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Morpho-physiological and anatomical characteristics of *Urochloa brizantha* cv. Marandu in silvopastoral and monoculture systems

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ABSTRACT. To gain insights into the forage morphological and anatomical characteristics in a silvopastoral system (SPS) with Bolsa de Pastor (*Zeyheria tuberculosa*) and palisadegrass 'Marandu' (*Urochloa brizantha*) monoculture (MONO). The SPS was established through natural regeneration of the tree species. Treatments were a SPS and MONO distributed in a completely randomized design with six replicates and repeated measures were the harvest periods. Response variables were morpho-physiological and anatomical characteristicss: green: dead material ratio, leaf blade: stem+sheath ratio, leaf area index, chlorophyll and carotenoid concentrations, proportions of non-lignified and achlorophyllous areas, lignified areas in stems, proportions of non-lignified and achlorophyllous areas, lignified and chlorophyllous areas in leaves, as well as cell length in longitudinal section of stem. Morpho-physiological patterns were altered ($p < 0.05$) under natural shading conditions due to higher photosynthetic efficiency in the SPS. There was no effect ($p > 0.05$) of the systems on anatomical patterns, proportions of non-lignified and achlorophyllous, lignified and chlorophyllous tissues, these proportions were influenced only by the periods of the year, both for stems and leaves. Cells of the internodes of the grasses of the studied systems had the same length. The SPS alters morpho-physiological characteristics of palisadegrass and increases the concentration of chlorophyll *a* and *b*.

Keywords: chlorophyll; pastures; plant physiology; shading.

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Introduction

In Brazil, livestock systems are predominantly based on grass monocultures and relies on fertilizer and herbicides to be feasible. Conversely, the lack of inputs and mismanagement may result in soil degradation and destruction of the remaining native vegetation (Dias-Filho, 2014). Silvopastoral systems (SPS) are an alternative method to restore degraded pastures and diversify the income in livestock systems (Jose & Dollinger, 2019; Murgueitio, Chará, Barahona, & Rivera, 2019). Silvopastoral system consist of combinations of trees and shrubs with pastures and animals in the same area, simultaneously or staggered over time (Jose, Walter, & Mohan Kumar, 2019). Palisadegrass (*Brachiaria brizantha* [Hochst. ex A. Rich.] R. Webster cv. Marandu) is the most cultivated warm-season perennial grass in Brazil (Jank, Barrios, Do Valle, Simeão, & Alves, 2014; Ministério da Agricultura, Pecuária e do Abastecimento [MAPA], 2018). Palisadegrass is widely used due to its adaptation on different soil and climatic conditions, and is commonly used in SPS (Gomes et al., 2019; Oliveira et al., 2021).

According to Malaviya, Baig, Kumar, and Kaushal (2020) and Cruz et al. (2021), leaves of plants growing under conditions of low light, such as forages grown in SPS have greater concentration of chlorophylls than those growing in full sunlight. Moreover, such environmental conditions may modify other chemical, morphological, and

productive characteristics of forage tissues under either natural (Gomes et al., 2019, 2022; Santos et al., 2018) or artificial (Guenni, Romero, Guédez, Bravo de Guenni, & Pittermann, 2018; Pang et al., 2019a; Pang et al., 2019b) shading conditions. These changes in morpho-physiological and anatomical patterns can modify herbage mass and nutritive value, which may affect stocking rates, voluntary intake, and animal performance of livestock grazing in SPS (Paciullo et al., 2021; Silva et al., 2021a; Silva et al., 2021b; Sousa et al., 2015).

The objective of this study was to evaluate morpho-physiological and anatomical characteristics of palisadegrass, under the influence of the tree species Bolsa de Pastor (*Zeyheria tuberculosa*) in a tropical region.

Material and methods

Experimental area and location

The experiment was conducted in a SPS located in Lagoa Santa, state of Minas Gerais, Brazil (19°35'36''S, 43°51'56'W; 747 m altitude). The soil at the experimental site is classified as ferralsols in the European Soil Classification System (Tóth et al., 2008) and the chemical properties before the initiation of the study were: pH (H_2O) = 4.72 $cmol_c \cdot dm^{-3}$, P = 1.99 ($mg \cdot dm^{-3}$), Ca^{2+} = 1.61 $cmol_c \cdot dm^{-3}$, Mg^{2+} = 1.46 $cmol_c \cdot dm^{-3}$, K = 1.25 $cmol_c \cdot dm^{-3}$, SB = 4.41 $cmol_c \cdot dm^{-3}$, CEC = 10.69 $cmol_c \cdot dm^{-3}$, OM = 33.82 $g \cdot kg^{-1}$.

