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Physiological and production responses of four grasses from the genera *Urochloa* and *Megathyrsus* to shade from *Melia azedarach* L.

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Abstract This research investigated the effect of shade from *Melia azedarach* L. on the physiology, production, and forage quality of *Megathyrsus maximus cv*. Tanzania and cv. Mombaza, and the *Urochloa* hybrids Oaxaca and Yacaré. Evaluations were made during the rainy (August 2013) and windy (February 2014) seasons under sun and shade. Mombaza and Tanzania produced more forage biomass (4683 ± 4529 and 4279 ± 4745 kg DM ha^{-1} harvest^{-1}; $P < 0.05$) than hybrids, and there was more biomass during the rainy (8236 ± 4257 kg DM ha^{-1} harvest^{-1}; $P < 0.0001$) than the windy season, although biomass declined by 44 % ($P < 0.05$) under shade. Leaf crude protein was similar among grasses ($P = 0.516$), although Mombaza and Tanzania had higher neutral detergent (49.2 and 50.2 %, respectively; $P < 0.05$) and acid detergent fiber fractions (34.4 and 34.1 %, respectively; $P < 0.05$), making them less digestible (61.7 and 61.6 %, respectively; $P < 0.05$) than the hybrids. Overall, nutritional quality increased during the windy season ($P < 0.05$) and under shade

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($P < 0.05$). Assimilation of CO_2 was greater during the rainy season ($P < 0.0001$) and under sun (19.1 ± 8.2 vs. 8.6 ± 4.4 µmol m^{-2} s^{-1}; $P < 0.05$). The most favorable conditions for biomass production occurred during the rainy season, although nutritional quality was better during the windy season. Shade affects photosynthetic rate and production, and promotes the nutritional quality of all grasses. Oaxaca and Yacaré appear to be more adapted to shade by responding with greater production stability and better forage nutritive quality.

Introduction

Melia azedarach L. is a multipurpose tree with timber potential, is deciduous, has rapid growth, can reach up to 15 m in height (Sánchez 2011), and has foliage characteristics which can be integrated into agroforestry systems associated with feed crops. The combination can be a means of diversifying production, allowing grasses to grow during the early years of tree life, and then timber production over the medium and long term. This allows families to diversify land use and increase system productivity (González-Hernández and Rozados-Lorenzo 2008). The association between trees and grasses can improve production and the nutritional quality of grasses (Murgueitio 2001; Ibrahim et al. 2007).

Tropical grasses of the genera *Urochloa* (formerly *Brachiaria*) and *Megathyrsus* (formerly *Panicum*) with C$_4$ metabolic pathways have their own physiological characteristics that provide adaptive potential for their growth and improved nutritional quality under moderate shade (<50 %). The genus *Megathyrsus* has much morphological diversity. The species are erect, from 0.5 to 3.5 m high depending on the cultivar, and have the ability to tolerate limited light conditions by changing their physiology, decreasing biomass production, and improving nutritive quality. The physiological adjustments have led to a forage availability decrease of *M. maximus* by 20 % when it is associated with scattered trees of *Gliricidia sepium* (Medinilla-Salinas et al. 2013), yet when associated with *Eucalyptus tereticornis*, it has produced more biomass under younger stands (<5 m high) that provide less shade

(Mahecha et al. 2007). However, herbage may not decrease when associated with browse species such as *Leucaena leucocephala* (Alonso et al. 2006), as shade in those systems is temporal, depending on the utilization patterns of the pastures. In most associations, where trees are either cultivated or regenerated and scattered through the pasture, grasses of this genus gain nutritive value (Obispo et al. 2008; Pandey et al. 2011). *Urochloa* species and its hybrids grow semierect from 0.6 to 2 m in height (Olivera et al. 2006), which suffer morphological changes such as increasing their leaf area index and height under limited light conditions (<50 % shade) (Carrilho et al. 2012; Ortega 2012). *Urochloa decumbens* has limited growth under scattered trees in the savannas (Gómez et al. 2012), but it tolerates 30–50 % shade under those conditions. *Urochloa mutica* decreased herbage biomass by 50 % under shade from *Cocos nucifera* trees relative to open spaces (Pandey et al. 2011). As with *Megathyrsus*, reduced herbage biomass in *Urochloa* species may be compensated by gaining nutritive quality (Hernández and Guenni 2008; Pandey et al. 2011).

Grass responses, even when they have tolerance to limited light, may vary depending on environmental conditions (Belsky 1994; Peri 2005) and associated woody species (Belsky 1992; Singh and Upadhyaya 2001). Association of grasses in *Urochloa* and *Megathyrsus* with *M. azedarach* may be an adequate combination and alternative for diversifying land use, which in turn can improve family income over the short (under canopy forage production) and medium terms (quality wood production), making the systems more sustainable over time (Lemus 2008).

