5–37.9	2.76	3.46	0.0178	0.1885
ADL (%)	4.18	4.25	3.59–4.70	3.95–4.59	0.51	0.44	0.0263	0.772
ME (MJ/kg)	7.99	8.60	7.59–8.37	8.29–8.96	0.72	0.39	0.8363	0.0863
IVOMD (%)	55.7	59.7	52.5–58.9	57.1–62.6	5.32	3.04	0.7455	0.1753
Dhurrin (ppm)	639	233	37–2,298	7–767	78.1	50.2	<0.0001	<0.0001
Plant height (m)	2.28	2.59	1.90–2.70	1.75–3.62	0.53	0.35	0.3603	<0.0001
Ratoon score[1]	NA[2]	3	NA	1–5	NA	0.8483	NA	<0.0001
Number of tillers	5	3	1–19	1–14	1.80	1.40	<0.0001	<0.0001
Dry biomass yield (t/ha)	22.87	8.47	17.32–33.82	3.20–17.42	12.14	1.49	0.8165	<0.0001

[1] Scale: 1: 81–100% stubble tillering, 2: 61–80% stubble tillering, 3: 41–60% stubble tillering, 4: 21–40% stubble tillering and 5: <21% stubble tillering.

[2] NA = not applicable.

Forage quality traits

Nitrogen concentration ranged from 2.23 to 2.89% (mean 2.56%) in the main crop and from 2.06 to 2.72% (mean 2.40%) in the ratoon (Table 2). Similarly, NDF concentration varied from 56.1 to 59.8% (mean 58.0%) in the main crop and from 52.6 to 60.7% (mean 55.6%) in the ratoon. The ADF concentrations also varied between sorghum lines in the main crop (35.2–42.1%; mean 37.9%) and in the ratoon (31.5–37.9%; mean 34.1%) (Figure 1). Acid detergent lignin concentrations varied from 3.59 to 4.70% (mean 4.18%) in the main crop and from 3.95 to 4.59% (mean 4.25%) in the ratoon. Metabolizable energy concentrations were similar in the main and ratoon crops (mean values 7.99 and 8.60 MJ/kg DM) with significant differences between lines. Mean in vitro organic matter digestibility for the main crop was lower than for the ratoon (55.7 vs. 59.7%) with significant ($P<0.05$) differences between lines. The *dhurrin* concentration in the main crop was higher than in the ratoon crop (639 vs. 233 ppm, respectively) (Figure 2). There was extreme variation in *dhurrin* concentration in different sorghum lines with the commercial hybrid Seredo (2,298 ppm) recording the highest concentration in the main crop and IS 23143 recording the lowest (38 ppm).