Treatments consisted of the SPS or a monoculture of palisadegrass (MONO) distributed in a completely randomized design with six replicates. Six paddocks (16 m^2 each; experimental unit) were established and fenced. The Bolsa de Pastor trees were established in 1982 from natural regeneration of trees. The excessive trees and undesirable species were eliminated and the remaining tree stand was oriented to maintain a minimum distance of 4 m between trees, which resulted in a density of 160 $trees \cdot ha^{-1}$ (Viana, Maurício, Matta-Machado, & Pimenta, 2002). The vegetation of the area used for the MONO was removed and the area was prepared for sowing. Based on soil analysis results, phosphate rock and dolomitic limestone were manually applied at 1.2 and 1.5 $t \cdot ha^{-1}$, respectively. Palisadegrass was planted in 1990 and seeds were manually sown at a seeding rate of 10 $kg \cdot ha^{-1}$ viable seeds and 2 cm depth. From the establishment until the beginning of this study, pastures were used to feed beef and dairy cattle. Rainfall data were collected at 10 km from the experimental area. Seven harvestings were made over one year, as shown in Table 1.

Table 1. Experimental periods and rainfall (mm) during the trial.

Period	Rainfall pattern	Date	Days	Rainfall (mm)
P1	Wet	(Nov. 24 to Dec. 25)	31	325.0
P2	Wet	(Dec. 26 to Jan. 25)	31	185.5
P3	Wet	(Jan. 26 to Feb. 25)	31	259.7
P4	Wet	(Feb. 26 to Mar. 28)	31	157.3
P5	Transition from wet to dry season	(Mar. 29 to Apr. 28)	31	88.4
P6	Dry	(Apr. 29 to Jul. 28)	92	11.7
P7	Transition from dry to wet season	(Jul. 29 to Nov. 23)	118	99.1
Total	----	----	365	1126.7

Characteristics evaluated

In November 2008, forage was manually harvested with a cleaver at 30 cm stubble height at the initiation of the experimental period. Canopy height within the sampling area was measured according to Almeida, Maraschin, Harthmann, Ribeiro Filho, and Setelich (2000) in four points per experimental unit using a graduated ruler. Three 1 m^2 samples per experimental unit were harvested using a metal frame (Paladines, 1992), thereafter with a 31 days regrowth interval during the growing season. In the dry season, forage was harvested twice in July and November due to limited herbage mass. The remaining forage was harvested at the same stubble height and removed from the experimental unit, after sample collection. The harvested forage was weighed, dried in a forced-air oven at 55°C (Association of Official Analytical Chemists [AOAC], 1990) and used for forage mass calculation. Two subsamples were taken and according to Chacón, Stobbs, and Haydock (1977), green and dead material, leaf blade (Lb), and stem + sheath (SS) were quantified, and green: dead and Lb:SS ratios were calculated.

Sections with 2 cm^2 of the middle third of the leaf blade from fully expanded green leaves were harvested, weighed, and extracted in 80% acetone for measuring the chlorophyll and carotenoid concentrations. Extracts obtained were filtered through fast filter paper and stored in test tubes in a dark environment. Subsequently, the optical density of filtrates was read at 663, 645, and 470 nm using a Genesys 10S® ultraviolet/visible spectrophotometer (Thermo Fisher

Scientific, USA). Values obtained at these density readings were used for determining the chlorophyll (*a* and *b*) and carotenoids concentrations, according to the method described by Lichtenthaler (1987).

Photosynthetically active radiation (PAR) was measured with a quantometer (LI-1400 DataLogger, Li-Cor Biosciences, USA). Six readings per experimental unit were taken every hour after forage harvesting from 07:00 to 18:00h. Leaf area index (LAI) was estimated indirectly using a LAI-2000 plant canopy analyzer (Li-Cor Biosciences, USA). Measurements in the SPS were taken at 08:00h, at the forage sampling points from a reference point outside the canopy of trees and from five readings below the SPS canopies in their two layers (close to the ground level and above the forage). In contrast, MONO measurements were taken at 08:00h, at the forage sampling points, totaling one reading above and five readings under the forage canopy, taken at the ground level. From these readings, the device estimated the leaf area index of pastures through procedures described by Welles and Norman (1991).

In each plot, representative tillers of the population were collected to measure the different proportions of lignified (LIG), chlorophyllous (CHLO) and non-lignified (N-LIG) and achlorophyllous (ACHLO) plant tissues (Figure 1). These proportions were measured in cross sections of leaves (middle-third of the first leaf of the second node) and longitudinal and cross sections of stems (middle third of the internode between the second and third nodes). Slides were prepared using a Leica RM-2145 rotary microtome (Leica Microsystems, Germany). Subsequently, they were dissolved in water and stained with 0.0125% aqueous solution of basic fuchsin, 0.5% basic safranin and 1.0% Astra blue solution (Bukatsch, 1972), which allowed distinguishing tissues with different characteristics. Once the double staining was performed, slides were washed in running water, oven-dried at 40°C, subjected to conventional slide mounting and covered with coverslips.