The present research evaluated the physiology and productive responses of four tropical grasses, two cultivars of *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs (Mombaza and Tanzania) and *Urochloa* hybrids Oaxaca [CIAT 1794; a tri-hybrid of *Urochloa ruziziensis* (R. Germ. & Evrard) Crins x *U. decumbens* (Stapf) R.D. Webster × *U. brizantha* (Hochst. ex A. Rich.) R.D. Webster] and Yacaré (CIAT 1752; *U. brizantha* CIAT 16320 × hybrid from the fourth cycle of sexual reproduction), under shade from the timber tree *M. azedarach*, under subtropical weather conditions. The working hypothesis was that the grasses reduce their photosynthetic capacity under limited light conditions, reducing their production of forage biomass while improving their nutritional quality, regardless of season.

Fig. 1 Average monthly precipitation and temperature (from 1980 to 2009) for the region where the experiment was conducted (CONAGUA 2013)
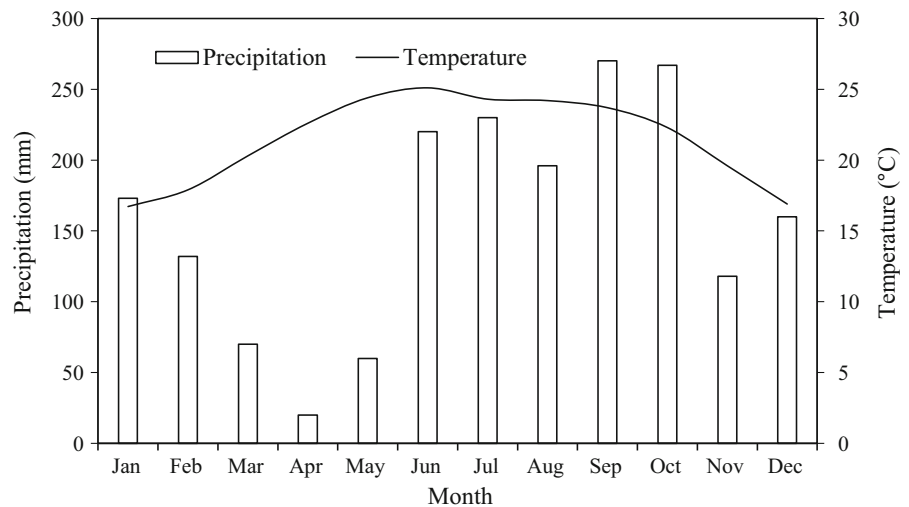
Materials and methods

Location and experimental site characteristics

The experiment was conducted in the municipality of Tlapacoyan, Veracruz, Mexico (between 19°56′26″ and 19°56′48″ N, and 97°15′55″ and 97°15′34″ W), at 847 masl. The prevailing climate is Af, which corresponds to regular warm humid with rains all year (García 1981), and an average annual temperature and rainfall of 18 °C and 1977 mm, respectively (CONAGUA 2013; Fig. 1). The experimental site is located in a mountainous area with slopes and sandy-loamy soils. Here, about 26 % of annual precipitations falls from May through July and 24 % from December through January, considered as the rainy and windy sampling seasons in this research. Mean temperatures were 24.6 and 17.2 °C during these same sampling seasons; the soil under the tree canopy had pH 5.1 and 5.5 % organic matter, and in the open area had pH 5.2 and 6.6 % organic matter. The leaf area index of the canopy during the experiment ranged from 0.35 ± 0.18 to 0.32 ± 0.08 m^2 foliage per m^2 (rainy and windy seasons, respectively).

Experimental design

The experiment was established in June 2010 under conditions of full shade to full sun. A *M. azedarach* plantation with 1000 trees ha^{-1} (2.5 m between trees and 4 m between rows) was used for the first

condition. This site was established in 2003, and at the beginning of the experiment, tree diameter at breast height was 11.0 cm. For an insolated area without trees, a location approximately 20 m from the planting of *M. azedarach* was used. In both conditions, plots of 2.5-m wide and 5-m long were established (with the longest side in favor of the slope), where tufts of grass were planted 50 cm apart in a complete randomized block design (considering a slope of 25 %) in a split-plot arrangement with four treatments and three replicates. The treatments were cultivars Tanzania and Mombaza (*Megathyrsus*) and the *Urochloa* hybrids Oaxaca (CIAT 1794; a tri-hybrid of *U. ruziziensis* (R. Germ. & Evrard) Crins × *U. decumbens* (Stapf) R.D. Webster x *U. brizantha* (Hochst. ex A. Rich.) R.D. Webster, and Yacaré (CIAT 1752; *U. brizantha* CIAT 16320 x hybrid from the fourth cycle of sexual reproduction). Large plots corresponded to sunny or shaded conditions and subplots to the grass cultivars Mombaza, Tanzania, and hybrids Oaxaca and Yacaré.