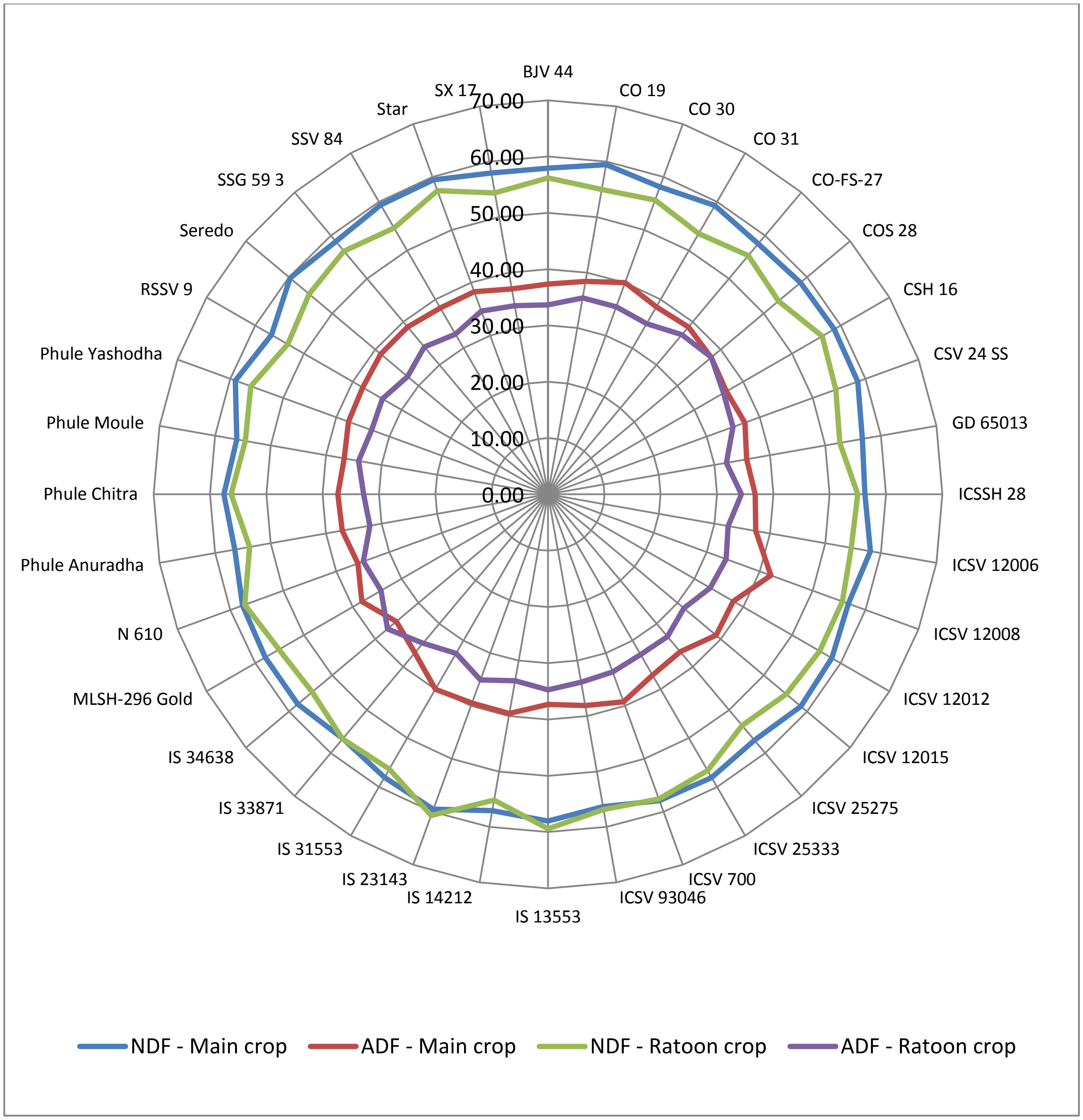

Figure 1. Ranges of neutral detergent fiber (NDF %) and acid detergent fiber (ADF %) concentrations of 36 sorghum lines in main and ratoon crops.

