PHOTOSYNTHETIC LIGHT RESPONSE OF THE C_4 GRASSES *Brachiaria brizantha* AND *B. humidicola* UNDER SHADE

Moacyr Bernardino Dias-Filho*

Embrapa Amazônia Oriental, C.P. 48 - CEP: 66017-970 - Belém, PA. - CNPq Fellow - <moacyr@cpatu.embrapa.br>

ABSTRACT: Forage grasses in tropical pastures can be subjected to considerable diurnal and seasonal reductions in available light. To evaluate the physiological behavior of the tropical forage grasses *Brachiaria brizantha* cv. Marandu and *B. humidicola* to low light, the photosynthetic light response and chlorophyll contents of these species were compared for plants grown outdoors, on natural soil, in pots, in full sunlight and those shaded to 30 % of full sunlight, over a 30-day period. Both species showed the ability to adjust their photosynthetic behavior in response to shade. Photosynthetic capacity and light compensation point were lower for shade plants of both species, while apparent quantum yield was unaffected by the light regime. Dark respiration and chlorophyll a:b ratio were significantly reduced by shading only in *B. humidicola*. *B. humidicola* could be relatively more adapted to succeed, at least temporarily, in light-limited environments.
Key words: carbon assimilation, chlorophyll content, light compensation point, pasture

RESPOSTAS FOTOSSINTÉTICAS DAS GRAMÍNEAS C_4 *Brachiaria brizantha* E *B. humidicola* SOB SOMBREAMENTO

RESUMO: Gramíneas forrageiras em pastagens tropicais podem sofrer reduções consideráveis na disponibilidade diária e anual de luz. Com o objetivo de avaliar a comportamento fisiológico das gramíneas forrageiras tropicais *Brachiaria brizantha* cv. Marandu e *B. humidicola* ao sombreamento, as respostas fotossintéticas e os teores de clorofila dessas espécies foram comparados em plantas cultivadas em solo natural, em vasos, a pleno sol e a 70 % de interceptação da luz solar, durante um período de 30 dias. Ambas as espécies mostraram-se capazes de ajustar o comportamento fotossintético ao sombreamento. A capacidade fotossintética e o ponto de compensação de luz foram menores nas plantas sombreadas de ambas as espécies, enquanto que a eficiência quântica aparente não foi significativamente afetada pelo regime de luz. A respiração no escuro e a razão clorofila a:b foram significativamente reduzidas pelo sombreamento somente em *B. humidicola*. *B. humidicola* poderia ser considerada relativamente mais adaptada à ambientes sujeitos a redução temporária na disponibilidade de luz.
Palavras-chave: assimilação de carbono, conteúdo de clorofila, ponto de compensação de luz, pastagem

INTRODUCTION

Although light is normally not considered an important limiting resource in tropical pastures, forage grasses in this environment can be subjected to considerable reductions in available light (Humphreys, 1991). This condition is usually a consequence of the intentional introduction of tree species, or the proliferation of woody weeds in active pasture areas, the establishment of pastures in plantations, or, even the diurnal and seasonal reduction in the amount of sunshine due to cloud cover. In Brazil, shading of pasture areas by plantation agriculture has the potential to become more common, since agroforestry systems are being recommended as alternatives to reclaim the productivity of degraded pasture areas (Carvalho, 1998; Dias-Filho, 1998). On the other hand, the reduction in the amount of irradiance reaching a pasture canopy can also be brought about by climatic conditions. For example, in the north of Brazil (1°28'S), the daily mean sunshine duration, during a three-year period, was found to be reduced by 60%, in the wet season (Dec to Apr), relative to the dry season (May to Nov) (Dias-Filho, 2000).

Brachiaria brizantha (Hochst. ex A. Rich.) Stapf cv. Marandu and *B. humidicola* (Rendle) Schweick are considered important forage grass species throughout tropical America and, particularly, in Brazil (e.g., Argel & Keller-Grein, 1996). These species have been classified as having a medium tolerance to shade (Humphreys, 1991; Shelton et al., 1987). However, there is no comparative study examining their photosynthetic responses to light-limited environments.

According to their phenotypic plasticity, plants are able to change their biochemical, physiological and morphological characteristics in response to environmental variation (Schlichting, 1986). The nature of this response usually determines a species' ability to succeed or not under temporary or permanent environmental stress. Studying the phenotypic plasticity of tropical forage grasses to shading will add to the understanding of grass potential and management, in agroforestry systems, and in regions with extended cloudy weather. The objective of this study was to evaluate the photosynthetic behavior of *B. brizantha* and *B. humidicola* to reduced light environment.