Experiment procedure

Grass responses were evaluated during the rainy and windy seasons (summer and winter, respectively) (Fig. 1). Grasses were harvested each season at 10 cm from the ground for uniform grass growth (May 25 and December 15, 2013, for rainy and windy seasons, respectively). At 61 days during the rainy season, and 60 days during the windy season (the difference of

1 day was due to weather conditions), variables were measured according to the evaluation protocol and biomass was harvested on July 22, 2013 (rainy season) and February 14, 2014 (windy season), under sunny and shaded conditions. No fertilizers were applied, nor were animals allowed to graze.

Variables evaluated

Forage biomass production and its morphological components (leaves, stems, and dead material) were evaluated, as were nutritive quality of the dry matter and photosynthetic efficiency of the grasses. During the windy season, in addition to the above variables, grass leaf area also was measured. Forage biomass was estimated at 61 days during the rainy season and 60 days of regrowth during the windy season. Harvests were accomplished by harvesting all biomass within four clumps to 10 cm above ground level within a quadrat of 1 m². A subsample of 400 g was used for analysis of leaves, stems, and dead material, and the remaining quantity was used for laboratory analysis. All samples were dried in a forced air oven at 65 °C for 48 h.

A curve of photosynthetic activity of grasses was made to determine peak photosynthetic efficiency, for which readings of photosynthesis were recorded using an Lc Pro + (ADC Bioscientific, Hertfordshire, England) every 2 h over a period of 11 h (07:00–18:00 h). The resulting curve was used to determine the moment for evaluating photosynthesis between 11:00 and 13:00 h. These measurements were performed the day before grass harvest, and included the net CO_2 assimilation rate (A_N), transpiration (E), and stomatal conductance (g_s). The measurements were made on three randomly chosen tillers within each experimental plot, taking the reading on the fourth leaf of each tiller (counting from the apex to the base). As well, plant water use efficiency (WUE) was calculated by dividing A_N by E.

Grass leaf area was measured under sunny and shaded conditions one day before biomass harvest. For this, in each experimental plot, four clumps and three tillers within each clump were randomly selected, and the total area of extended leaves within the selected tillers was estimated using an LI-3000C (Portable Area Meter, Lincoln, Nebraska, USA).

The nutritive quality of forage biomass was analyzed by measuring the content of crude protein (CP) using the macro-Kjeldahl method (AOAC 1980). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured using the Ankom filter bag method (F57 filter bag model, ANKOM Technology, Macedon, NY, USA) and an Ankom²⁰⁰ (Ankom Technology, Macedon, NY, USA; ANKOM 2010). Lignin was measured using the precipitate method with 72 % H_2SO_4 (AOAC 1997) and filter bags. *In vitro* dry matter digestibility (IVDMD) was determined using the pepsin-cellulase enzyme method and ANKOM filter bags (Model F57, ANKOM Technologies, Macedon, NY, USA; Jones and Hayward 1975; Clarke et al. 1982), and a circular agitation incubator (Model-ISO-45). Even though in vitro techniques can overestimate digestibility of forage dry matter relative to the in vivo technique, the pepsin-cellulase method has the strength that cellulose and pepsin are stable, providing homogeneity throughout sample runs, and this fact can ensure that all differences among samples are due to treatments.

Statistical analysis

Total biomass and its component variables (leaves and stems), and photosynthetic activity (A_N, g_s, E, and WUE) were analyzed using a completely randomized block split-plot design. All variables, except CO_2 assimilation (A_N), were transformed to natural logarithms to reduce variance and achieve normality, after which they were analyzed using the GLM procedure in SAS (SAS 2010). Dead matter was not analyzed because it represented less than 1 % of the total biomass. The statistical model included the effects of grass, condition (sun or shade), season, block, condition*block, condition*grass, season*grass, season*condition, and season*condition*grass; the plot error term was condition and for the model was block*condition. Mean comparisons were performed using the Tukey-adjusted least squares method (LSMeans) at α = 0.05.

