

# Leaf anatomy of 'Marandu' grass cultivated in plant arrangements in agrosilvopastoral systems

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**Abstract** – The objective of this work was to evaluate the effects of different arrangements of eucalyptus plants on the morphoanatomical characteristics of leaf blades of 'Marandu' grass [*Urochloa brizantha* (Syn. *Brachiaria brizantha*)] plants cultivated in agrosilvopastoral systems. The experiment was set in field conditions in a randomized complete block design with four replicates, in which the plots were represented by planting spacings of eucalyptus (12x2 and 12x4 m), and the subplots, by the distance between 'Marandu' grass and eucalyptus rows (6, 4, and 2 m). A control was added, composed by 'Marandu' grass cultivated in full sun. Variables of the leaf anatomy of 'Marandu' grass were evaluated for shading interference by trees. The distance of 'Marandu' grass plants from the eucalyptus lines interfered with the leaf blade anatomy of the forage, and the highest modifications occurred in linear measures and tissue proportions in the arrangements with the highest eucalyptus density. The shading interference of eucalyptus on the anatomical characteristics of leaf blades of 'Marandu' grass depends on the adopted arrangement. This occurs due to the grass adaptation to the low-light incidence, with a consequent reduction in its physiological activity and growth.

**Index terms:** *Urochloa brizantha*, agroforestry systems, forest-livestock-crop integration, morphoanatomical characteristics.

## Anatomia foliar de capim-marandu cultivado em arranjos de plantio em sistemas agrossilvipastoris

**Resumo** – O objetivo deste trabalho foi avaliar os efeitos de diferentes arranjos de plantas de eucalipto sobre as características morfoanatômicas de lâminas foliares de plantas de capim 'Marandu' [*Urochloa brizantha* (Syn. *Brachiaria brizantha*)] cultivadas em sistemas agrossilvipastoris. O experimento foi estabelecido em condições de campo, em um delineamento de blocos ao acaso, com quatro repetições, em que as parcelas foram representadas pelos espaçamentos de plantio de eucalipto (12x2 e 12x4 m), e as subparcelas, pela distância entre o capim 'Marandu' e as fileiras de eucalipto (6, 4 e 2 m). Adicionou-se um controle apenas com capim 'Marandu', cultivado a sol pleno. As variáveis de anatomia foliar do capim 'Marandu' foram avaliadas quanto à interferência do sombreamento pelas árvores. A distância entre as plantas de capim 'Marandu' e as linhas de eucalipto interferiram na anatomia da lâmina foliar da forragem, e as maiores modificações ocorreram nas medidas lineares e proporções teciduais nos arranjos com maior densidade de eucalipto. A interferência do sombreamento pelo eucalipto nas características anatômicas das folhas de capim 'Marandu' depende do arranjo adotado. Isto ocorre em razão da adaptação do capim à baixa incidência de luz, com a consequente redução de sua atividade fisiológica e crescimento.

**Termos para indexação:** *Urochloa brizantha*, sistemas agroflorestais, integração lavoura-pecuária-floresta, características morfoanatômicas.

### Introduction

The species *Urochloa brizantha* (Syn. *Brachiaria brizantha*) has a broad edaphoclimatic adaptation, and is easily spread due to the large amount of seed produced and their dormancy, which facilitates their

dispersion over time and space. Its leaves are linear and numerous, usually densely hairy, especially the cultivar 'Marandu' (Gobbi et al., 2011), that is widely used in crop-livestock-forestry integrated systems, mainly in intercropping with corn and eucalyptus (Santos et al., 2015).

One of the main obstacles in the management of agrosilvopastoral and silvipastoral systems is the alteration of the growth environment for the species that constitute the understory. The shade provided by the trees promotes significant changes in the microclimate, which may affect the morphology, anatomy, chemical, and bromatological composition of forage plants, which, in turn, may have effect on the growth, productivity, and quality of forage (Gobbi et al., 2011).

