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BIOLOGY

Effects of storage on vis-NIR-SWIR reflectance spectra of Mombasa grass leaf samples

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ABSTRACT: *Vis-NIR-SWIR reflectance spectra of leaf samples, collected in the laboratory, allow the calibration of predictive models to quantify their physicochemical attributes in a practical manner and without producing chemical residues. This technique should enable the development of management strategies for intensification of pasture use. However, spectral analysis performed in the laboratory may be affected by the deterioration of plant material during transport from the field to the lab, so storage methods are necessary. This research aimed to evaluate the effects of different storage methods on the spectral response of Mombasa grass leaves. Three methods were evaluated: (i) artificially refrigerated environment, (ii) humid environment, and (iii) without microenvironment control. These methods were tested in five different storage times: 2 hours, 4 hours, 8 hours, 24 hours and 48 hours. The spectral behavior of the leaves still inserted in the plant was used as a quality reference. Results showed notable changes at the earliest storage time for the treatment without microenvironment control. Both methods with microenvironment control stabilized the occurrence of spectral changes over 48 hours of the samples storage, thus both were suggested for this species.*
Key words: *diffuse reflectance spectroscopy, hyperspectral sensors, forage, precision agriculture.*

Efeitos do armazenamento no espectro de reflectância vis-NIR-SWIR de amostras foliares de capim Mombaça

RESUMO: *Espectros de reflectância vis-NIR-SWIR de amostras foliares, coletados em laboratório, permitem a calibração de modelos preditivos para quantificação de seus atributos físico-químicos de maneira prática e sem produção de resíduos químicos. Esta técnica permite o desenvolvimento de estratégias de manejo para a intensificação do uso de pastagens. Contudo, análises espectrais realizadas em laboratório podem ser afetadas pela deterioração do material vegetal durante o transporte do campo ao laboratório, fazendo-se necessário a utilização de métodos de armazenamento. O presente trabalho objetivou avaliar o efeito de diferentes métodos de armazenamento na resposta espectral de folhas de capim Mombaça. Avaliou-se três métodos: (i) ambiente refrigerado artificialmente; (ii) ambiente úmido; e (iii) ao ar livre, sem controle do microambiente; assim como, cinco diferentes tempos de armazenamento: 2 horas, 4 horas, 8 horas, 24 horas e 48 horas. O comportamento espectral das folhas ainda inseridas na planta foi utilizado como referência de qualidade. Os resultados mostraram alterações pronunciadas para o armazenamento ao ar livre já nos primeiros intervalos de tempo. Ambos métodos com controle de microambiente permitiram estabilizar a ocorrência de alterações espectrais ao longo das 48h de armazenamento das amostras, sendo ambos sugeridos para esta espécie.*
Palavras-chave: *espectroscopia de reflectância difusa, sensores hiperespectrais, forragem, agricultura de precisão.*

INTRODUCTION

The Brazilian territory is occupied by 160 million hectares of pasture (PARENTE & FERREIRA, 2018), which is mostly used in extensive livestock. This production system is characterized by low land-use efficiency with low rates of stocking density (RESTLE et al., 1998), which is estimated by 1.32 animals per hectare (IBGE, 2017; ABIEC, 2018). The higher the animal stocking rate, the more efficient the production system is. This intensification is made possible mainly by the use of adequate forage plants with a high production capacity of dry matter and nutritional quality (DIAS-FILHO, 2014). The Panicum maximum cv. Mombasa is one of the most used forages in livestock production systems in Brazil

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(FREITAS et al., 2012; CARVALHO et al., 2017), which is characterized by high potential productivity and quality (DE ALENCAR et al., 2010). In addition, Precision Agriculture (PA) approaches to site-specific grazing had been suggested for optimized pasture management (SCHELLBERG et al., 2008).

In the PA context, remote sensing techniques are applied as an alternative to increasing the density of spatial information (MOLIN et al., 2015) allowing more detailed mapping and thus avoiding extra costs with sample analyses in traditional laboratories. Predicting physicochemical attributes of forages by diffuse reflectance sensors was proposed as an alternative for mapping its agronomic attributes in a practical way (ZHAO et al., 2007; PULLANAGARI et al., 2011, 2012). In this context, most researches that focused on vegetation assessment via diffuse reflectance spectroscopy (DRS) used spectral information in the visible region (vis: 350 a 700 nm), near-infrared (NIR: 700 a 1000 nm) and short-wave infrared (SWIR: 1000 a 2500 nm) (THENKABAIL et al., 2000).