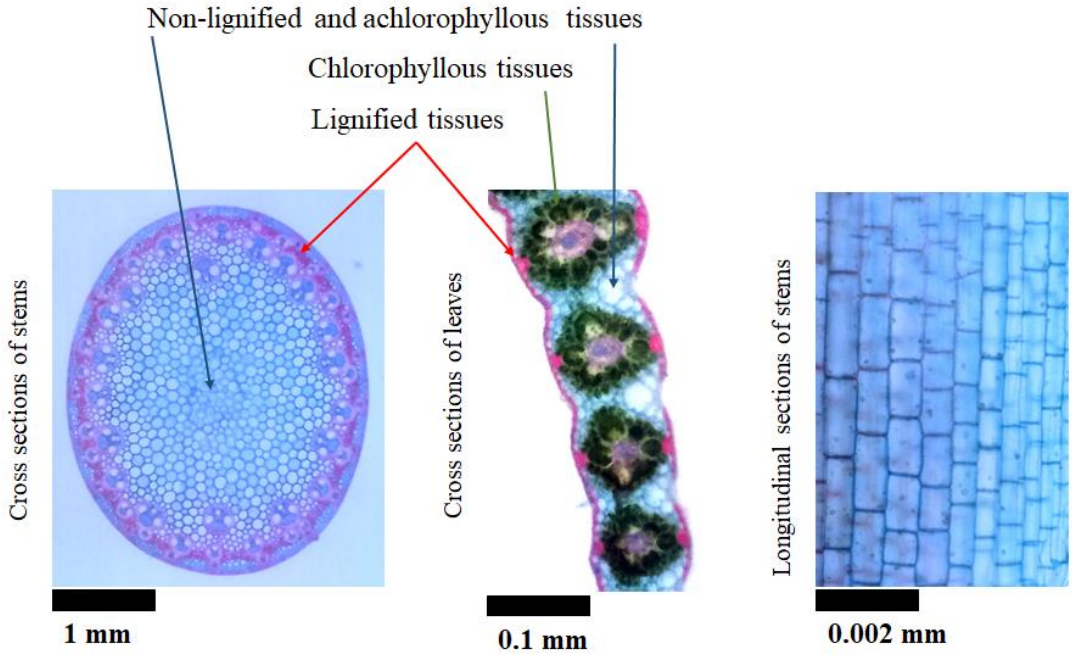

Figure 1. Illustration of the photos used to measure anatomical characteristics.

Then, photomicrographs of the sections were taken using an analog microscope. Photomicrographs were then developed, scanned and digitized, and tissue areas with different characteristics in the leaf and stem cross-sections, and cell length in the longitudinal section of the stem were measured by ImageJ® software.

Statistical analysis

A completely randomized design with six replicates and repeated measures was adopted to analyze the chemical composition, and productive and physiological characteristics of forages. The repeated measures consisted of the seven harvesting periods (P1 to P7): four during the wet season (P1 to P4), one during the transition period (wet-dry) (P5), one during the dry period (P6), and one during the transition period (dry-wet) (P7).

Statistical analysis was performed using the SAS software (SAS Institute Inc., 2004). Data relative to chemical composition, the productive and physiological characteristics of forages were analyzed according to

a repeated-measures model with PROC MIXED of SAS. The sphericity test was applied to check if this assumption was met.

If the sphericity test was not significant ($p > 0.05$) (Situation 1), the univariate structure was chosen (same as PROC GLM); on the other hand, if the sphericity test was significant ($p < 0.05$) (Situation 2), an analysis of variance was applied using mixed models analysis of variance (PROC MIXED procedure), and the variance-covariance matrix was estimated using a restricted maximum likelihood function -2RLL (-2 Res Log Likelihood) and AIC (Akaike's Information Criterion) with -2RLL, AIC values closer to zero indicating a better fit (Wolfinger, 1993). The significance level adopted for analysis of variance was set at 0.05 (probability of Type I error).

Comparisons of means were performed using the Scott-Knott test, as suggested by Conrado, Ferreira, Scapim, and Maluf (2017), with a significance level of 0.05 (probability of Type I error). Regression studies between rainfall and production data were performed to contribute to the discussion of results. The significance level adopted for the analysis of variance was set at 0.05 (probability of Type I error).

Statistical analyses were run in SAS statistical software (SAS Institute Inc., 2004) according to the model as follows:

Statistical model of situation 1: $Y_{ijk} = \mu + S_i + e_{(a)}i + P_j + PS_{ji} + e_{(b)ijk}$, where: "$Y_{ijk}$" is the observation in the i-th system, j-th harvesting period and k-th replicate; "μ" is the overall mean; "S_i" is the effect of the i-th system, i = 1, 2; "$e_{(a)}i$" is the type A error; "P_j" is the effect of the j-th harvesting period, j = 1, 2, 3, ..., 7; "PS_{ij}" is the effect of interaction between harvesting period ×system; "$e_{(b)ijk}$" is the type B error.