Among the main environmental factors affecting plant growth and development are the availability of light radiation, water, temperature, and nutrients (Akula & Ravishankar, 2011). In forest environments, the wide variation of the availability of incident radiation is a limiting factor to the growth of seedlings and young individuals of certain species (Valladares & Niinemets, 2008). The leaf is the primary photosynthetic organ that generally shows phenotypic plasticity in relation to light radiation, and its availability and quality can cause substantial effects on size and thickness and, thus, on the development of the organ (Valladares & Niinemets, 2008; Sanches et al., 2009). When developing under full sun, leaves are generally thicker and have a greater photosynthetic capacity per unit area because of the increased amount of enzymes, and increased stomatal conductance in comparison to shaded leaves (Valladares & Niinemets, 2008). However, the potential of acclimation to light is variable between species, as some of them may increase their photosynthetic capacity when transferred from low to high irradiance, or not, according to the anatomical plasticity (Taiz & Zieger, 2013). Therefore, anatomical evaluation of plant responses to different shading levels are of great importance for the development of management systems to use agronomic, zootechnical, and arboreal species in multicrop systems, such as in agroforestry ones, also known as crop-livestock-forest. The lack of knowledge on the anatomical and micromorphological changes resulting from the exposure of forage species to different levels of shading, in agrosilvopastoral systems, motivated the development of this work.

The objective of this work was to evaluate the effects of different arrangements of eucalyptus trees on the anatomical characteristics of leaf blades of 'Marandu' grass plants intercropped with maize in agrosilvopastoral systems.

## Materials and Methods

The experiment was carried out in an area with an agrosilvopastoral system established seven years before, with a clonal hybrid of *Eucalyptus grandis* x *E. urophylla* (*Urograndis*), clone 3336, and forage of the genus *Urochloa* (Syn. *Brachiaria*), in the Universidade Federal de Viçosa, in the state of Minas Gerais, Brazil (20°46'38"S 42°51'40"W). Due to the begging of pasture degradation, the area was renewed in November 2013, and corn (*Zea mays* L.) was cultivated in consortium with 'Marandu' grass. The soil of the experimental area is a Red Yellow Latosol (Oxisol), clayey with smooth and wavy relief, with the following chemical characteristics: pH in H<sub>2</sub>O, 5.60; P, 1.13 mg dm<sup>-3</sup> (Mehlich-1); K, 41.00 mg dm<sup>-3</sup>; Ca<sup>+2</sup>, 3.33; Mg<sup>+2</sup>, 0.57; Al<sup>+3</sup>, 0.03 cmol<sub>c</sub> dm<sup>-3</sup> (KCl 1 mol L<sup>-1</sup>); CTC (T), 5.99 cmol<sub>c</sub> dm<sup>-3</sup>; sum of bases, 2.34 cmol<sub>c</sub> dm<sup>-3</sup>; H+Al, 3.65 cmol<sub>c</sub> dm<sup>-3</sup>; 2.37 dag kg<sup>-1</sup> of organic matter; and 20.35 mg dm<sup>-3</sup> of remaining P.

A completely randomized block experimental design was applied with four replicates. The treatments consisted of 'Marandu' grass, intercropped with corn and eucalyptus in an agrosilvopastoral systems, in split plots, in which the plots were represented by eucalyptus planting spacings (12x2 and 12x4 m) and, the subplots, the distance between 'Marandu' grass and eucalyptus rows (6, 4, and 2 m). In addition, a control was added with 'Marandu' grass monoculture (grown in full sun). Corn was the double-cross BM207, with planiform leaves.

The experimental units were 12 m wide by 12 m long, covering 144 m<sup>2</sup> area, consisting of two eucalyptus rows, interspersed with 'Marandu' grass and corn grown in the understory, in the intercropped plots. The monoculture plots were 12 m wide by 10 m long, covering 120 m<sup>2</sup> area.

Desiccation was carried out in the experimental area, with 4 L ha<sup>-1</sup> glyphosate and 2.0 L ha<sup>-1</sup> 2,4-D, with 100 L ha<sup>-1</sup> spray volume, thirty days before the sowing of corn and 'Marandu' grass to form a mulch for the sowing with no-tillage.