Using DRS to analyze leaves in laboratory conditions allows acquisition of spectra without canopy effects, presenting better characterization of chemical constituents, such as protein concentration, cellulose, lignin, starch, chlorophyll and others (ASNER et al., 1998; NETO et al., 2017). Recent advances in spectrometers of DRS vis-NIR-SWIR expanded the use of portable instruments with reduced weight and size (GAŁUSZKA et al., 2015). These advances are popularizing the DRS as a complementary method to the traditional laboratory analyses; some studies already mention its use associated with traditional methods, suggesting the concept of hybrid laboratory (DEMATTÊ et al., 2019).

In situ spectral measurements of leaves are not always viable due to the difficulty of controlling illumination conditions and lack of access with portable spectrometers (FOLEY et al., 2006). Therefore, samples are collected in the field, stored and transported to a controlled environment (spectral analysis laboratory) where measurements can be performed. The main limitation of this approach is the possibility of leaf spectral response alteration. These alterations can be caused by loss of water content, degradation of leaf structures, and biochemical alterations (HUNT & ROCK, 1989; WONG & GAMON et al., 2015). Therefore, it is important to adopt storage methods that preserve characteristics of the plant material (LEE et al., 2014a), under the risk of compromising spectral measurements in case that this procedure is overlooked. One of the main objectives of storage methods is to maintain the water content of leaves (FOLEY et al., 2006). Thus, it is recommended to use methods that maintain the microenvironment moist and thus reducing leaf dehydration (SOUSA et al., 1996), or methods that artificially reduce the temperature of the microenvironment reducing leaf transpiration and metabolism (DAUGHTRY & BIEHL, 1985; SIMS & GAMON, 2003; LEE et al. 2014b).

Researches that analyzed the spectral response of leaves along time (SANCHES et al. 2003, SCHUH et al. 2016) and in different storage procedures (DAUGHTRY & BIEHL 1985, SOUSA et al. 1996, FOLEY et al. 2006, LEE et al. 2014b) presented distinct results based on plant species and adopted methodology. These researches indicated the need to conduct new experiments for different plant species. It should also be noted that, so far, no studies have been developed for Panicum maximum cv. Mombasa leaves; thus, it is necessary to extrapolate information generated for other species, which may not be compatible. Besides that, the adoption of PA techniques in Brazil has recently increased (MOLIN, 2017), and is expected to expand to pasture management (BOGREKCI & LEE, 2005; PULLANAGARI et al., 2012; ZHAO et al., 2007).

Given the potential for application of sensing techniques in tropical forages and the lack of consensus over a standard method for storing leaf samples for spectral measurements, this research aimed to (i) define the most suited methodology to minimize time effects when storing Mombasa leaf samples, and (ii) estimate the maximum storage time in which the spectral response of the collected sample will not alter significantly.

MATERIALS AND METHODS

The fieldwork started with selecting 15 leaves of Panicum maximum (cv. Mombasa) in three experimental plots with homogeneous conditions of soil, fertilizer, management, and irrigation. The sampling was made by extracting the medium third of the leaf "+1", which is the first leaf presenting a visible intersection between the leaf blade and the leaf sheath. The leaf spectra were measured before extracting the leaves showing no symptoms of stress. This first spectral measurement (T0) was used as a reference for comparison with other measurements obtained along the storage procedures. The field experiments were performed between 8 and 9 AM with air temperature and humidity of 23 ºC and 82.9 %, respectively.

Shortly after the first spectral measurement, the leaves were detached and submitted to three different storage methods; the samples of the same

experimental plot were assigned to a given storage method. The storage methods were: (i) storage in translucent plastic bags kept open and stored in a thermal box with ice, keeping the leaves out of direct contact with ice (G) (LEE et al., 2014a); (ii) storage in sealed translucent plastic bags with moist cotton fragments and stored in a thermal box (A) (SOUSA et al., 1996); and (iii) samples kept in open-air condition throughout the experiment without any microenvironmental control (AL). In the laboratory, the spectra of each sample were measured after five different storage periods using the moment of leaf abscission as a reference. The storage periods were: 2 hours (T2), 4 hours (T4), 8 hours (T8), 24 hours (T24) e 48 hours (T48). The T2 interval was defined as the minimum time required for sample collection and transport to the laboratory for spectral analysis.