Statistical model of situation 2: $Y_{ijk} = \mu + S_i + \delta_{ik} + e_{(a)ik} + P_j + PSji + e_{(b)ijk}$, where, "$Y_{ijk}$" is the observation in the i-th system, j-th harvesting period and k-th replicate; "μ" is the overall mean; "S_i" is the effect of the i-th system, i = 1, 2; "δ_{ik}" is the random effect of the k-th experimental unit on the i-th system; $e_{(a)}i$ is the type A error associated with the i-th system and k-th replicate.; "P_j" is the fixed effect of the j-th harvesting period, j = 1, 2, 3, ..., 7; "PS_{ij}" is the effect of interaction between harvesting period × system; "$e_{(b)ijk}$" is the random error associated with the i-th system, j-th harvesting period and k-th replicate.

Results and discussion

Photosynthetically active radiation data

There was an effect of period on shading percentage ranging from 47.2 to 60.2 (Table 2). The greatest proportion of shading occurred between P4 and P6, which seems contradictory, because *Z. tuberculosa* was deciduous at this period (April to July), reducing canopy density. However, PAR in full sun was lower at this period due to the translation movement of the earth, which increases the Earth's tilt with respect to the sun. Consequently, sunlight passes through a thicker layer of atmosphere.

According to Guenni, Seiter, and Figueroa (2008), palisadegrass subjected to 71% artificial shading showed lower biomass compared to 43 and 0%. Shading values in the SPS were close to 55% in most harvesting periods. Although PAR strongly influences forage yield, other factors also contribute to forage production. Sousa et al. (2010) reported that forage mass of palisadegrass under 74% natural shading was reduced by only 15%, demonstrating that other aspects may interfere with plant response to PAR. The greater humidity provided by the SPS may result in more beneficial conditions for forage production compared to MONO (Baliscei et al., 2013). However, several studies have shown that severe PAR restriction decreased forage mass in tropical pastures (Abraham et al., 2014; Lelis et al., 2018; Santiago-Hernández et al., 2016).

Table 2. Photosynthetically active radiation (PAR) and shading percentage in silvopastoral (SPS) and monoculture (MONO) systems during different experimental periods.

	PAR (μmol·photons·sec^{-1}·m^{-2})									
System	Period							Mean	p-value[1]	SEM
	P1	P2	P3	P4	P5	P6	P7			
SPS	715.4aD	600.8aC	571.3aC	473.2aB	369.9aA	367.6aA	652.1aD	535.8	System < 0.001	
MONO	1354.9bC	1304.8bC	1290.6bC	1119.2bB	928.3bA	845.6bA	1258.8bC	1157.5	Period = 0.002	42.696
Mean	1035.2	846.6	931.0	796.2	649.1	606.6	955.5	846.6	Interaction <0.001	
Shading[2] (%)	47.20	53.95	55.73	57.72	60.15	56.53	48.20	54.21		

Means followed by different lowercase letters in the same column are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); Means followed by different uppercase letters in the same row are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); P1 to P4 = wet periods; P5 = transition period (wet-dry); P6 = dry period and P7 = transition period (dry-wet); SEM = Standard error of the mean; [1]Probability of type I error by Fisher' test; [2](PAR in MONO system - PAR in SPS system) × 100/PAR in MONO system.

Morpho-physiological characteristics

The Lb:SS ratio (Table 3) did not vary ($p > 0.05$) between treatments during the wet season. However, the SPS had greater Lb:SS ratio ($p < 0.05$) than MONO in the transition periods and dry season. Greater Lb:SS ratio in the dry season can be partially explained by less severe water deficit conditions in the SPS compared to the MONO, due to a favorable micro-climate provided by trees in these systems (Baliscei et al., 2013). Furthermore, leaf blade growth rate may be greater in the MONO due to greater sunlight exposure (Taiz, Zeiger, Møller, & Murphy, 2015).

Period had a significant effect ($p < 0.05$) on Lb:SS ratio. In both treatments, the Lb:SS ratio was lower ($p < 0.05$) in the wet season than in the other periods (Table 3). Effect of period on Lb:SS ratio likely occurred due to reduced leaf growth under water deficit conditions, since the leaf is the main transpiration organ and, consequently, responsible for water loss in plants (Taiz et al., 2015).

Table 3. Leaf blade: stem+sheath and green: dead material ratios, canopy height, leaf area index of forage in silvopastoral (SPS) and monoculture (MONO) systems during different experimental periods.