MATERIAL AND METHODS

Plant material and growing conditions

Seeds of *Brachiaria brizantha*, and *B. humidicola* were germinated on sand and then planted individually in pots with 3.5 kg (dry mass) of soil (2:1; natural soil to sand). Pots were fertilized with a water soluble fertilizer solution of 48 mg kg^{-1} of P (K_2HPO_4), 50 mg kg^{-1} of N, 25 mg kg^{-1} of K, 10 mg kg^{-1} of S, 2 mg kg^{-1} of Zn, 1 mg kg^{-1} of Cu, 0.5 mg kg^{-1} of B and 0.2 mg kg^{-1} of Mo. All plants were grown outdoors, under a black polypropylene shade fabric. Light extinction by the shade fabric measured with a Li-Cor quantum sensor (Li-Cor, Inc., Lincoln, NE, USA) on a clear day was 70%. Ten days after planting, half of the pots were moved out of the shade net (full sun) and the other half stayed under the permanent shaded conditions. The light extinction value in the shade treatment is similar to that encountered under the canopy of most tree species in pastures in the region. Plants received ample water and were fertilized once a week with 50 mL of a water soluble fertilizer solution per pot (15:30:15; N, P_2O_5, K_2O; 3.5 g L^{-1}).

Leaf Gas exchange and chlorophyll content

Gas exchange parameters were measured 31 days after the beginning of the experiment. A photosynthesis (A) versus irradiance (PFD) response curve, was measured outdoors, on a sunny day, between 9.00 and 11.00 h local time, on the most recent, fully expanded intact leaf of four plants per treatment using a portable photosynthesis system (Model LI-6200, LI-COR, Inc., Lincoln, NE, USA). Gas exchange parameters were calculated on a leaf area basis. The light levels were obtained by intercepting the solar radiation with neutral-density filters. After exposure to the lowest PFD, the plants were left for 15 min in the dark and measurements were made to obtain dark respiration values. Four replicate plants per treatment and one leaf per plant were sampled.

At each harvest period, leaf blades were processed for chlorophyll content following the method described by Arnon (1949).

Photosynthetic light response curves and statistical analysis

The shape of the photosynthetic light response curve in each light regime was modeled by fitting data to a nonrectangular hyperbola (Lambers et al., 1998) by means of the nonlinear least squares curve-fitting procedure of STATISTICA 5.5 for Windows (StatSoft, Inc., Tulsa, USA). This model may be expressed as follows:

$$A = \frac{\alpha * Q + A_{\max} - \sqrt{(\alpha * Q + A_{\max})^2 - 4 * \alpha * Q * k * A_{\max}}}{2k} - R_d$$

where α is the quantum yield of photosynthesis, Q is the photosynthetic active radiation (PFD), A_{max} is the light-saturated rate of gross CO_2 assimilation, k is the curvature factor, R_d is leaf-level "dark" respiration, and A is the photosynthetic rate. Because in the present study the *in situ* photosynthetic responses were of interest, Q is the incident radiation rather than absorbed radiation. Also, quantum yield is the "apparent" quantum yield, rather than the maximum "physiological" quantum yield expressed on the basis of absorbed light.

Differences in light response curve parameters due to light regime were examined by calculating apparent quantum yield (α), and light compensation point. When appropriate, the calculated values were compared between treatments by two-sample *t* test, using the Dunn-Sidak adjustment to the probabilities procedure of SYSTAT 7.0.1 for Windows (SPSS Inc., Chicago, USA). Photosynthesis versus PFD response data provided direct estimates of the highest measured value of photosynthetic rate (A_{max}). Values of a and light compensation point were calculated according to Dias Filho & Dawson (1995): the slope of photosynthesis versus incident irradiance between 50 and 200 µmol m^{-2} s^{-1}. The light compensation point was calculated as the ratio, R_d/α.

Differences in total leaf chlorophyll content and chlorophyll a:b ratio, within species and between treatments were assessed by two-sample *t* test, using the Dunn-Sidak adjustment to the probabilities.