The corn hybrid sowing was held in November, and cultivated between November 2013 and February 2014, by distributing 6 seed m<sup>-1</sup>, with 1.0 m row spacing, in 8 m in the center between rows of trees, in the intercropped plots. The fertilizer used in seeding was 400 kg ha<sup>-1</sup> of 8-28-16 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) formulation. In the same time, the forage species was sown in and

between the corn rows, at 2 cm soil depth, using a multiple seeder (Semeato, model SHM 11/13). For all treatments, 4 kg ha<sup>-1</sup> viable pure seed of 'Marandu' grass were used. Nitrogen topdressing was applied 30 days after corn emergence at 100 kg ha<sup>-1</sup>, using ammonium sulfate as source.

For the anatomical evaluations of 'Marandu', in February, four leaf blades were collected per subplot, two of which from a 'Marandu' plant cultivated within the corn row, and the other two ones from plants cultivated between corn rows. The youngest fully expanded leaf in the tiller was collected, with the total of 96 collected leaves.

The middle portion of the leaves was cut into segments of approximately 1 cm in length, which were fixed in FAA50 (formaldehyde:glacial acetic acid: ethanol, 5:5:90) for 48 hours (Johansen, 1940). Then, the samples were removed from the fixative solution, washed, and stored in ethanol solution at 70%. For the preparation of the permanent slides, leaf portions were dehydrated in an ethanol series, and embedded in glycol methacrylate (Historesin, Leica Instruments, Heidelberg, Germany). Transverse leaf sections of 5 µm thickness were obtained with the assistance of a manual rotary microtome (model 820, AO Scientific Instruments) and subsequently disposed upon histological slides, stained with toluidine blue (O'Brien et al., 1964), and assembled with Permount. Five slides were made for each sample unit.

Two scanned images were obtained from the material contained in each of the slides by averages of light microscopy Primo Star (Zeiss, Berlin, Germany), coupled with a digital camera Axio Cam ERc5s (Zeiss, Berlin, Germany).

Linear measures and area (tissue percentage) were determined using the software Image-Pro Plus 4.1. The

following linear variables and tissue percentage were obtained: adaxial epidermis thickness (DET), abaxial epidermis thickness (BET), mesophyll thickness (MEST), leaf blade thickness (LBT); percentage of vascular bundle (VB%), percentage of sheath cells of the vascular bundle (BSC%), percentage of adaxial epidermis (ED%), percentage of abaxial epidermis (EB%), percentage of bulliform cells (BLF%) and percentage of mesophyll cells (MES%). To determine leaf blade thickness (LBT), five fragments fixed in FAA50 were included in 2-hydroxyethyl-methacrylate, sectioned at 7 µm, and stained with toluidine blue.

The characterization of the experimental units with eucalyptus trees during harvest is described on Table 1. The following information was determined: the number of trees per hectare as a function of spacing; the average values of tree heights; the breast height diameter (cm), 1.30; and the estimation of wood volume (m<sup>3</sup> ha<sup>-1</sup>) using the Schumacher & Hall (1933) formula.

The wood volume per hectare was determined for the cultivated system (trees + crop + pasture), as well as the heights of corn and 'Marandu' grass plants. Concomitantly, the average shading exerted by trees on corn and 'Marandu' grass canopies was estimated 110 days after sowing, with the aid of a LI-COR device model LAI 20001, when corn plants were in the stage from dough to dent (corn silage point) by the time of the anatomical harvest. Readings were taken under diffuse light conditions (overcast weather, early morning, or late afternoon), following the recommendations for device use (Welles & Norman, 1991). To determine the shading imposed by trees, the percentage of reduction of photosynthetically active radiation (PAR) was evaluated above the corn and 'Marandu' grass canopies, and compared to plants grown in full sun. The PAR measures were taken with

**Table 1.** Number of trees per arrangement, total height, breast height diameter (BHD), volume of wood per tree (Voltre), volume of wood per hectare of cultivated system (Volha), corn height, 'Marandu' grass (*Urochloa brizantha*) height, photosynthetically active radiation (PAR, µmol m<sup>-2</sup> s<sup>-1</sup>), and percentage of shade (%) above corn and the 'Marandu' grass canopies and in full sun, at 110 days after sowing the species in an agrosilvopastoral system.