Leaf blade: stem+sheath ratio										
System	Period							Mean	p-value[1]	SEM
	P1	P2	P3	P4	P5	P6	P7			
SPS	5.34aB	5.22aB	5.02aB	4.62aB	2.12bA	2.79bA	2.33bA	3.92	System = 0.032	
MONO	5.66aB	5.03aB	4.89aB	4.54aB	1.65aA	1.36aA	1.65aA	3.57	Period < 0.001	0.451
Mean	5.40	5.13	4.96	4.58	1.89	2.08	1.99	3.74	Interaction = 0.023	
Green: dead ratio										
SPS	15.5bC	12.5bC	10.1bC	9.89bC	5.38aB	1.27aA	5.03aB	8.54	System = 0.021	
MONO	10.9aC	8.68aC	7.12aC	6.55aC	6.35aB	1.27aA	4.95aB	6.55	Period = 0.012	0.667
Mean	13.23	10.61	8.63	8.22	5.87	1.27	4.99	7.54	Interaction < 0.001	
Canopy height (cm)										
SPS	62.0bB	63.3bB	65.0bB	66.7bB	65.0bB	55.3bA	53.7bA	61.6	System = 0.352	
MONO	50.0aB	50.0aB	49.7aB	48.3aB	47.6aB	42.7aA	43.0aA	47.3	Period= 0.024	2.453
Mean	56.0	56.7	57.3	57.5	56.3	49.0	48.3	54.5	Interaction = 0.542	
Leaf area index										
SPS	3.33bB	3.47bB	3.97bB	3.02bB	3.36bB	1.35aA	1.53aA	2.86	System < 0.001	
MONO	2.01aB	2.51aB	2.15aB	2.09aB	2.18aB	1.39aA	1.51aA	1.98	Period = 0.003	0.272
Mean	2.67	2.99	3.06	2.56	2.77	1.37	1.52	2.42	Interaction < 0.001	

Means followed by different lowercase letters in the same column are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); Means followed by different uppercase letters in the same row are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); P1 to P4 = wet periods; P5 = transition period (wet-dry); P6 = dry period and P7 = transition period (dry-wet); SEM = Standard error of the mean; [1]Probability of type I error by Fisher's test.

During the wet season, the green: dead ratio in the SPS was higher ($p < 0.05$) than in MONO (Table 3). In the transition periods and dry season, the green: dead ratio of the SPS did not differ ($p > 0.05$) from the MONO. Harvesting period had a significant effect ($p < 0.05$) on the green: dead ratio. In both treatments, green: dead ratios were significantly greater ($p < 0.05$) in the wet season than in transition periods (P5 and P7), with greatest values in the dry season (P6). Greater values of green: dead ratio in the SPS during the growing season can be explained by the effect of shading on morphogenic characteristics, such as the number of days of full leaf activity (Santos et al., 2018), and also, shading induces a reduction in dead biomass (Gómez, Guenni, & Bravo de Guenni, 2013). This indicates higher tissue senescence rate in MONO. Furthermore, shading can inhibit bud activity and affect the formation of new leaves and tillers, leading to a reduced leaf metabolism, which increases the maintenance of semi-senescent tissues (Frank & Hofmann, 1994). Formation and maintenance of the living parts of plants depend on several genetic and environmental factors. Among environmental variables, PAR and water may be influential factors (Taiz et al., 2015). These factors were limiting during the dry season (Tables 1 and 2), which impairs the formation and maintenance of living plant tissues, reducing the green: dead ratio.

Canopy height was greater in the SPS ($p < 0.05$) (Table 3). Period had a significant effect ($p < 0.05$) on canopy height, with higher values ($p < 0.05$) in the wet season and transition period (wet-dry) (P5) than in the dry season and transition period (dry-wet) (P7). It is expected that palisadegrass growing under shading conditions would have greater canopy height than under full sunlight. Stem elongation is a compensatory mechanism for the reduced light intensity (Paciullo et al., 2017). This is also explained by the higher availability of water during the experiment, in periods of higher canopy height.

During the wet season and transition period (wet-dry), LAI in the SPS was greater ($p < 0.05$). However, in the dry season and transition period (dry-wet), the LAI of the SPS did not differ ($p > 0.05$) from the MONO (Table 3). Period had a significant effect ($p < 0.05$) on LAI and was greater ($p < 0.05$) in the wet season and transition period (wet-dry) than in the dry season and the transition period (dry-wet).

Leaf area index can interfere with critical ecological aspects, such as inter- and intra-specific competition between plants, carbon retention, and soil conservation. It can also be a crucial component of agroecosystem biogeochemical cycles (Bréda, 2003). Differences in LAI values found in this study are possibly related to changes in leaf appearance and elongation rates, which are influenced by the quantity and quality of light, among other environmental factors. These rates determine some structural canopy characteristics such as the number of leaves per tiller, leaf size, and tiller density, which are responsible for determining the LAI of the canopy (Bahmani, Thom, Matthew, Hooper, & Lemaire, 2003). Higher values of LAI in the SPS may be justified by thinner and longer leaves resulting from the limited light incidence (Santos et al., 2018).

When the LAI value is optimal, the interception of approximately all incident radiation with minimal self-shading will provide the maximum crop growth rate, defined as dry matter weight accumulated per unit area per unit time (Ludlow, Wilson, & Heslehurst, 1974). According to Portes, Ferreira, Peixoto, and Melo (2017), the optimal LAI for palisadegrass monoculture cultivated in the wet season is 6.51, but this LAI is only reached at 97 days of growth, when palisadegrass contains reduced nutritive value (Quintino et al., 2013). The 32-day LAI (range of 29 - 35, on average), proposed by Portes et al. (2017), corresponds to an LAI of 2.01, which is similar to that found in MONO during the wet season, but below the values reported in the SPS (Table 3).