Spectral measurements

The measurements were taken using the spectroradiometer FieldSpec 3® (ASD - Analytical Spectral Devices, Boulder, EUA) (Figure 1A) coupled with the peripheral device LeafClip (Figure 1B). The LeafClip is a contact probe used specifically for vegetation analysis, using a halogen lamp as a source of electromagnetic radiation and enabling spectral analysis in field and laboratory environments. The FieldSpec 3 is a hyperspectral sensor that measures radiance in the vis-NIR-SWIR regions. It presents a spectral resolution of 3 nm in the region from 350 to 1000 nm and of 10 nm between 1000 and 2500 nm.

Sensor calibration was performed at the beginning of each data collection and repeated every 30 leaves. For this procedure, the reflectance standard Spectralon® (Labsphere Inc, North Sutton, EUA) was used as a reference; it is coupled to LeafClip, providing a diffuse reflectance close to 100% for the analyzed spectral region. The reflectance curve of each sample was obtained by averaging 30 measurements taken automatically by the sensor.

Data analysis

The reflectance curves were qualitatively evaluated by descriptive analysis following a methodology adapted from DEMATTÊ et al. (2014). The descriptive analysis evaluates the spectra at its intensity, shape and absorption features. Principal component analysis (PCA) was also applied to reduce data dimensionality, allowing to evaluate the treatment effects more clearly (VISCARRA ROSSEL & BEHRENS, 2010; DEMATTÊ et al., 2016). This technique helps to reduce the high collinearity present in the spectral data, which provides an excessive amount of redundant information (VISCARRA ROSSEL & BEHRENS, 2010).

In spectral data, the PCA allows identifying possible groupings or classes of samples according to their spectral similarity. This is evaluated by the proximity between scores of the generated components (DEMATTÊ et al., 2016). Analyses were applied separately for each spectral range (vis, NIR, and SWIR) and for the entire spectrum using

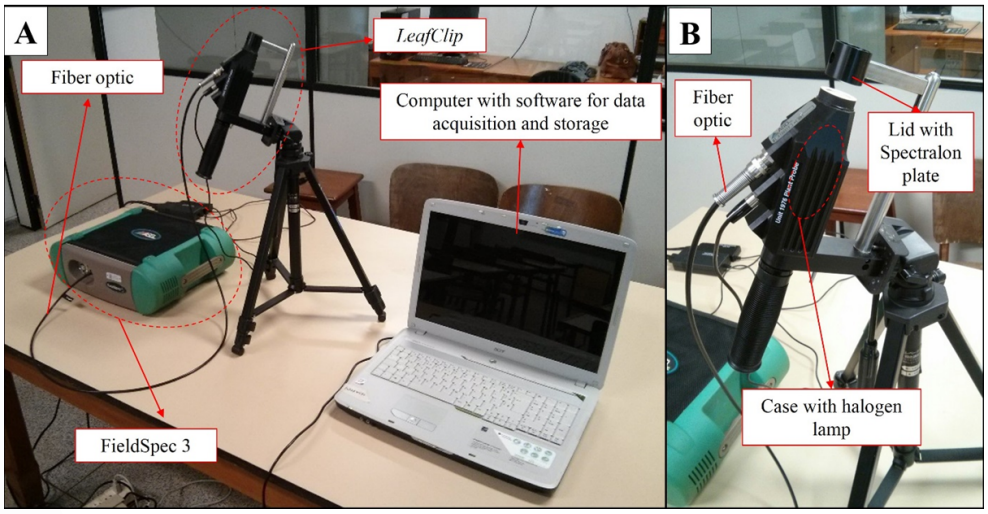

Figure 1 - (A) Set of equipment used: FieldSpec 3, computer and LeafClip; (B) LeafClip in detail.

Unscrambler 9.7 software (CAMO AS, Trondheim, Norway). Data from 350 to 419 nm were excluded from analysis due to the presence of noise.

The storage methods were evaluated by observing the reflectance deviation in relation to the T0 spectrum. The deviation was calculated to compensate for the effect of the experimental plots on the storage method. This was essential since even with the rigorous selection of homogeneous plots, as well as the calibration and spectral data collection procedures, there still was a significant difference between the T0 spectra of the different plots (Table 1).