Arrangement	Number of trees ha <sup>-1</sup>	Height (m)	BHD (cm)	Voltre (m <sup>3</sup> )	Volha (m <sup>3</sup> )	Corn height	'Marandu' grass height	Corn - PAR and shade (%)	'Marandu' - PAR and shade (%)	PAR of full sun (µmol m <sup>-2</sup> s <sup>-1</sup> )
12 x 2 m	416	35.22	23.24	0.435	181.3	2.79	83.2	719.7–41.3	512.5–58.2	1226
12 x 4 m	208	29.26	27.34	0.622	129.8	2.91	85.1	853.3–30.40	388.6–68.3	1226

the equipment AccuPAR Linear PAR/LAI ceptometer, Model PAR-80 (Decagon Devices), according to recommendations for the equipment use.

Data were subjected to analysis of variance and, when significant, to the Tukey's test, at 5% probability.

## Results and Discussion

Corn plants grown in the 12x4 m plots showed a higher exposure to radiation (30.4% shade) than those grown in the 12x2 m plots (41.3% shade), which caused higher heights of corn plants (Table 1) and higher shade over 'Marandu' grass plants grown under the corn canopy (68.3 shade). These results corroborate those by Domingues et al. (2017), who found a higher corn production in less shaded areas.

When assessing adaxial epidermis thickness (DET), 'Marandu' grass plants, grown in the plots with eucalyptus in the 12x4 and 12x2 m arrangements, showed no difference for the forage cultivation distances (2, 4, and 6 m) compared to the control (monocrop). However, when comparing the effect of the arrangements within each spacing of 'Marandu' grass to the eucalyptus rows, there was a reduction of the DET values in the higher density arrangement (12x2 m plot, in all distances of the tree crop) (Table 2). It is noteworthy that the DET ranged from 7.87  $\mu\text{m}$  (in the treatment in which 'Marandu' grass was grown 2 m of eucalyptus rows) to 9.32  $\mu\text{m}$  (in the distance of 2 m in the 12x4 m arrangement), 15.56% thinner. Contrasting to these results, Baliza et al. (2012) found lower values of adaxial epidermis thickness (DET) in coffee plants grown in full sun, than under artificial shade of 35, 50, 65, and 90%.

Regarding thickness of the abaxial epidermis (BET) of 'Marandu' grass leaf blades, there was no difference between the forage cultivation distances and the control (full sun), in the plots where eucalyptus plants were grown in 12x4 and 12x2 m arrangements. When evaluating the planting distances of the tree species, in different *Eucalyptus* arrangements, for 'Marandu' grass leaf anatomy, there was also no difference in BET. These results differ from what was found by Baliza et al. (2012). The thickness of abaxial epidermis (BET) was lower in coffee leaves cultivated in full sun than under 35% shading. The authors report that shade promotes distension of the epidermis cells by action of auxin, which results in thicker epidermis.

The thickness of the mesophyll (TMES) of 'Marandu' grass leaf blades collected at different distances from the eucalyptus row (2, 4, and 6 m) did not show differences in comparison to the control within the two eucalyptus planting arrangements (12x4 and 12x2 m). However, for the effect of the arrangements within the distances of collection of 'Marandu' grass plants, it was found that at 6 m distance in the arrangement of 12x4 m these plants showed higher TMES values than in the 12x2 m arrangement. For the other distances, no changes

**Table 2.** Adaxial epidermis thickness (DET,  $\mu\text{m}$ ), abaxial epidermis thickness (BET,  $\mu\text{m}$ ), mesophyll thickness (MEST,  $\mu\text{m}$ ), leaf blade thickness (LBT,  $\mu\text{m}$ ) of 'Marandu' grass (*Urochloa brizantha*) cultivated 2, 4 and 6 m away from the eucalyptus rows<sup>(1)</sup>.