Concentration of chlorophyll *a* in the SPS was greater ($p < 0.05$) (Table 4). In the SPS, the chlorophyll *a* was higher ($p < 0.05$) in the wet season and the transition period (dry-wet) than in the other periods (Table 4). There were no significant effects of period ($p > 0.05$) on chlorophyll *a* concentration between forages harvested, regardless of the period. Concentration of chlorophyll *b* in the SPS was greater than in MONO throughout the experimental period (Table 4). Period had a significant effect ($p < 0.05$) on chlorophyll *b*, which showed greater ($p < 0.05$) values in the wet season and transition period (dry-wet) than in the dry season and in the transition period (wet-dry).

Table 4. Concentrations of chlorophyll *a* and *b*, chlorophyll *a/b* ratio and carotenoid concentration in *U. brizantha* leaves in silvopastoral (SPS) and monoculture (MONO) systems during different experimental periods.

System	Period							Mean	p-value[1]	SEM
	P1	P2	P3	P4	P5	P6	P7			
Chlorophyll *a* (μg·cm^{-2})										
SPS	2.48bB	2.49bB	2.55bB	2.50bB	1.92bA	1.93bA	2.48bB	2.34	System < 0.001	
MONO	1.29aA	1.28aA	1.27aA	1.21aA	1.29aA	1.27aA	1.18aA	1.26	Period = 0.025	0.139
Mean	1.89	1.89	1.91	1.86	1.61	1.60	1.83	1.80	Interaction = 0.028	
Chlorophyll *b* (μg·cm^{-2})										
SPS	0.87bB	0.86bB	0.90bB	0.88bB	0.73bA	0.74bA	0.93bB	0.84	System < 0.001	
MONO	0.40aB	0.40aB	0.40aB	0.38aB	0.50aA	0.49aA	0.44aB	0.43	Period = 0.041	0.036
Mean	0.64	0.63	0.65	0.63	0.62	0.62	0.69	0.64	Interaction < 0.001	
Chlorophyll *a/b* ratio										
SPS	2.85aA	2.90aA	2.83aA	2.84aA	2.63aB	2.61aB	2.67aB	2.76	System = 0.042	
MONO	3.18bA	3.20bA	3.18bA	3.18bA	2.58aB	2.59aB	2.68aB	2.94	Period = 0.003	0.083
Mean	3.02	3.05	3.01	3.01	2.61	2.60	2.67	2.85	Interaction = 0.034	
Carotenoids (μg·cm^{-2})										
SPS	0.59bB	0.61bB	0.63bB	0.35bA	0.36bA	0.39bA	0.64bB	0.48	System < 0.001	
MONO	0.44aB	0.41aB	0.38aB	0.26aA	0.26aA	0.30aA	0.50aB	0.37	Period = 0.012	0.019
Mean	0.52	0.51	0.51	0.26	0.29	0.35	0.57	0.43	Interaction = 0.378	

Means followed by different lowercase letters in the same column are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); Means followed by different uppercase letters in the same row are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); P1 to P4 = wet periods; P5 = transition period (wet-dry); P6 = dry period and P7 = transition period (dry-wet); SEM = Standard error of the mean; [1]Probability of type I error by Fisher's test.

Response to the amount of light is the result of a sequence of environmental signals and their respective receptors. In the specific case of photosynthesis, light signal is received by pigments (chlorophyll and carotenoids), and the response is directly proportional to the amount of energy received (PAR) up to the light saturation point of a given leaf (Taiz et al., 2015). Cruz et al. (2021) reported a linear increase in the concentration of chlorophyll *a* and *b* in leaves as shading percentage increased. Plants growing under conditions of low radiation develop more

grana (Shao et al., 2014), which are a set of stacked membranous discs (thylakoid) containing chlorophyll and located on chloroplasts (Taiz et al., 2015).

Another theory is that in tropical plants exposed to high light conditions, chlorophyll is continuously synthesized and destroyed (photo-oxidation); however, under higher light intensities, a greater degradation occurs, and equilibrium is reached at a lower concentration. Therefore, shaded leaves have greater chlorophyll concentrations than those exposed to full sunlight (Malaviya et al., 2020). Souza et al. (2016) observed an increase in the concentration of chlorophyll *a* in palisadegrass in response to 50% natural shading. Corroborating with this, Mishra, Tiwari, and Bhatt (2010) reported an increase in total chlorophyll concentration due to shading in tropical forages.

According to Shao et al. (2014), the concentrations of chlorophylls *a* and *b* increase disproportionately in response to natural shading, with a relatively greater increase in chlorophyll *b*. Thus, the greater relative proportion of chlorophyll *b* may be advantageous under shading since it allows for greater light absorption efficiency due to the energy uptake of chlorophyll *b* at other wavelengths (typical of naturally shaded environments). It effectively participates in photochemical reactions, ensuring greater values of photosynthetic rate and forage accumulation. These results are in accordance with Gomes et al. (2019), who stated that one of the physiological characteristics of shaded leaves is the lower amount of secondary pigments and the lower chlorophyll *a*/*b* ratio compared to leaves in full sun.