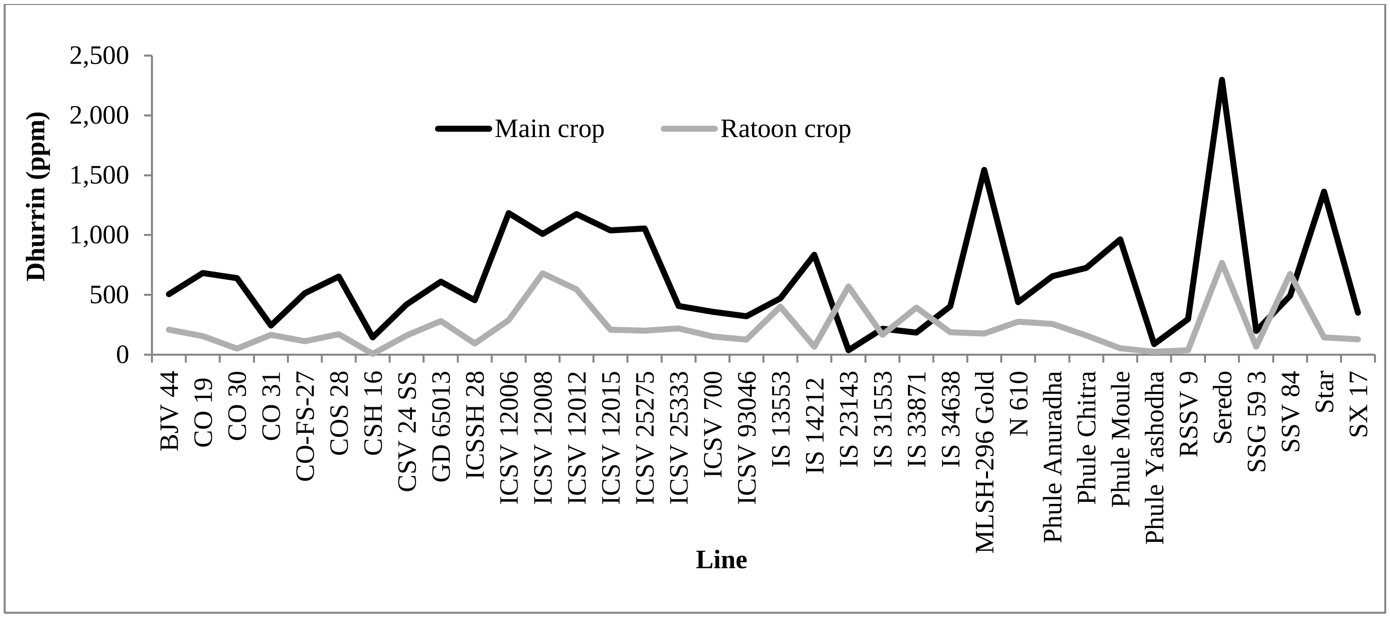

Figure 2. Variability of *dhurrin* concentrations of 36 sorghum lines in main and ratoon crops.

Similarly in the ratoon crop, concentration in Seredo was highest (767 ppm), while CSH 16 exhibited the lowest *dhurrin* concentration (7 ppm). Across the different lines of sorghum evaluated in the experiment, highest N concentrations were recorded in *bmr* line N 610 (2.90%), the forage line SSG 59 3 (2.86%) and SX 17 (2.81%). Highest ADF concentrations were recorded by ICSV 12008 (42.1%), CO 31 and IS 34638 (40.0 %). The lowest ADL concentrations were observed in MLSH-296 Gold (3.59%), ICSV 700 (3.75%) and ICSSH 28 (3.83%). ME concentration was highest in N 610 (8.38 MJ/kg DM), Phule Yashodha (8.36 MJ/kg DM) and SX 17 (8.29 MJ/kg DM). Similarly, IVOMD was highest in N 610 (58.9%), SX 17 (58.4%) and Phule Yashodha (58.3%).

Correlations

The only significant correlations (P<0.05) between parameters for main and ratoon crops were: positive correlation (r = 0.384) between *dhurrin* concentrations in main and ratoon crops; positive correlation (r = 0.806) between tiller numbers in main and ratoon crops; negative correlation (r = -0.407) between ratoon score in ratoon crop and number of tillers in main crop; and negative correlation between number of tillers in ratoon crop and ratoon score in the ratoon crop (r = -0.501) (Table 3).

Table 3. Correlations between main and ratoon crops for plant height, dry biomass yield, *dhurrin* concentration, ratooning score and number of tillers.

	PHMC[1]	DBMC	DHMC	NTMC	PHRC	DBRC	DHRC	RSRC	NTRC
PHMC	0								
DBMC	0.011	0							
DHMC	-0.324	0.067	0						
NTMC	-0.045	0.173	-0.003	0					
PHRC	0.101	0.034	-0.253	-0.101	0				
DBRC	0.163	0.296	-0.146	0.095	0.055	0			
DHRC	-0.089	-0.235	0.384*[2]	-0.127	-0.012	0.175	0		
RSRC	0.116	-0.127	-0.107	-0.407*	0.106	-0.012	0.084	0	
NTRC	-0.015	0.099	-0.056	0.806**	0.087	0.093	-0.093	-0.501**	0

[1] Plant height main crop: PHMC; dry biomass yield main crop: DBMC; *dhurrin* main crop: DHMC; no. of tillers main crop: NTMC; plant height ratoon crop: PHRC; dry biomass yield ratoon crop: DBRC; *dhurrin* ratoon crop: DHRC; ratoon score ratoon crop: RSRC; no. of tillers ratoon crop: NTRC.

[2] Significant correlations at the P<0.05 (*) and P<0.01 (**) levels.