Collection distance of 'Marandu' grass plants	Eucalyptus arrangement (spacing between plants in the row)	
	12x4 m	12x2 m
Adaxial epidermis thickness (DET)		
2 m	9.32*Aa	7.87Ab
4 m	9.66Aa	8.96Aa
6 m	9.89Aa	8.28Ab
Control	9.31A/A	
CV (%)	8.28	
Abaxial epidermis thickness (BET)		
2 m	10.14Aa	8.89Aa
4 m	9.94Aa	9.48Aa
6 m	10.06Aa	9.05Aa
Control	9.60A/A	
CV (%)	9.34	
Mesophyll thickness (MEST)		
2 m	147.06Aa	145.35Aa
4 m	150.29Aa	154.81Aa
6 m	162.55Aa	131.73Ab
Control	155.90 A/A	
CV (%)	16.49	
Leaf blade thickness (LBT)		
2 m	169.71Aa	149.66Bb
4 m	176.00Aa	137.18Bb
6 m	158.96Aa	152.32Bb
Control	176.40A/A	
CV (%)	5.97	

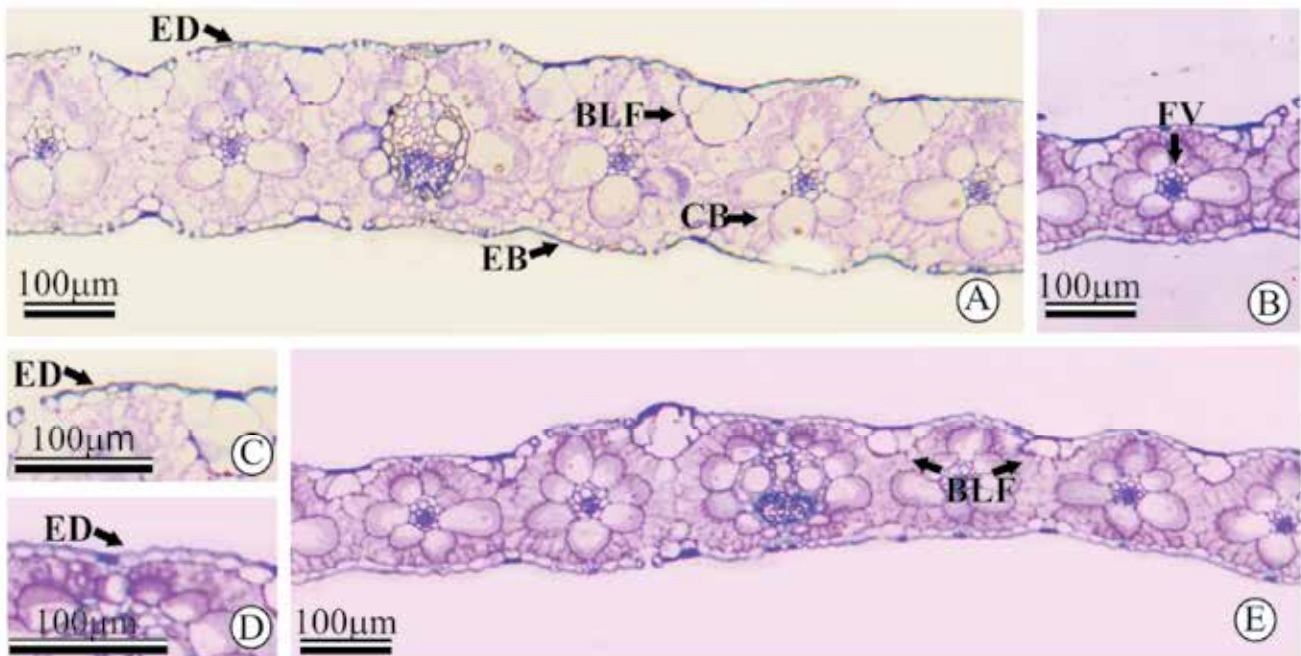
<sup>(1)</sup>Means followed by equal letters, uppercase in the columns and lowercase in the lines, do not differ by Tukey's test, at 5% probability.

were observed between arrangements (Table 2). Jiang et al. (2011) allocated sorghum (*Sorghum bicolor*) under four shade treatments (full sun, fully shaded, with young leaves shaded, and young leaves not shaded). The authors found different results from those found in the present study. Sorghum plants showed mesophyll thickness of adaxial and abaxial sides responses differentially to shading; while adaxial mesophyll thickness decreased about 16 to 23% in comparison to plants grown under full sun, mesophyll thickness in the abaxial side was less than 10% in either treatment (Jiang et al., 2011).