RESULTS AND DISCUSSION

The shape of the light response curves of photosynthesis (Figure 1) shows that, relative to plants grown in the sun, shaded plants of both species tended to display higher assimilation rates under lower light levels and lower rates under higher light levels. Similar responses have been described for the C_4 grass *Panicum maximum* (Dias-Filho, 1995), and for the tropical weeds *Ipomoea asarifolia* (Dias-Filho, 1999) and *Rolandra fruticosa* (Dias-Filho & Chagas Júnior, 2000).

Apparent quantum yield (α) was unaffected by the light regime on both species but tended to be higher on shaded plants (Table 1). Many studies have shown that α is normally not affected by light regime during growth (e.g., Avalos & Mulkey, 1999; Dias-Filho, 1997; Groninger et al., 1996; Horton & Neufeld, 1998). Light saturated photosynthesis rate (A_{max}) tended to be higher in sun plants of both species (Figure 1, Table 1). It could be speculated that the difference in A_{max} between sun and shade plants was mainly a result of leaf-level morphological acclimation to the light environment (i.e., reduction in the amount of photosynthetic tissue per unit of leaf area in shade plants), observed in this study and previously reported by Dias-Filho (2000).

The rate of dark respiration (R_d) covaried with A_{max}. In *B. humidicola*, R_d was 43% lower ($P < 0.01$) in

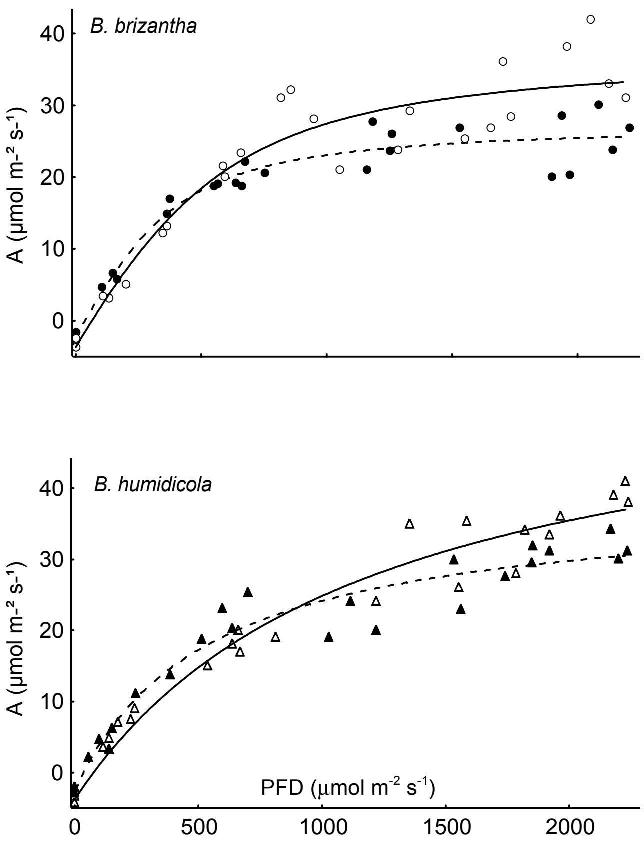

Figure 1 - Light (PFD) response curves of photosynthesis (A) of *B. brizantha* and *B. humidicola* grown under full sun- (open symbols and solid lines) or shade (closed symbols and dashed lines).

Table 1 - Photosynthetic parameters of *Brachiaria brizantha* and *B. humidicola* under sun and shade regimes. Symbols and units: A_{max} is light saturated photosynthetic rate; α is apparent quantum yield; R_d is dark respiration, R_d/α is light compensation point. Values are means (± s.e.), n= 4.

Parameter	Sun	Shade	*P* value[1]
Brachiaria brizantha			
A_{max} (μmol m^{-2} s^{-1})	42.0	30.0	-
α (mol CO_2 mol photon^{-1})	0.044 (0.002)	0.052(0.003)	0.15
R_d (μmol m^{-2} s^{-1})	3.15 (0.45)	2.37 (0.32)	0.34
$R_{d/\alpha}$ (μmol m^{-2} s^{-1})	71.28 (2.08)	45.78 (2.25)	<0.001
Brachiaria humidicola			
A_{max}	41.0	34.4	-
α	0.055 (0.004)	0.067 (0.007)	0.53
R_d	3.78 (0.26)	2.51 (0.23)	0.03
$R_{d/\alpha}$	68.97 (4.09)	37.10 (4.87)	0.01

[1]Differences between light regimes were analyzed by two-sample *t* test, using Dunn-Sidak adjustment to the probabilities.

shade plants; in *B. brizantha*, there was also a tendency for reduction of R_d under shade (ca. 25%), however, the difference between treatments was not statistically significant (Table 1). For both species, shade plants had lower light compensation point than plants grown in the sun (Figure 1, Table 1), a result of their lower dark respiration rates per unit of leaf area, that was particularly evident in *B. humidicola*. Low dark respiration rate and low light compensation point are well-known attributes of shade tolerance (Boardman, 1977). A low light compensation point is advantageous because it may help maintain a positive carbon balance under low light levels.