Discussion

Agronomic results

This study has shown that the sorghum lines that we tested have great potential for production of good quality forage and show sufficient variation to allow selection within breeding programs for further improvement. Quality differences were not as great as dry biomass yield differences, but there were large differences in *dhurrin* concentration, indicating that there are much greater risks in feeding some lines than in feeding others. While there were marked differences between lines in both main crop and ratoon crop dry biomass yields, total yield (main + ratoon) is probably the most relevant. Lines like ICSSH 28, IS 31553 and COS 28 seemed the most promising in terms of total production and had *dhurrin* and NDF concentrations which were generally below average. The much higher dry biomass yields in the main crop than in the ratoon may be related to the change in seasonal conditions for growth of the 2 crops and possibly depletion of nutrient levels in the soil. Escalada and Plucknett (1975b), Srinivasa et al. (2011) and Afzal et al. (2012) suggested that higher levels of inputs (nitrogen application) are needed to prevent production differences between main and ratoon crops of sorghum. It was of interest that the ratoon crop was taller than the main crop; thus the higher yields in the main crop were a function of a greater number of tillers and possibly thicker tillers. Despite the lower yields produced, ratooning of sorghum crops for forage production has the advantages of rapid tiller initiation and early maturity but requires more fertilizer application than a corn crop (Ketterings et al. 2004). However, these traits are supplementary to the main objective, i.e. high DM yield of forage for livestock (Undersander et al. 1990; Whish and Bell 2008; Saberi 2014).

Forage quality traits

A shortcoming of this study was that leaf and stem were not separated to assess the yields and quality parameters of these plant parts independently. In our environment farmers chop the fodder and feed it to livestock as a mixture of leaf and stem, which annuls the leaf:stem separation effect. High quality silage can be produced from sorghum by making 2 harvests per season, as opposed to making a single cut at physiological maturity (McCormick et al. 1995). Although the number of tillers produced declines in each succeeding ratoon crop, acceptable yields can be obtained by increasing the plant population (Escalada and Plucknett 1975a). All lines evaluated in the current study recorded N concentrations (both in main and ratoon crops) above that required for effective rumen microbial activity (1–1.2%), a value below which feed intake can be affected (Van Soest 1994; Rai et al. 2012). A total of 26 lines in the main crop and 20 lines in the ratoon recorded N concentrations above 2.4%. The average N, NDF and ADF concentrations were higher in the main crop than in the ratoon crop, possibly mainly due to the relative advantage of fertilizer applied and more favorable weather environment during the growth period of the main crop. Contrastingly, ADL concentration was higher in the ratoon crop than in the main crop. Sweet sorghum lines have recorded high ADF and low ADL, so breeding studies to improve these lines by enhancing the fodder quality traits will expand utilization of dual-purpose lines (Blümmel and Reddy 2006). These differences in quality parameters were not significant across main and ratoon crops, as reported earlier by Srinivasa et al. (2011), even with the various fertilizer levels applied during crop growth. Harvesting the crop immediately post flowering rather than at physiological maturity will produce better quality forage, due to low lignin levels (McCormick et al. 1995). Moreover, the current evaluation was performed in vitro only, and animal feeding trials which measure intake, feed preferences/acceptance, digestibility and absorption are needed to take these preliminary results closer to the adoption stage (Miron et al. 2007).

Interestingly, mean *dhurrin* concentrations in the main crop far exceeded those in the ratoon crop (mean 639 vs. 233 ppm). However, the extreme variation between lines in *dhurrin* concentrations, especially in the main crop (37–2,298 ppm), indicates the great potential for selecting lines which are safe for feeding fresh to livestock as either the plant crop or as a ratoon crop. None of the lines recorded levels of *dhurrin* regarded as lethal (>1,000 ppm DM basis; Smitha Patel et al. 2013) in the ratoon crop. *Dhurrin* is the main anti-nutritional factor in sorghum, but is known to act as a nitrogen reserve once the crop has overcome the influence of abiotic stress (Park and Coats 2002). It limits the flexibility of using sorghum as a fodder due to its toxic effect when sorghum containing high concentrations is fed to livestock. However, since the *dhurrin* concentration in sorghum decreases with increase in maturity and the enzyme is deactivated by the process of ensiling as well (Wheeler and Mulcahy 1989), this issue can be managed when fodder is conserved for feeding later. The positive relationship between *dhurrin* concentrations in main and ratoon crops indicates that a particular line will have a consistent relative concentration whether fed as a plant or ratoon crop. The absence of significant correlation between *dhurrin* concentration and biomass yield (Table 3) indicates that attempts to lower *dhurrin* concentrations by breeding should not necessarily affect dry biomass yields of sorghum crops.