Leaf blade thickness (LBT) of 'Marandu' grass collected at different distances from the eucalyptus row, in the arrangement of 12x2 m, showed a variation in comparison to the control. The plots cultivated in full sun showed average LBT values of 176.40  $\mu\text{m}$ , while the intercropped plants showed lower values. For the plants cultivated in the arrangement of 12x4 m, no difference was observed between treatments. During the passage of radiation through the leaf, it passes through successive layers of cells (Figures 1 A and

E), causing a decline in the intensity (Niinemets & Sack, 2006). Jiang et al. (2011) noted that the shaded sorghum leaves were thinner, which is caused by the reduction in the palisade parenchyma of the leaves and could represent an attempt to increase diffusion of  $\text{CO}_2$  to the mesophyll (Gobbi et al., 2011).

As to the effect of the arrangements within the distances of collection of 'Marandu' grass, it was found that plants grown on plots where eucalyptus was planted in smaller spacing (12x2 m) showed lower values of LBT in the collection distances of 2, 4, and 6 m (Table 2). In general, leaves that grow in shaded environments show a decrease of the mesophyll thickness and increased leaf blade area (Gomes et al., 2008; Gondim et al., 2008), to obtain a larger surface of absorption and optimize capturing photons. The decrease of leaf blade thickness of 'Marandu' grass cultivated in higher levels of shade (12x2 m, 58.2%) may have occurred due to a difference in the distribution of photoassimilates. Under the influence of a certain shading range, plants use a higher proportion of photoassimilates to increase leaf area in an attempt to maximize the uptake of



**Figure 1.** Cross section of leaf blades of 'Marandu' grass (*Urochloa brizantha*), in different arrangements in an agrosilvopastoral system: A, control in full sun; B, arrangement 12x2 m at 2 m; C, arrangement 12x2 m at 6 m; D, arrangement 12x4 m at 6 m; E, arrangement 12x4 m at 2 m. BLF, bulliform cell; FV, vascular bundle; CB, sheath cell; ED, adaxial epidermis; EB, abaxial epidermis.

available light. They usually have thin leaves, higher-specific leaf area (SLA), and leaves with lower-mass density (Gobbi et al., 2011; Bosi et al., 2014).

Anatomical changes that occur in leaves developing under low-radiation conditions have an important role in the adaptation of the plant to the conditions imposed by the environment. Generally, these changes are related to increasing uptake and utilization of incident light, a resource that limits the growth in the shade, increasing the photosynthetic efficiency of the plant (Kirchner et al., 2010; Gobbi et al., 2011).

The percentage of vascular bundle (VB%) did not differ between treatments and the control in the cross section of leaf blades of 'Marandu' grown in the understory of eucalyptus plants cultivated in the plot arrangement of 12x4 m. However, in the arrangement 12x2 m, at the distance of 2 m for collecting the forage, a decrease of 15.21% was observed in VB% in comparison to the plots in full sun, with no significant difference from the other treatments. When comparing the values for the eucalyptus arrangements, within different distances of collection, there was only a difference between the arrangements in the 2 m distance of collection, whose values are 2.42 and 1.84%, respectively, for the arrangements of 12x4 and 12x2 m (Table 3).

Therefore, the shading provided a reduction of the proportion of vascular bundles. Similar results were observed by Gobbi et al. (2011), who described a decrease of the proportion of vascular and support tissues in *Brachiaria*, as well as reduction of thickness of cell wall as a function of shading. Assessing the anatomical responses of two temperate climate grasses for the reduction of light intensity, Wilkinson & Beard (1975) found a lower proportion of vascular and support tissues in *Poa pratensis* plants subjected to shading.