After this compensation, the effect of the treatments was evaluated in seven different spectral bands (SOUSA et al., 1996). These spectral bands were centered at the main spectral features of vegetation (CURRAN, 1989). Three bands were positioned in the visible region (centered at 480, 550 and 670 nm), related to the absorption of electromagnetic radiation by leaf pigments: two in the NIR region (centered at 740 and 910 nm), related to cell structure; and two in the SWIR region (centered at 1450 and 1940 nm), related to absorption due to leaf water content (FOLEY, et al. 2006; JENSEN, 2009). We used a range from ± 10 nm in relation to the central wavelength. Variance analysis, Tukey test and regression analysis (linear and quadratic models) were applied to the data; p-value less than 5% was considered significant.

RESULTS AND DISCUSSION

The descriptive analysis of the spectra showed that treatments G and A (Figure 2A and B, respectively) presented less dispersion of the reflectance values over time compared to those of the AL treatment (Figure 3C). The dispersion observed in the AL treatment is evident for the NIR (mainly after 740 nm) and SWIR region, which showed an increasing albedo as the storage time rises. All spectra of the G and A treatments, as well as the visible region (up to 700 nm) of the AL treatment, showed greater spectral stability over time. Only slight variations can be observed in these spectra; however, they are presented in a random way, without presenting any relation with the storage time.

The spectral behavior of the AL treatment (Figure 3C), characterized by progressive increases in reflectance intensity as a function of the storage period, can be attributed to leaf water loss during sample storage (HUNT & ROCK, 1989; FOLEY et al., 2006). The water has the property to absorb the radiation. The radiation absorption caused by the leaf water content increases as the wavelength increases, being smoother in the visible region, and intensifying in the NIR and the SWIR regions (WOOLLEY, 1971; CARTER, 1991).

In addition to the effect on the albedo, the spectra obtained after 24 and 48 hours in the open-air storage condition (T24 and T48 of Figure 2C) were distinguished from the others by the presence of new spectral features. This more complex shape happens due to the attenuation of water absorption bands, located near 1190, 1450 and 2300 nm (SIMS & GAMON, 2003; FOLEY et al., 2006), which masked the features related to the protein, nitrogen and cellulose content, near 2020, 2220 and 2395 nm (CURRAN, 1989). In these same spectra, as illustrated by the red arrows in Figure 3C, it was observed that the angle formed between the inflection range (740 nm) and the beginning of the near-infrared (910 nm) increase. The spectral range from 740 to 800 nm is a region related to the emission of fluorescence by photosynthesizing agents for dissipation the excess of absorbed energy (GAMON & SURFUS, 1999). Changes in fluorescence characteristics over time are related to changes in the leaf photochemical activity (FOLEY, et al. 2006). According to GHOBADI et al. (2013) plants under water stress are also affected by secondary damage caused by oxidative stresses, which is harmful to lipids, proteins, and nucleic acids, leading to harmful effects such as chlorophyll degradation. The interpretation of vis-NIR-SWIR spectra is dynamic

Table 1 - Tukey test for the reflectance of the spectral bands obtained at T0, according to the storage method.

Storage method	----------Central wavelength of the spectral range (nm)----------						
	480	550	670	740	910	1450	1940
G	4.85 b	13.51 b	4.74 b	40.78 b	44.19 b	10.05 a	2.47 a
A	6.03 a	14.73 ab	5.69 a	42.25 b	45.61 b	10.28 a	2.94 ab
AL	6.70 a	15.84 a	6.38 a	43.8 a	47.37 a	11.31 a	3.95 a

Means followed by equal letters (vertical) do not differ statistically from each other by the Tukey test at 5%.