The nutritional quality variables (CP, ADF, NDF, IVDMD, and lignin) were transformed to natural logarithms to reduce variance and achieve normality, and were analyzed using the GLIMMIX procedure in SAS (SAS 2010). The model included the effects of grass, condition, season, grass*condition, grass*season, condition*season, and grass*condition*season. Blocks were considered as a random effect, while block*condition*grass was used as the error term.

Means comparison tests were performed using the least squares method (LSMeans).

Leaf area was analyzed as completely randomized blocks with split-plots using the GLM procedure in SAS. The effects included in the model were condition, grass, and grass*condition. When significant differences were found, means comparison tests were performed using a Tukey-test and $\alpha = 0.05$.

Results

Biomass and nutritional quality

The Mombaza and Tanzania cultivars produced more forage biomass ($P = 0.0012$), although the production from the Tanzania cultivar was similar ($P > 0.05$) to the Oaxaca and Yacaré hybrids, while Mombaza produced the least ($P < 0.05$). The difference between the grasses having the highest and lowest biomass production (Mombaza vs. Yacaré) was 2156 kg DM ha^{-1} harvest^{-1} (Table 1), but the difference between the hybrids and cultivars was small (277 and 404 kg DM ha^{-1} harvest^{-1}, respectively) and not significant ($P > 0.05$). Consequently, the biomass of leaves ($P < 0.0001$) and stems ($P = 0.0127$) also differed among grasses, with Mombaza and Tanzania having similar values for leaves ($P = 0.999$), as did Oaxaca and Yacaré ($P = 0.921$), although the last harvest was less than for the cultivars ($P < 0.0001$; Table 1). The Mombaza and Tanzania cultivars and the Oaxaca hybrid produced the same quantity of stems ($P > 0.05$), although the Yacaré hybrid had lower stem biomass than Mombaza ($P < 0.05$), but was similar to that for Oaxaca and Tanzania ($P > 0.05$; Table 1).

The condition*season interaction ($P = 0.0026$) affected total biomass yield, such that during the rainy season, it was 2.3 times more under shade than sun ($P < 0.05$). During the windy season, when total biomass production declined ($P < 0.0001$), production was similar under both conditions ($P > 0.05$). Between the rainy and windy seasons, the quantity decreased 3.3 and 5.3 times under shade and sun, respectively (Table 2). The same trend occurred with leaves ($P = 0.0002$) and stems ($P = 0.0297$). During the rainy season, leaf biomass ($P < 0.0001$) and that for stems ($P < 0.0001$) were greater than during the windy season. There were more leaves ($P < 0.05$) and stems ($P < 0.05$) produced under sun than shade during the rainy season, although quantities were similar under both conditions ($P > 0.05$; Table 2).

Although the leaf CP content did not differ among grasses ($P = 0.516$), there was less produced during the rainy season (13.2 ± 2.2 %) than during the windy season (18.0 ± 0.9 %; $P < 0.05$) due to an effect from season ($P < 0.0001$). Also, plants growing under full sun produced less protein (14.3 ± 3.3 %; $P < 0.0001$) than those under shade (16.8 ± 2.1 %). Similar to what happened with total biomass and leaf biomass, stems contained 4.5 % more CP during the windy season (11.0 ± 1.4 %) than during the rainy season (6.5 ± 0.8 %; $P < 0.05$), and had more protein under shaded conditions (9.1 ± 2.3 %) than under sun (8.3 ± 2.7 %; $P < 0.05$).

Grasses contained different quantities of NDF ($P < 0.0001$) and ADF ($P < 0.0001$) in leaves (Table 3). The Mombaza and Tanzania cultivars contained more fibrous fractions than the Oaxaca and Yacaré ($P < 0.05$) hybrids. Also, NDF in leaf dry matter differed between seasons ($P < 0.0001$), and was higher during the rainy season, but declined 43.8 % during the windy season ($P < 0.05$). The cultivars Mombaza and Tanzania had similar

Table 1 Total biomass and biomass of leaves and stems (kg DM ha^{-1} harvest^{-1}) for the grasses *Megathyrsus maximus cv*. Mombaza and Tanzania, and *Urochloa* hybrids Oaxaca and Yacaré (values are mean ± SD; *DM* dry matter)

Species	Total	Leaves	Stems
M. maximus cv. Mombaza	4683 ± 4529[a]	2703 ± 2282[a]	1774 ± 2528[a]
M. maximus cv. Tanzania	4279 ± 4745[a,b]	2854 ± 3074[a]	1279 ± 1713[a,b]
Urochloa hybrid Oaxaca	2804 ± 2594[b]	1232 ± 933[b]	1064 ± 1505[a,b]
Urochloa hybrid Yacaré	2527 ± 1672[b]	1233 ± 804[b]	811 ± 806[b]