Chlorophyll *a*/*b* ratio in SPS was lower ($p < 0.05$) than in MONO during the wet season (Table 4). The chlorophyll *a*/*b* ratio generally tends to decrease with reduced light intensity due to greater relative proportion of chlorophyll *b* in shaded environments. This because chlorophyll *b* molecule is degraded more slowly in shaded plants than chlorophyll *a* (Taiz et al., 2015). In the transition periods, and the dry season, the chlorophyll *a*/*b* ratio did not differ ($p > 0.05$) between systems (Table 4). Nonetheless, chlorophyll *a*/*b* ratio was lower ($p < 0.05$) in the wet season compared to the transition period and dry season (Table 4), possibly due to factors such as lower PAR incidence and a higher proportion of shading (Table 2) in the transition period and dry season. Such factors may increase chlorophyll *b* concentration by reducing chlorophyll *a*/*b* ratio.

Concentration of carotenoids was greater ($p < 0.05$) in the SPS (Table 4). In both treatments, carotenoids were greater ($p < 0.05$) in the first three periods of the wet season and in the transition period (dry-wet) than in the last period of the wet season, the transition period (wet-dry) and the dry season (Table 4). Carotenoids are essential components in the photosynthetic antenna complex, contributing to the absorption of incident radiation and dissipation of excess absorbed energy, among other functions (Taiz et al., 2015) and, in general, forage grasses and cover crops used as forage are rich sources of carotenoids (Maxin, Cornu, Andueza, Laverroux, & Graulet, 2020; Nozière et al., 2006). Under water or light stress conditions, carotenoids concentration in the leaves tends to increase (Shao et al., 2014), which corroborates the results found in this experiment.

Anatomical characteristics

There was no effect of treatment ($p > 0.05$) on the proportion of N-LIG and ACHLO tissues (Table 5). However, the proportion of N-LIG and ACHLO tissues was lower in the dry season than in the wet season, which may be explained by the increased proportion of LIG areas (Table 5). Increase in lignin concentration of perennial grasses in the dry season is an adaptive strategy for greater conservation of tissue moisture and chemical energy photoassimilated in the wet season (Gomes et al., 2019). According to some authors (Santos et al., 2016; Sousa et al., 2010), morphostructural alterations of some tropical forages is one of the responses associated with shading; however, literature evaluating anatomical modifications in the proportion of tissues is still scarce.

Leaves have three different areas: N-LIG and ACHLO areas, LIG areas, and CHLO areas. Forage in MONO had a similar proportion of N-LIG and ACHLO areas compared to forage in the SPS, with differences ($p > 0.05$) only in the proportion of areas during the P1 in the SPS (Table 6). This indicates that treatments did not influence the increase or reduction of these areas. Regarding LIG areas, no significant differences were detected between harvesting periods and forage production systems ($p > 0.05$; Table 6).

Proportion of CHLO area in the SPS did not vary during the experimental period, except for the P1 period (greatest rainfall). The proportion of CHLO in MONO pastures was lower in the transition period and dry season than in the wet season (Table 6). Valente et al. (2016) reported anatomical differences in leaves of tropical forage exposed to full sunlight or shaded, but these modifications were conditional to shading percentage. This study was conducted with a 54% shading, proportion lower than the one tested

by the aforementioned authors (69%). This difference possibly explains the non-anatomical differences in this study. The lack of modifications in the proportion of areas in leaves (N-LIG and ACHLO; LIG, and CHLO) strengthens the idea that there is no change in the chemical composition of the leaf blade tissue. Thus, changes in the nutritive value of forage are more related to the proportion of leaves in the canopy (Lee, 2018).

Table 5. Proportion of non-lignified and lignified areas in stem cross-sections of forage in silvopastoral (SPS) and monoculture (MONO) systems during different experimental periods.

Non-lignified and achlorophyllous area (%)										
Systems	Periods							Mean	p-value[1]	SEM
	P1	P2	P3	P4	P5	P6	P7			
SPS	70.1aA	71.6aA	66.9aB	66.4aB	68.4aB	61.8aC	62.8aC	66.9	System = 0.563	
MONO	68.9aA	68.3bA	66.6aA	66.9aA	66.5aA	62.8aB	64.8aB	66.4	Period = 0.041	1.52
Mean	69.5	70.0	66.8	66.7	67.5	62.3	63.8	66.6	Interaction = 0.039	
Lignified area (%)										
SPS	29.9aA	27.4bA	33.1aA	33.6aA	31.6aA	38.2aB	37.2aB	33.2	System = 0.452	
MONO	31.2aA	32.7aA	33.4aB	33.1aB	33.5aB	37.3aC	35.2aC	33.6	Period = 0.029	1.617
Mean	30.5	30.0	33.3	33.4	32.5	37.8	36.2	33.4	Interaction = 0.047	

Means followed by different lowercase letters in the same column are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); Means followed by different uppercase letters in the same row are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); P1 to P4 = wet periods; P5 = transition period (wet-dry); P6 = dry period and P7 = transition period (dry-wet); SEM = Standard error of the mean; [1]Probability of type I error by Fisher's test.