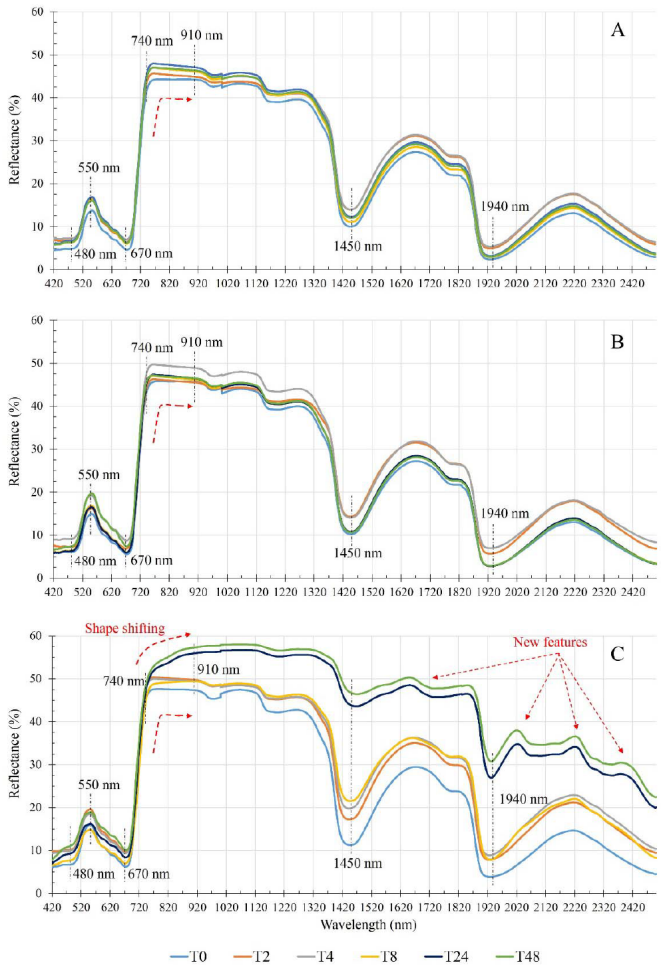

Figure 2 - Spectral curve of the leaf sample still inserted in the plant (T0) and after different storage period (T2, T4, T8, T24 and T48); this behavior was presented for the three storage methods evaluated: using ice (A), using wet cotton (B) and open-air, without any microenvironment control (C).

since their features are characterized by being broad, subtle and with some overlaps of absorptions (e.g., water feature attenuating and masking features of proteins, cellulose, among others) (WOOLLEY, 1971). Besides that, the leaf degradation, which generally alters the leaf constituents, is easily observed by qualitative evaluations of the spectrum.

The comparative results between the storage methods of the seven selected spectral bands are presented in Table 2. The visible ranges (480, 550 and 670 nm) and the first NIR range (740 nm), which are less influenced by the water content, showed a greater similarity between the three methods for all different storage periods. In general, only observing the range of 910 nm (NIR) and the SWIR ranges (1450 and 1940 nm), it is possible to verify a distinction of the AL treatment in relation to the others, with significant deviations of reflectance, mainly after 24 hours of storage. However, among the G and A treatments, it is not possible to observe a clear statistical behavior that differentiates the performance of both, which, in turn, would allow defining the best storage method. Corroborating with this analysis, neither of these two methods presented regression analyses with significant behavior, showing the absence of the time effect on the reflectance.