[a,b] Means with different letters in each column are statistically different ($P < 0.05$)

Table 2 Total forage biomass and biomass of leaves and stems for the grasses *Megathyrsus maximus cv*. Mombaza and Tanzania, and *Urochloa* hybrids Oaxaca and Yacaré under sunny and shaded conditions, during the rainy and windy seasons (values are mean ± SD; *DM* dry matter)

Condition	Rainy	Windy
Total biomass (kg DM ha^{-1} harvest^{-1})		
Sun	8236 ± 4257[aA]	1376 ± 350[aB]
Shade	3612 ± 1470[bA]	1071 ± 219[aB]
Leaf biomass (kg DM ha^{-1} harvest^{-1})		
Sun	4487 ± 2672[aA]	703 ± 340[aB]
Shade	2208 ± 970[bA]	623 ± 124[aB]
Stem biomass (kg DM ha^{-1} harvest^{-1})		
Sun	3261 ± 2296[aA]	188 ± 92[aB]
Shade	1334 ± 726[bA]	145 ± 45[aB]

[a,b] Means with different letters between rows are significantly different ($P < 0.05$)

[A,B] Means with different letters between columns are significantly different (P < 0.05)

quantities of ADF ($P > 0.05$) and were superior to the *Urochloa* hybrids ($P < 0.05$). The *Urochloa* hybrids had similar ADF content ($P > 0.05$) (Table 3). Leaf ADF across all grasses differed by season ($P < 0.0001$), having more during the rainy season (36.8 ± 5.5 %) than during the windy season (24.4 ± 2.9 %; $P < 0.05$). Shade promoted less ADF ($P = 0.0478$) in plants (30.0 ± 6.7 %; $P < 0.05$) than under full sun (31.2 ± 8.5 %).

Similar to the results with fibers, grasses also had different IVDMD ($P < 0.0001$). Oaxaca and Yacaré were 6–8 % more digestible than Mombaza and Tanzania ($P < 0.05$; Table 3). At the same time, Mombaza and Tanzania had similar digestibility ($P > 0.05$). Based on condition ($P = 0.0320$), digestibility was lowest under full sun (64.4 ± 11.2 %) than under shade (65.7 ± 9.5 %; $P < 0.05$). Also, dry matter digestibility

was higher during the windy season (74.1 ± 2.8 %), but decreased by 18.1 % during the rainy season (56 ± 6.2 %; $P < 0.05$).

A grass*season interaction affected leaf lignin content ($P = 0.0018$). During the rainy season when there was a greater percentage of lignin ($P < 0.0001$), the content was similar in all grasses ($P > 0.05$). During the windy season, when there was a lower percentage of lignin ($P < 0.0001$), the Oaxaca and Yacaré hybrids were similar, but the higher percentages were with the Mombaza and Tanzania cultivars ($P < 0.05$; Table 4).

The NDF and ADF fractions for stems only varied among seasons ($P < 0.0001$ for both), regardless of grass type. During the rainy season, there was more NDF (73.0 ± 2.6 %; $P < 0.05$), which declined by 35.7 % during the windy season (67.7 ± 3.5 %), and similarly, there was more ADF (48.2 ± 3.6 %; $P < 0.05$) which declined during the windy season (36.7 ± 3.3 %). Consequently, IVDMD was greater during the windy season (58.8 ± 4.5 %; $P < 0.0001$), than during the rainy season where it declined 9.3 % (49.5 ± 3.1 %; $P < 0.05$).

The stem lignin content also varied due to grass*season interaction ($P = 0.001$). During the rainy season, the percentage of lignin was similar among all grasses ($P > 0.05$), yet during the windy season, the Oaxaca and Yacaré hybrids were similar to each other ($P > 0.05$), but were greater than the Mombaza and Tanzania cultivars ($P < 0.05$; Table 4).

Grass morphology and physiology

Grass leaf area differed among species ($P < 0.0001$). The Mombaza and Tanzania grass cultivars had greater leaf area (137.9 ± 33.0 and 124.5 ± 27.2 cm^2 g^{-1}; $P < 0.05$), approximately three times greater than the

Table 3 Neutral detergent fiber (NDF), acid detergent fiber (ADF), and in vitro digestibility of leaf dry matter (IVDDM) for the grasses *Megathyrsus maximus cv*. Mombaza and Tanzania, and the *Urochloa* hybrids Oaxaca and Yacaré (values are mean ± SD)