Therefore, the smaller spacing of eucalyptus plants possibly promoted a decrease of fiber content because of a reduction in the proportion of vascular bundles (Figure 1 B). Tissues like the sclerenchyma and the xylem, formed by thickened secondary cell wall, are the ones that contribute to the low quality of forages (Gobbi et al., 2011). These tissues form a solid multicellular block inside the rumen, which results in particles of large size that are poorly digested because of lignification and problems in the accessibility of rumen microorganisms to the surface of the cell wall (Gobbi et al., 2011). Some authors have found an

**Table 3.** Percentages of the vascular bundle, and of sheath cells of the vascular bundle, adaxial epidermis, abaxial epidermis, bulliform cells, and mesophyll cells of leaf blades of 'Marandu' grass (*Urochloa brizantha*) cultivated 2, 4, and 6 m away from the eucalyptus rows<sup>(1)</sup>.

Distance of 'Marandu' grass plants	Eucalyptus arrangement (spacing between plants in the row)	
	12x4 m	12x2 m
Percentage of vascular bundle (VB%)		
2 m	2.42*Aa	1.84Ab
4 m	2.17Aa	2.07Aa
6 m	2.73Aa	2.00Aa
Control	2.17 A/A	
CV (%)	33.36	
Percentage of sheath cells of the vascular bundle (BSC%)		
2 m	4.58Ab	3.37Ba
4 m	4.62Aa	5.25ABa
6 m	5.08Aa	5.92Aa
Control	4.82 A/B	
CV (%)	39.09	
Percentage of adaxial epidermis (ED%)		
2 m	8.37Ab	13.05Aa
4 m	8.88Aa	7.52Ba
6 m	11.35Aa	8.01Ba
Control	7.32 A/B	
CV (%)	38.09	
Percentage of abaxial epidermis (EB%)		
2 m	8.76Ab	14.01Aa
4 m	9.68Aa	8.72Ba
6 m	10.34Aa	8.60Ba
Control	8.40 A/B	
CV (%)	39.28	
Percentage of bulliform cells (BLF%)		
2 m	1.55Aa	2.35Aa
4 m	1.46Aa	1.60Aa
6 m	1.75Aa	1.52Aa
Control	1.81 A/A	
CV (%)	37.07	
Percentage of mesophyll cells (MES%)		
2 m	74.32Aa	57.37Bb
4 m	73.18Aa	74.81Aa
6 m	68.74Aa	74.93Aa
Control	75.45 A/A	
CV (%)	14.58	

<sup>(1)</sup>Means followed by equal letters, uppercase in the columns and lowercase in the lines, do not differ by Tukey's test, at 5% probability.

increased digestibility of forage plants grown in the forest understory by reducing the fiber content (Barro et al., 2008; Lopes et al., 2017).

Regarding the percentage of vascular bundle sheath cells (BSC%), no difference was verified between distances of forage collection in the plots where eucalyptus was grown in the arrangement of 12x4 m (68.3% shading). However, in the densest arrangement of tree species, 'Marandu' grass plants grown at 2 m from the eucalyptus rows showed lower-BSC% values than plants grown at 6 m from the trees and grown in full sun (Table 3). Contrasting with the present study, Gobbi et al. (2011) found a higher portion of BSC in signal grass leaves under intense shade (50%), which might represent the need to increase photosynthetic efficiency under light restriction condition.

The sheath of the vascular bundle, a typical feature of C<sub>4</sub> grasses, plays an important role in the process of those species photosynthesis because their cells contain the Rubisco enzyme, which – through the Calvin cycle – is responsible for refixation of the CO<sub>2</sub> initially fixed by the PEP carboxylase enzyme in the mesophyll cells. This two-step process, which consists of C initial uptake in the mesophyll, and later photosynthetic C reduction in the vascular bundle sheath cells, is important for the inhibition of photorespiration, by concentrating CO<sub>2</sub> at the site where the Rubisco enzyme operates (Majeran & van Wijk, 2009; Jiang et al., 2011).