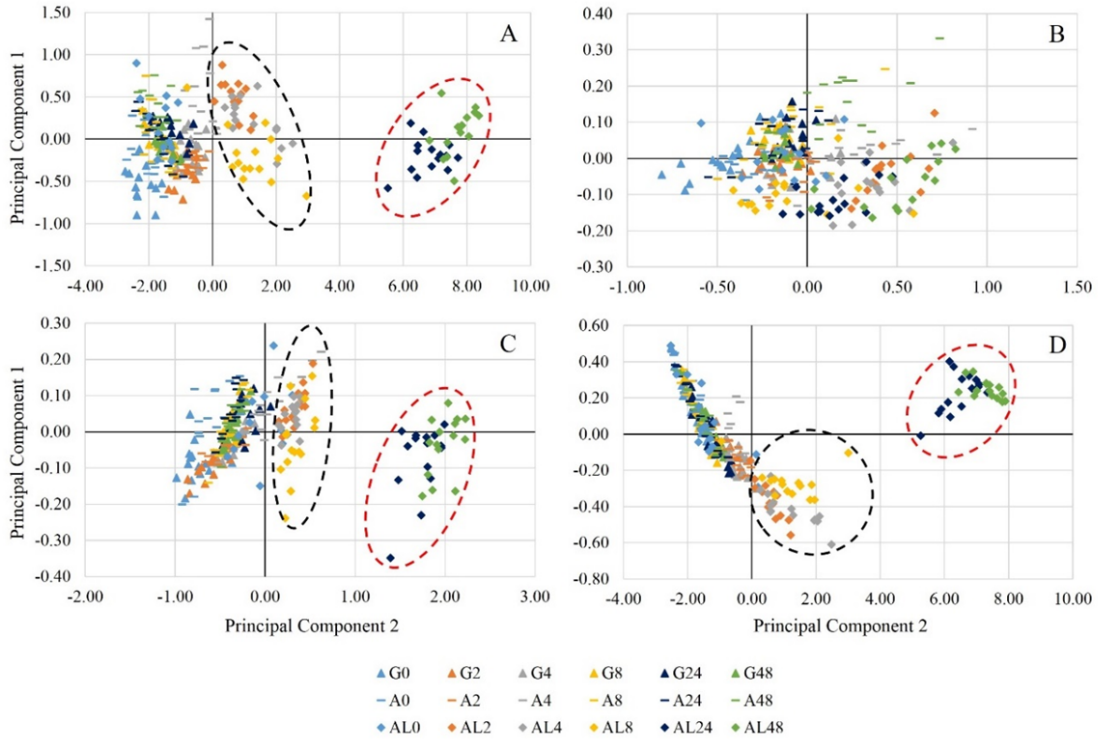

Figure 3 - Scatter plots of the PC 1 and 2 scores for each treatment. Scatter plots using the entire spectrum from 420 to 2500 nm (A) and fragmenting the spectrum in the visible region from 420 to 700 nm (B), in the NIR region from 700 to 1000 nm and in the SWIR region from 1000 to 2500 nm (D). The ellipses showed groupings of similar spectra.

Conversely, the regression analyses were significant for the AL method in the bands centered at 910, 1450 and 1940 nm, wavelengths that clearly showed gain of reflectance over the storage periods.

Concerning the principal components analysis, PC1 and 2 were responsible for explaining more than 90% of the total data variance. The scatter plots of the scores of these two components allowed grouping similar treatments based on their spectral similarity (Figure 3 A, B, C, and D); and this analysis confirms what was observed in the individual evaluation of the bands. The visible region did not show clusters between the different treatments (Figure 3B), indicating the low spectral variability in this region. Conversely, when evaluating the scores generated for the entire spectrum and for the NIR and SWIR regions (Figure 3A, C and D, respectively), the separation of the AL treatment from T2 is clear, with an emphasis on T24 and T48 (indicated by red ellipses - Figure 3A, B and C). This behavior highlights the greater sensitivity of the NIR and SWIR regions for the treatments applied, and a reduced sensitivity of the visible region. This also highlights the spectral degradation of the AL treatment, as well as the spectral similarity of the G and A treatments for all storage periods.

Regarding the maximum storage time, the Tukey test between the reflectance of T0 and T2 (Table 3) shows that changes in reflectance values were already observed on T2, the first spectral measurement after in situ data collection. This result showed that spectral changes in Mombasa grass leaf samples occurred within the first two hours of storage.

Although, a significant change in reflectance values was observed in the first two hours of storage, treatments G and A did not present drastic changes, as occurred in AL. For the samples stored using microenvironment control (G and A), the greatest variations occurred in the first 4 hours of storage, with a tendency to stabilize the reflectance after 8 hours of storage (Table 2).

The maximum storage period varies between methods and species. LEE et al. (2014b) observed that cotton and soybean leaves stored using ice had their integrity prolonged; however, at 30 minutes

Table 2 - Average of the reflectance deviation from T0 for the different methods and times of storage; Tukey test between methods; and regression analysis for the reflectance deviation as a function of storage period.