Conclusion

While dry biomass yield differences between lines were observed in both main and ratoon crops, there was little quality difference between lines except for the anti-nutritional compound *dhurrin*. The lines ICSSH 28, IS 31553 and COS 28 were consistently high yielding and could be tested more widely to verify these findings. Use of these lines to develop higher yielding varieties in a forage program would seem appropriate. The wide variation in *dhurrin* concentration in the various lines and absence of a strong relationship between *dhurrin* concentration and dry biomass yield indicates a significant potential to breed superior lines with lower *dhurrin* concentrations without jeopardizing yield.

Acknowledgments

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References

Afzal M; Ahmad A; Ahmad AH. 2012. Effect of nitrogen on growth and yield of sorghum forage (*Sorghum bicolor* (L.) Moench cv.) under three cutting systems. Cercetari Agronomice in Moldova 45(4):57–64. DOI: 10.2478/v10298-012-0065-y

Akabari VR; Parmar HP. 2014. Heterosis response and combining ability for green fodder yield and its quality traits in forage sorghum. Journal of Progressive Agriculture 5:9–14. (Available at: https://goo.gl/rF0DzC).

Bean B; McCollum T. 2006. Summary of six years of forage sorghum variety trials. Pub. SCS-2006-04. Texas Cooperative Extension and Texas Agricultural Experiment Station, College Station, TX, USA. (Available at: https://goo.gl/EuE0aU).

Bean BW; Baumhardt RL; McCollum FT III; McCuistion KC. 2013. Comparison of sorghum classes for grain and forage yield and forage nutritive value. Field Crops Research 142:20–26. DOI: 10.1016/j.fcr.2012.11.014

Blümmel M; Reddy BVS. 2006. Stover fodder quality traits for dual-purpose sorghum genetic improvement. International Sorghum and Millets Newsletter 47:87–89. (Available at: http://oar.icrisat.org/1117/).

Dahlberg J; Berenji J; Sikora V; Latkovic D. 2011. Assessing sorghum [*Sorghum bicolor* (L) Moench] germplasm for new traits: Food, fuels & unique uses. Maydica 56(2):85–92. (Available at: https://goo.gl/oX2rLw).

De Nicola GR; Leoni O; Malaguti L; Bernardi R; Lazzeri L. 2011. A simple analytical method for *dhurrin* content evaluation in cyanogenic plants for their utilization in fodder and biofumigation. Journal of Agricultural and Food Chemistry 59:8065–8069. DOI: 10.1021/jf200754f

Escalada RG; Plucknett DL. 1975a. Ratoon cropping of sorghum: II. Effect of daylength and temperature on tillering and plant development. Agronomy Journal 67:479–484. DOI: 10.2134/agronj1975.00021962006700040007x

Escalada RG; Plucknett DL. 1975b. Ratoon cropping of sorghum: I. Origin, time of appearance, and fate of tillers. Agronomy Journal 67:473–478. DOI: 10.2134/agronj1975.00021962006700040006x

Gill JR; Burks PS; Staggenborg SA; Odvody GN; Heiniger RW; Macoon B; Moore KJ; Barrett M; Rooney WL. 2014. Yield results and stability analysis from the sorghum regional biomass feedstock trial. Bioenergy Research 7:1026–1034. DOI: 10.1007/s12155-014-9445-5

Hassan SA; Mohammed MI; Yagoub SO. 2015. Breeding for dual purpose attributes in sorghum: Effect of harvest option and genotype on fodder and grain yields. Journal of Plant Breeding and Crop Science 7:101–106. DOI: 10.5897/JPBCS2015. 0498

Hoveland CS; Monson WG. 1980. Genetic and environmental effects on forage quality. In: Hoveland CS, ed. Crop quality, storage, and utilization. American Society of Agronomy and Crop Science Society of America, Madison, WI, USA. p. 139–168. DOI: 10.2135/1980.cropquality.c6

Ketterings QM; Godwin G; Cherney JH; Beer S; Kilcer TF. 2004. Nitrogen management for brown midrib sorghum sudangrass: Results of two years of studies at the Mt. Pleasant Research Farm. What's Cropping Up? 14(2):5–6. (Available at: https://goo.gl/Brne9Z).