Table 6. Proportion of non-lignified and achlorophyllous areas, lignified areas and chlorophyllous areas in leaf cross-sections of forage in silvopastoral (SPS) and monoculture (MONO) systems during different experimental periods.

Non-lignified and achlorophyllous area (% total leaf cross-sectional area)										
System	Period							Mean	p-value[1]	SEM
	P1	P2	P3	P4	P5	P6	P7			
SPS	36.7aA	46.5aB	43.7aB	46.7aB	46.2aB	50.7aB	46.4aB	45.4	System = 0.659	
MONO	40.0aA	45.7aA	51.4aA	43.5aA	43.2aA	52.4aA	43.5aA	45.7	Period = 0.041	1.754
Mean	38.3	46.1	47.5	45.1	44.7	52.1	44.9	45.5	Interaction = 0.025	
Lignified area (% total leaf cross-sectional area)										
SPS	26.1	23.2	20.5	23.2	21.9	22.9	23.0	23.1a	System = 0.543	
MONO	23.4	25.7	25.4	24.2	25.7	24.7	24.2	24.8a	Period = 0.729	1.137
Mean	24.7	24.4	23.0	23.7	23.8	24.3	23.6	23.9	Interaction = 0.417	
Chlorophyllous area (% total leaf cross-sectional area)										
SPS	37.3aA	30.8aB	31.7aB	30.8aB	29.3aB	28.4aB	30.6aB	30.7	System = 0.562	
MONO	38.6aA	32.9aB	31.6aB	30.4aC	30.8aC	22.9aD	30.4aC	31.2	Period = 0.046	1.440
Mean	38.0	31.8	31.7	30.6	30.0	23.7	30.5	31.0	Interaction = 0.032	

Means followed by different lowercase letters in the same column are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); Means followed by different uppercase letters in the same row are significantly different by Scott-Knott test at a significance level of 0.05 (probability of Type I error); P1 to P4 = wet periods; P5 = transition period (wet-dry); P6 = dry period and P7 = transition period (dry-wet); SEM = Standard error of the mean; [1]Probability of type I error by Fisher's test.

Reduced proportions of CHLO area during the wet-dry transition periods and dry season, can be because these periods have climate restrictions related to photosynthetic activity and plant development, in MONO and SPS. Plants under shading have increased concentration of total chlorophylls (Taiz et al., 2015) as observed in this study. Moreover, the number of cells and the amount of CHLO tissues also increased with natural shading. The literature corroborates these results, emphasizing that this aspect is typical of tropical Poaceae plants, the so-called anatophysiological plasticity (Costa et al., 2015; Paciullo et al., 2011; Souza et al., 2016).

The stem cell length did not differ ($p > 0.05$) between treatments, and was not affected by the System × Period interaction ($p > 0.05$) (Table 7). Stem etiolation possibly occurred due to greater meristem cell proliferation at stem nodes of forages in the SPS. Anatomical studies on longitudinal sections of the stem in shaded plants are essential to elucidate the process of etiolation in tropical grasses (Lelis et al., 2018; Sousa et al., 2010). This process may be linked to the balance of plant hormones with stem cell elongation, including auxin (Bartel, 1997; Mutai, Njuguna, & Ghimire, 2017) and hormones, such as gibberellins and cytokines (Zaman, Kurepin, Catto, & Pharis, 2016), which trigger cell division and differentiation in plants (Taiz et al., 2015).

Table 7. Mean cell length in the cross-sectional area of the middle third of the forage stem in silvopastoral (SPS) and monoculture (MONO) systems during different experimental periods.

Mean cell length in the cross-sectional area of the stem (µm)										
Systems	Periods							Mean	p-value[1]	SEM
	P1	P2	P3	P4	P5	P6	P7			
SPS	1.85	1.87	1.85	1.87	1.88	1.87	1.86	1.87	System = 0.342	
MONO	1.71	1.86	1.77	1.72	1.92	1.71	1.72	1.73	Period = 0.582	0.115
Mean	1.78	1.87	1.81	1.80	1.90	1.74	1.79	1.80	Interaction = 0.198	

P1 to P4 = wet periods; P5 = transition period (wet-dry); P6 = dry season and P7 = transition period (dry-wet); SEM = Standard error of the mean.
[1]Probability of type I error by Fisher's test.

Conclusion

The SPS alters morpho-physiological characteristics of palisadegrass and increases the concentration of chlorophyll *a* and *b*. The proportion of chlorophyllous cells in forage leaves in the SPS remains constant throughout the year, even in the transition and dry periods, which does not occur in the MONO. Etiolation in the SPS is due to cell multiplication and not to the increase in stem cell length.

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