Storage method	Storage periods					Linear reg.	Quad. reg.
	T2	T4	T8	T24	T48		
480 nm							
G	1.89 b	2.42 a	1.48 a	1.82 a	1.57 b	ns	ns
A	1.24 b	2.90 a	0.39 a	0.23 b	1.32 b	ns	ns
AL	3.47 a	3.28 a	0.98 a	2.56 a	4.32 a	ns	ns
550 nm							
G	2.43 ab	3.17 a	2.91 a	3.16 a	2.33 a	ns	ns
A	1.26 b	4.29 a	1.92 a	1.57 ab	4.74 a	ns	ns
AL	3.57 a	2.55 a	-1.11 a	0.22 b	2.97 a	ns	ns
670 nm							
G	1.75 b	2.37 a	1.45 a	1.70 a	1.61 b	ns	ns
A	1.26 b	3.15 a	0.49 a	0.38 b	1.88 b	ns	ns
AL	3.42 a	3.13 a	0.81 a	2.20 a	3.94 a	ns	ns
740 nm							
G	1.99 ab	3.25 ab	3.27 a	3.88 a	2.87 a	ns	ns
A	0.99 b	4.49 a	1.93 ab	1.99 a	2.62 a	ns	ns
AL	3.10 a	2.23 b	0.62 b	1.96 a	3.47 a	ns	ns
910 nm							
G	0.64 b	1.89 a	1.87 ab	2.81 b	2.12 b	ns	ns
A	- 0.14 b	3.22 a	0.50 b	0.80 c	0.84 b	ns	ns
AL	2.40 a	2.12 a	2.06 a	8.59 a	9.93 a	<0.01	<0.01
1450 nm							
G	3.96 b	4.00 b	1.07 b	2.25 b	1.98 b	ns	ns
A	4.07 b	3.82 b	0.48 b	0.59 b	0.44 b	ns	ns
AL	6.00 a	8.60 a	10.30 a	32.57 a	35.46 a	<0.01	<0.01
1940 nm							
G	2.56 b	2.96 b	0.49 b	0.79 b	0.59 b	ns	ns
A	2.81 b	4.10 ab	-0.06 b	-0.09 b	0.00 b	ns	ns
AL	4.10 a	5.15 a	4.17 a	23.39 a	27.20 a	<0.01	<0.01

Means followed by equal letters (vertical) do not differ statistically from each other by the Tukey test at 5%.

after their cutting there was a slight mischaracterization of the spectral response. Analyzing birch leaves, DAUGHTRY & BIEHL (1985) concluded that such leaves could be stored in a cold environment for several days without compromising the spectrum since they presented variations of less than 5% in relation to their initial state. In turn, SOUSA et al. (1996), analyzing eucalyptus leaves, observed the first significant changes in their spectral response, stored together with wet cotton, occurred after 6 hours.

It is believed that the maximum storage time for leaf samples of Mombasa grass should be within the first two hours after leaf abscission. However, collecting, storing, transporting and analyzing within this interval of time is, in practice, not feasible. Thus, approaches that involve the calibration of predictive models in green leaves of Mombasa grass, with spectral acquisition performed in the laboratory, should take into account this storage effect. To reduce the random error due to the transport of foliar samples from the field to the laboratory, we suggest standardizing the method and the period of storage. Although, both methods with microenvironment control (G and A) did not allow preserving the spectral characteristics of the leaf still inserted in the plant, they stabilized these changes over 48 hours of storage, for all spectral regions.

CONCLUSION

In order to transport leaf samples of Panicum maximum (cv. Mombasa) for spectral

Table 3 - Reflectance deviation from that obtained at T0, according to time and storage method.

----------G----------							
Storage periods	----------Central wavelength of the spectral range (nm)----------						
	480	550	670	740	910	1450	1940
0	4.85 b	13.51 b	4.73 b	40.78 b	44.19 a	10.05 b	2.47 b
T2	6.73 a	15.94 a	6.49 a	42.77 a	44.83 a	14.01 a	5.03 a
----------A----------							
Storage periods	----------Central wavelength of the spectral range (nm)----------						
	480	550	670	740	910	1450	1940
0	6.03 b	14.73 b	5.69 b	42.25 b	45.61 a	10.28 b	2.94 b
T2	7.27 a	15.96 a	6.95 a	43.23 a	45.47 a	14.36 a	5.75 a
----------AL----------							
Storage periods	----------Central wavelength of the spectral range (nm)----------						
	480	550	670	740	910	1450	1940
0	6.70 b	15.85 b	6.38 b	43.84 b	47.37 b	11.31 b	3.95 b
T2	10.17 a	19.42 a	9.79 a	46.94 a	49.76 a	17.31 a	8.05 a

Means followed by equal letters (vertical) do not differ statistically from each other by the Tukey test at 5%.

analysis, two methods with microenvironment control were tested andreported equally efficient and superior to open-air storage, allowing to stabilize reflectance values up to 48 hours.

In all storage methods tested, we observed slight spectral changes immediately after the first interval of time evaluated (2 hours after leaf abscission); this did not allow determining the maximum storage time for leaf samples in which the Mombasa grass would maintain its original spectral characteristics.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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