Species	NDF (%)	ADF (%)	IVDMD (%)
M. maximus cv. Mombaza	49.2 ± 23.1[a]	34.4 ± 7.9[a]	61.7 ± 10.6[b]
M. maximus cv. Tanzania	50.2 ± 24.2[a]	34.1 ± 7.5[a]	61.6 ± 11.4[b]
Urochloa hybrid Oaxaca	42.8 ± 22.2[b]	26.9 ± 6.0[b]	69.2 ± 9.1[a]
Urochloa hybrid Yacaré	43.0 ± 22.4[b]	26.8 ± 5.6[b]	67.7 ± 8.8[a]

[a,b] Means with different letters in each column are statistically different ($P < 0.05$)

Table 4 Lignin in leaves and stems of the grasses *Megathyrsus maximus cv*. Mombaza and Tanzania, and *Urochloa* hybrids Oaxaca and Yacaré, during the rainy and windy seasons (values are mean ± SD)

Species	Leaves (%)		Stems (%)	
	Rainy	Windy	Rainy	Windy
M. maximus cv. Mombaza	6.7 ± 1.4[aA]	2.7 ± 0.4[bB]	5.6 ± 1.1[aB]	3.2 ± 0.5[bA]
M. maximus cv. Tanzania	6.2 ± 1.2[aA]	2.7 ± 0.3[bB]	5.5 ± 0.7[aB]	3.2 ± 0.4[bA]
Urochloa hybrid Oaxaca	5.9 ± 1.3[aA]	3.5 ± 0.3[aB]	6.4 ± 1.0[aA]	6.6 ± 0.6[aA]
Urochloa hybrid Yacaré	5.6 ± 0.9[aA]	3.4 ± 0.2[aB]	6.5 ± 1.0[aA]	6.2 ± 1.2[aA]

[a,b] Means with different letters among rows are statistically different ($P < 0.05$)

[A,B] Means with different letters between columns are statistically different ($P < 0.05$)

Oaxaca and Yacaré hybrids (48.1 ± 9.8 and 43.3 ± 10.8 cm^2 g^{-1}; $P < 0.05$).

The CO_2 assimilation (A_N) differed between conditions depending on season ($P = 0.0122$). During the rainy season, when there was greater uptake of CO_2 ($P < 0.0001$), there was more sun ($P < 0.05$), and shade was 2.2 times less (Table 5). During the windy season there was less uptake ($P < 0.0001$), but it was similar between conditions ($P > 0.05$). Likewise, stomatal conductance (g_s) affected the season*condition interaction ($P < 0.008$). During the rainy season, stomatal conductance was higher under sun than in shade (declining 50 %; $P < 0.05$), while during the windy season, when conductance declined ($P < 0.0001$), it was similar between the sun and shade conditions ($P < 0.05$).

Transpiration (E) of grasses was different between conditions ($P = 0.0008$). Plants under full sun

Table 5 Net CO_2 assimilation rate (A_N) and stomatal conductance (g_s) for the grasses *Megathyrsus maximus cv*. Mombaza and Tanzania, and *Urochloa* hybrids Oaxaca and Yacaré associated with *Melia azedarach* under sun and shade during the rainy and windy seasons (values are mean ± SD)

Condition	Rainy	Windy
A_N (µmol CO_2 m^{-2} s^{-1})		
Sun	19.1 ± 8.2[aA]	3.8 ± 3.8[aB]
Shade	8.6 ± 4.4[bA]	2.8 ± 1.0[aB]
g_s (mol H_2O m^{-2} s^{-1})		
Sun	0.14 ± 0.07[aA]	0.05 ± 0.01[aB]
Shade	0.07 ± 0.04[bA]	0.05 ± 0.02[aB]

[a,b] Means with different letters between rows are statistically different ($P < 0.05$)

[A,B] Means with different letters between columns are statistically different ($P < 0.05$)

transpired more (2.43 ± 1.9 mol H_2O m^{-2} s^{-1}; $P < 0.0008$), while under shade they transpired less (1.24 ± 0.8 mol H_2O m^{-2} s^{-1}). Furthermore, during the rainy season, the grasses transpired more (2.75 ± 1.7 mol H_2O m^{-2} s^{-1}; $P < 0.05$) than during the windy season (0.92 ± 0.62 mol H_2O m^{-2} s^{-1}). Season also influenced WUE, and plants were more efficient during the rainy season (5.3 ± 2.5 µmol CO_2 mol^{-1} H_2O; $P = 0.0042$) than during the windy season (3.2 ± 1.4 µmol CO_2 mol^{-1} H_2O).