Acclimation to low PFD is frequently related to low chlorophyll a:b ratios (Björkman, 1981; Pearcy & Sims, 1994). In the present study, shade caused a significant ($P < 0.01$) reduction in the chlorophyll a and chlorophyll b ratio in *B. humidicola* (Figure 2). On the other hand, based on the ratio between chlorophyll a and chlorophyll b, *B. brizantha* appeared to be relatively more shade tolerant, as that ratio was not significantly affected by shade (Figure 2). This could indicate a higher adaptation of the light harvesting system of *B. brizantha* to shade. However, an opposite hypothesis could also be proposed, and this behavior could be interpreted as a reduced plasticity (i.e., failure to adapt) of the light harvesting system of *B. brizantha* to respond to low light. Finally, because reduction in chlorophyll a:b in shade leaves is known to cause a significant savings in the nitrogen investment needed for light capture (Pearcy, 1999), *B. humidicola* could be considered a more efficient species than *B. brizantha* under shade. Total chlorophyll content per dry mass, followed the pattern generally observed in other species (e.g., Schiefthaler et al., 1999; Johnston & Onwueme, 1998; Borah & Baruah, 1995), it showed a tendency to be lower on leaves of shaded plants. This reduction, however, was not statistically significant in *B. humidicola* (Figure 3).

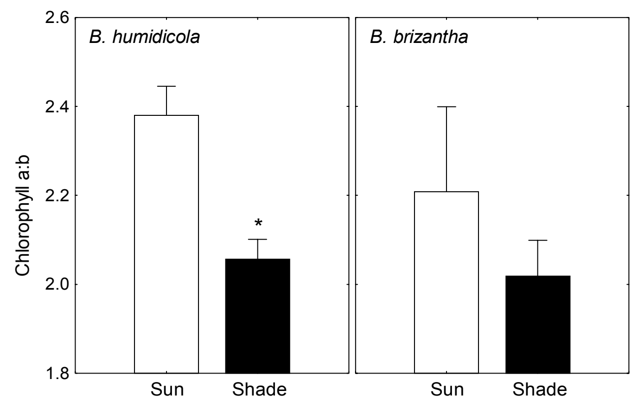

Figure 2 - Chlorophyll a:b ratio for *B. brizantha* and *B. humidicola* grown under full sun or shade. Bars are means ± 1 s.e. (n = 3). An asterisk indicates a significant difference (two-sample *t* test) between treatments and within each species.

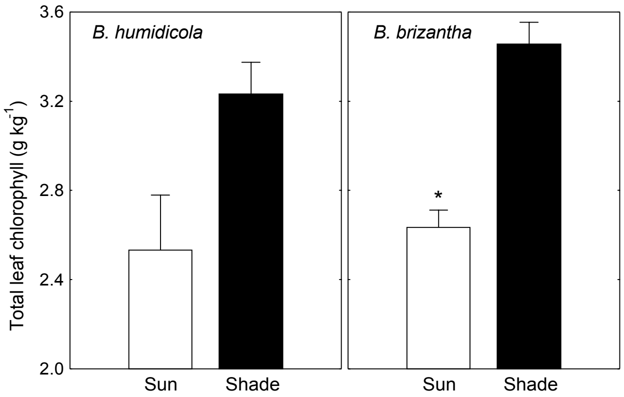

Figure 3 - Total leaf chlorophyll content for *B. brizantha* and *B. humidicola* grown under full sun or shade. Bars are means ± 1 s.e. (n = 3). An asterisk indicates a significant difference (two-sample *t* test) between treatments and within each species.

CONCLUSIONS

Both *B. brizantha* and *B. humidicola* showed the ability to adjust their photosynthetic behavior in response to shade, mainly by decreasing their light compensation points. However, the photosynthetic capacity of both species was reduced under permanent shade. Apparently, *B. humidicola* could be relatively more adapted to succeed, at least temporarily, in light-limited environments.

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