Krishnamurthy L; Serraj R; Hash CT; Dakheel AJ; Reddy BV. 2007. Screening sorghum genotypes for salinity tolerant biomass production. Euphytica 156:15–24. DOI: 10.1007/s10681-006-9343-9

Kurle JE; Sheaffer CC; Crookston RK; Peterson RH; Chester-Jones H; Lueschen WE. 1991. Popcorn, sweet corn, and sorghum as alternative silage crops. Journal of Production Agriculture 4:432–436. DOI: 10.2134/jpa1991.0432

Manjunatha SB; Angadi VV; Palled YB; Hosamani SV. 2014. Nutritional quality of multicult fodder sorghum (CoFS-29) as influenced by different row spacings and nitrogen levels under irrigated condition. Research in Environment and Life Sciences 7:179–182. (Available at: https://goo.gl/6O3YkS).

McCormick ME; Morris ME; Ackerson BA; Blouin DC. 1995. Ratoon cropping forage sorghum for silage: Yield, fermentation, and nutrition. Agronomy Journal 87:952–957. DOI: 10.2134/agronj1995.00021962008700050030x

Menke KH; Steingass H. 1988. Estimation of the energy feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research and Development 28:7–55.

Miron J; Zuckerman E; Adin G; Solomon R; Shoshani E; Nikbachat M; Yosef E; Zenou A; Weinberg ZG; Chen Y; Halachmi I; Ben-Ghedalia D. 2007. Comparison of two forage sorghum varieties with corn and the effect of feeding their silages on eating behavior and lactation performance of dairy cows. Animal Feed Science and Technology 139:23–39. DOI: 10.1016/j.anifeedsci.2007.01.011

Park DS; Coats JR. 2002. Cyanogenic glycosides: Alternative insecticides? The Korean Journal of Pesticide Science 6:51–57. (Available at: https://goo.gl/VxGHVG).

Rai KN; Blümmel M; Singh AK; Rao AS. 2012. Variability and relationships among forage yield and quality traits in pearl millet. European Journal of Plant Science and Biotechnology 6:118–124. (Available at: http://oar.icrisat.org/6576).

Saberi AR. 2014. Yield and water use efficiency (WUE) responses of forage sorghum ratoon crop under varying salinity and irrigation frequency. African Journal of Plant Science 8(12):554–559. DOI: 10.5897/AJPS2014.121

Sarfraz M; Ahmed N; Farooq U; Ali A; Hussain K. 2012. Evaluation of sorghum varieties/lines for hydrocyanic acid and crude protein contents. Journal of Agricultural Research 50:39–47. (Available at: http://eprints.icrisat.ac.in/4553).

Singh S; Shukla GP. 2010. Genetic diversity in the nutritive value of dual purpose sorghum hybrids. Animal Nutrition and Feed Technology 10:93–100.

Smitha Patel PA; Alagundagi SC; Salakinkop SR. 2013. The anti-nutritional factors in forages – A review. Current Biotica 6:516–526. (Available at: https://goo.gl/IjXNRM).

Srinivasa Rao P; Jayalakshmi M; Kuma CG; Kamal A; Reddy BVS. 2011. Response of fertilizer treatments on agronomic and biochemical traits in main and ratoon crops of sweet sorghum (*Sorghum bicolor* (L.) Moench) cultivar ICSV 93046. In: The IAPSIT International Sugar Conference, New Delhi, India. p. 1−11. (Available at: http://goo.gl/dASeIj).

Undersander DJ; Durgan BR; Kaminski AR; Doll JD; Worf GL; Schulte EE. 1990. Kochia. Alternative Field Crops Manual. University of Wisconsin Cooperative Extension Service, Department of Agronomy, Madison, WI, USA. (Available at: https://goo.gl/HTGKnu).

Van Soest PJ. 1994. Nutritional ecology of the ruminant. 2nd Edn. Cornell University Press, New York, USA.

Wheeler JL; Mulcahy C. 1989. Consequences for animal production of cyanogenesis in sorghum forage and hay – A review. Tropical Grasslands 23:193–202. (Available at: https://goo.gl/BkRtDf).

Whish J; Bell LW. 2008. Trade-offs for ratooning sorghum after harvest to provide forage for grazing. In: Unkovich M, ed. Proceedings of the 14th Australian Agronomy Conference, 21–25 September 2008, Adelaide, South Australia. (Available at: https://goo.gl/IpVDPq).

Willey RW. 1990. Resource use in intercropping systems. Agricultural Water Management 17:215–231. DOI: 10.1016/0378-3774 (90)90069-B

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