Discussion and conclusions

The best conditions for grass growth and biomass production were during the rainy season, both under sun and shade. During this period, temperatures ranged between 25 and 35 °C, which is considered optimal for grass growth (Candido et al. 2006; Sage and Kubeins 2007; Ramírez et al. 2009), such that more stems develop more leaves, thus promoting earlier plant maturity (Hodgson 1990; Lamaire 2001; Festo et al. 2003; Ramírez et al. 2010). However, during the windy season, low temperatures and greater cloud cover changed the conditions for growth, reducing the production of leaves and stems (Gerdes et al. 2000; Nascimento et al. 2002; Cruz et al. 2011). Changing from the rainy to the windy season, total biomass production declined 83 % in sun and 70 % in shade, leaf quantity declined 84 and 72 % in sun and shade, respectively, and stem quantity declined 94 and 89 % in sun and shade, respectively. Such changes in weather promote reductions in growth rate, gas exchange, stomatal closure, and the absorption of atmospheric CO_2. These reductions affect photosynthetic activity

and the production of dry matter (Basurto et al. 2008), because growth depends on increased photosynthetically active area for the formation and growth of stems and leaves (Boschman et al. 2003; Ramírez et al. 2009). Ramírez et al. (2010) stated that dry matter yield is related to increases in photosynthesis and thus the synthesis of structural carbohydrates. During the rainy season, leaf area was greater, permitting the net CO_2 assimilation rate to increase, but declined during the windy season from 15.3–5.8 μmol CO_2 m^{-2} s^{-1} in sun and shade, respectively. Likewise, transpiration declined by 1.83 mol H_2O m^{-2} s^{-1} during the windy season and by 1.19 mol H_2O m^{-2} s^{-1} under shade. In the grasses evaluated, WUE was directly related to the time of stomatal opening and stomatal resistance; while plants absorb CO_2 for photosynthesis, water is lost through transpiration (Pereira-Netto 2002). During the windy season, when conditions changed, plants modified their metabolic processes, closing their stomata and reducing CO_2 capture.

Under shade, grasses developed longer and wider leaves (although leaves were thinner) than those produced under greater light exposure, a response due to increased cell elongation as an important adaptive mechanism (Penton 2000; Dias-Filho 2001; Rego and Possamai 2006). This occurs because under low light conditions, grasses change their morphology (etiolation) by elongating their stems and leaves to increase plant height (Alonso et al. 2006) and leaf area to intercept more light for photosynthesis. The first increase in adaptation is in the competitive ability to intercept light, while the second reduces respiration rate (Piñeros et al. 2011). When plants have enough light for photosynthesis, biomass production increases. This response was observed during the rainy season with the grasses growing under sun, yet under shade production declined 56 % during summer and 22 % during winter, indicating that the growth of these grasses under the canopy of older *M. azedarach* trees might be compromised.

The cultivars Mombaza and Tanzania (genus *Megathyrsus*) had superior responses in total production, and leaf and stem production compared to the hybrids Oaxaca and Yacaré (genus *Urochloa*). This was related to the morphogenic characteristics of bunchgrass growth, with greater height and biomass production relative to decumbent species (Smith and Whitelam 1990; Avellaneda et al. 2008; Ramírez et al. 2010). These grasses are taller which is positively related to the amount of forage produced (Castillo et al. 2009) and is also associated with greater stem accumulation (Ramírez et al. 2009). The hybrids Oaxaca and Yacaré are shorter and semi-erect, providing lower biomass production, which is related to their reduced leaf area.

Grass nutritive quality improved during the windy season compared to the rainy season. This difference between seasons was due to the accompanying changes in environmental conditions that affected plant growth rate, increasing the time needed to reach maturity. In the area where the experiment was conducted, rainfall varies slightly between the rainy and windy seasons, which could rule out precipitation as a limiting factor. Yet it could be attributed to increased cloudiness and cooler temperatures than during summer (Fig. 1). These factors help determine changes in the morphology, physiology, and nutritional quality of grasses (Santos et al. 2011; Verdecia et al. 2012) by limiting growth and delaying plant maturity. If plant growth declines, there is less cell wall function in leaves and stems, and variability in their structural components (Lyons et al. 2001; Nascimento and Adese 2004). Low temperatures that limit grass growth help maintain carbohydrate reserves for a longer time (Cruz et al. 2011). High cloud cover altered the amount of photosynthetically active radiation for grasses, thus reducing CO_2 assimilation (A_N) by 80 % in sun and 67 % in shade. Likewise, stomatal conductance (g_s) declined 64 % in sun and 29 % in shade. Adaptations in the photosynthetic process influenced grass metabolism (Piñeros et al. 2011), increasing the CP content by 4.8 % and 4.5 % in leaves and stems, respectively, during the windy season. This increase in protein was due to a greater content of nonstructural carbohydrates in the grass cell walls (Zelada 1996). The NDF content decreased by 44 % in leaves and 36 % in stems, indicating that the grasses contained less cellulose, hemicellulose, and lignin during the windy season (Nascimento and Adese 2004), while leaf ADF content decreased by 12.4 % and by 11.2 % in stems. Likewise, grass lignin content in leaves declined ($P = 0.0018$; Table 3) and in stems ($P = 0.0010$), thus favoring digestibility. However, during the rainy season, the quantity of structural components in the cell walls increased, because the grasses used them to reduce the effects of heat stress (Lyons et al. 2001; Juárez et al. 2009). These adaptive mechanisms are