It is worth noting that these BSC% values were 4.58 in the arrangement of 12x4 m and 3.37 in the arrangement of 12x2 m (differing from each other within the collection distance of 2 m from forage trees), and the value observed for the control was 4.82. Within the collection distances, there were no differences between the arrangements for the other treatments (Table 3). However, Deinum et al. (1996) found no significant variation in the proportion of vascular bundle sheath in *Urochloa brizantha* and *Megathyrsus maximus* leaves subjected to different levels of light intensity.

In the plots with eucalyptus in the arrangement of 12x4 m, 'Marandu' grass showed no differences for the percentage of adaxial epidermis (ED%) between the different cultivation distances of the forage in the trees rows and the control. However, in the arrangement of 12x2 m, the cultivation distance of 2 m caused a higher value of ED% than the other distances and the plants in full sun. As to the effect of arrangements within each

distance of 'Marandu' grass from the trees, it was noted that the denser arrangement of the trees increased the average values of ED% at 2 m of grass cultivation, and that value varied from 8.37% in the arrangement 12x4 m (30.4% shade) to 13.05% in the arrangement of 12x2 (41.3% shade) respectively (Figures 1 C and D).

Similar results to those found for ED% were observed for abaxial epidermis (EB%). In the plots cultivated with 'Marandu' grass, in the 12x4 m arrangement, there was no difference of EB% for the different distances of the grass and the control. In the 12x2m arrangement, the 'Marandu' grass cultivated 2 m away from the eucalyptus rows showed higher-EB% values (14.1%). In the arrangement factor within the different distances of forage cultivation, only the 2 m distance showed a difference between the arrangements, and the values were 8.76% for the 12x4 m arrangement and 14.01% for the 12x2 m one (Table 3). Gobbi et al. (2011) evaluated the anatomical characteristics of *B. decumbens* 'Basilisk' and *Arachis pintoi*, and found no changes for ED% and EB% in plants grown at 50 and 70% levels of shade, in comparison to plants grown in full sun. Their results differ from those of the present study. Murphy et al. (2012) suggest that increasing values of epidermis percentage are proportionally related to leaf sizes, which are larger under intense shading conditions.

Bulliform cell percentages (BLF%) did not change in the different cropping arrangements of tree species and growing distances from 'Marandu' grass in relation to the rows of the forage species (Table 3). This result may indicate that the leaf transpiration area in response to environmental conditions was not affected. The bulliform cells are bulky epidermal cells responsible for the curling of leaves (Alvarez et al., 2008).

When assessing the percentage of mesophyll (MES%) of the leaf blades of 'Marandu' grass plants (Figures 1 A and E), it was found that plants grown in plots where the trees were planted in the arrangement of 12x4 m showed no difference between the evaluated distances and the control. However, in the arrangement of 12x2 m with eucalyptus, 'Marandu' cultivated 2 m away from the eucalyptus rows showed a lower average value of MES% than the other treatments. When evaluating the effect of eucalyptus arrangements within each distance of 'Marandu' grass cultivation, it was found that only the distance of 2 m showed a significant difference between the arrangements, and

the average values for MES% were 74.32 and 57.37% in the 12x4 and 12x2 m arrangements, respectively (Table 3). This result is opposite to those of Gobbi et al. (2011), who found no changes for MES% in *B. decumbens* 'Basilisk' and *Arachis pintoi* under shade and in full sun. The lower values of MES% could be related to the lesser stress caused by high-light and heat under the tree canopy.

### Conclusions

1. The shade imposed by the trees do not impact adaxial and abaxial epidermis thickness, mesophyll thickness, and percentage of bulliform cells in 'Marandu' grass [*(Urochloa brizantha* (syn. *Brachiaria brizantha*)].

2. Moderate shade provides the leaf blade thinness, reduces the proportion of mesophyll cells and the proportion of vascular bundles, and increases the percentage of epidermal tissues (abaxial and adaxial) in 'Marandu' grass.

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