used by grasses to counteract climate change which affects digestibility, reducing it by 18 % in leaves and 9.3 % in stems, causing increases in lignin and their indigestible complexed molecular forms (Padilla et al. 2009). Increased lignin in the grass cell walls makes them less digestible, since lignin is responsible for the incomplete digestion of cellulose and hemicellulose (Pirela 2005; Homen et al. 2010).

Biomass production and grass nutritive value are modified when grown under tree canopies, yielding changes in grass morphology and physiology and improving their digestibility (Lacorte et al. 2004; Ruiz et al. 2010). Under shade, where biomass production was lower than in sun, leaf protein content increased 2.5 % and stem protein content increased 0.8 %. However, ADF declined by 1.2 %, causing digestibility to increase 1.3 % in sun due to the substantial changes in the quantity and quality of light they received, reducing the photosynthetic process (Noguera-Talavera et al. 2009). Although dry matter digestibility was assessed using an in vitro technique, which have been considered to overestimate results obtained by the in vivo technique (Damiran et al. 2008), our findings are comparable to those from Mahecha et al. (2007) and Obispo et al. (2008) who estimated between 62 and 65 % dry matter digestibility in *M. maximus* strains under shaded conditions. However, comparisons should be cautiously taken because of all the possible differences in the analyzed matter due to forage management in each experiment.

We harvested grasses at a lower height than has been performed in other research (Mahecha et al. 2007) which, in turn, could lower herbage nutritive quality. However, in our experiment, fiber fractions (ADF and NDF) of grasses were relatively low denoting good nutritive quality (Vargas 2002), and we analyzed the dry matter of leaves apart from stems (Table 3). Nonetheless, the Mombaza and Tanzania cultivars had higher fiber fractions than the Oaxaca and Yacaré hybrids. This may be genetically related to the type of growth for *Megathyrsus,* as it is a tall grass it produces more stalks which generally contain less CP and more cell wall components which reduce digestibility (Verdecia et al. 2012). Naturally, digestibility is a variable associated with NDF; as NDF increases, digestibility decreases. Whereas NDF values exceeding 65 % indicate low nutritional value (Vargas 2002), grasses evaluated in the present study had an acceptable quality index below this value.

Under shade, grass photosynthetic processes were altered and they assimilated less CO_2. This, in turn, altered the production of leaves and stems. Grass nutritional quality improved under these conditions, increasing the content of CP and reducing the fiber content, making the dry matter more digestible. Therefore, shade from *M. azedarach* creates conditions that benefit the chemical and nutritional quality of the grass tissues evaluated.

All grasses evaluated had the same photosynthetic efficiency under the canopy of *M. azedarach*, but the *Megathyrsus* cultivars were superior to the *Urochloa* hybrids in producing forage biomass. While all grasses contained the same amount of CP, the Oaxaca and Yacaré hybrids were less fibrous and more digestible than the Mombaza and Tanzania cultivars. Therefore, *Urochloa* can grow better under the canopy of *M. azedarach*, losing little biomass production, while increasing nutritional quality.

Under the regular warm and humid climate conditions in which this research was conducted, the most favorable weather conditions occurred during the rainy season, so the assimilation of CO_2 by grasses is more efficient during the rainy season. Under these conditions, the amount of leaf and stem biomass is greater, while during the windy season, due to low temperatures and increased cloudiness, production decreases considerably. However, during the windy season, grass nutritional quality improves significantly; as CP content increases, the dry matter is less fibrous and the quantity of lignin in the cell walls decreases, significantly improving the nutritional quality during winter, which coincides with the windy season.

The association of grasses with *M. azedarach* is possible, at least until seven years of age, which was the age of the trees when this experiment was performed. Although grass biomass production declined under shade, the *Megathyrsus* cultivars had good production during the rainy season, yet the *Urochloa* hybrids were less sensitive to the effect of season, having better quality and losing less production during the windy season.

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