Photosynthesis, Transpiration, Stomatal Conductance, Chlorophyll Fluorescence and Chlorophyll Content in *Brosimum alicastrum*

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Abstract

Photosynthesis, stomatal conductance, transpiration, chlorophyll content and chlorophyll fluorescence were quantified in *Brosimum alicastrum* Swartz, developed in its natural habitat. Light curves show that maximum fixation of CO_2 was 5 µmol $m^{-2}s^{-1}$, photosynthetic efficiency was 0.67 while the photosystem II was found to saturate at a photonic flux density (PFD) of 500 µmol $m^{-2}s^{-1}$, at 15 h. Since this species is shade tolerant, it initiates the photosynthetic process with low levels of light, presenting a light compensation point of 24 µmol $m^{-2}s^{-1}$. A high correlation was found between photosynthesis and transpiration. Highest water use efficiency occurred after at 15 hours at a temperature of 30°C, while chlorophyll levels remained constant throughout the whole diurnal cycle. The results obtained suggest that, during the daylight hours and at higher temperatures, this species can carry out photosynthesis with high water use efficiency. Based on the results, the conclusion is that this species is capable of adapting to a diversity of environmental conditions.

Keywords: *Brosimum alicastrum*, tropical dry forest, stomatal conductance, chlorophyll fluorescence, water use efficiency.

Introduction

Light conditions under which plants develop in the forest understory can change rapidly, and the adaptation presented by these plants, in response to selection pressure to use the light efficiently is what allows them to exist in such variable environmental conditions (Walters y Reich, 1999).

In tropical forests, early or pioneer successional species develop efficiently in a wide variety of environments. As these species grow in an unpredictable and variable habitat, they have greater plasticity than those that develop in a more homogenous habitat (late successional species).

In contrast with the reports published on species growing in temperate zones, little is known about the photosynthetic response of tropical species to different light conditions, (Koch *et al.*, 1994). Plants growing in the understory probably experience the most variable light conditions and, as they are under selective pressure to use the light efficiently, a rapid response is required in the activation of photosynthesis and stomatal aperture (Ögren & Sundin, 1996). Moreover, in the Mediterranean Ceacero, *et al.* (2012) worked in an experimental plot of oak with restoration treatments determined that tree shelters exercised a protection effect on seedling limiting the photoinhibition processes and protected plants showed major photochemical efficiency.

Brosimum alicastrum, is a perennial evergreen tree of the tropical forest which has been classified as a late successional species (Oberbauer, 1985; Strauss-Debenedetti, 1989), tolerant to shade (Montgomery & Chazdon, 2002; Peters, 1989; Walters & Reich, 1999), and widely distributed in the tropical regions of Mesoamerica and South America.

Reports have estimated a photosynthetic rate of 5 µmol m^{-2} s^{-1} in plantlets developed under greenhouse conditions (Peters, 1989). Strauss-Debenedetti, (1989) and Montgomery (2004) reported it to be a species with low photosynthetic plasticity in a light gradient of 0 to 70%, with its highest photosynthetic value at 6 µmol m^{-2} s^{-1}. Ramos & Grace (1990) found that plantlets developed in growth chambers with light (800 µmol m^{-2} s^{-1}) and shade (80 µmol m^{-2} s^{-1}) present differences in their growth rate without any effect on their photosynthetic rate.

No reports were found in the literature on photosynthesis, transpiration, stomatal conductance, chlorophyll fluorescence or chlorophyll content, under "*in situ*" field conditions in adult plants; thus the present study was proposed. We determine the photosynthetic efficiency and water use efficiency in the *Brosimum alicastrum* (Ramón) developed under field conditions, evaluating CO_2 fixation, transpiration, stomatal conductance and chlorophyll fluorescence.

Materials and Methods

Plant material and growth conditions

Three 3-year-old *Brosimum alicastrum* trees with a height of 3 m were selected for this study, all of which were grown under the same field conditions with sun exposure at an average temperature of 28°C. These trees presented similar characteristics and were developed from germinated seeds, cultivated under conditions of weekly irrigation in the botanical garden of the Center for Scientific Investigation of Yucatan, A. C., Mérida, Yucatán, México (20º 59' N; 89º 38' W). The study was conducted in June 2011, at the end of the dry season.

CO_2 fixation and transpiration

Mature, fully exposed leaves were selected to carry out the measurements of gas exchange, transpiration, stomatal conductance and intercellular CO_2 concentration. A portable photosynthesis analyzer (IRGA, LI-COR 6400, Nebraska, USA) was used. The CO_2 concentration was maintained at 380 ppm throughout all the measurements.

Incident light, air temperature, leaf temperature and vapor pressure deficit (VPD) were measured during the determination of gas exchange, using the sensors of photon flux density (PPFD) and temperature integrated in the leaf chamber of the IRGA.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured in mature leaves exposed to full sunlight and in the shade. Quantum efficiency of the photosystem II (PSII) and the electron transport rate (ETR) were determined using a pulse-amplitude-modulated fluorometer (Mini-PAM, Walz, Effeltrich, Germany). Temperature and photon flux density were measured with a thermocouple and a micro quantum sensor, respectively, integrated in the Mini-PAM leaf clip.

Light response curves

Light response curves were carried out at 15 h on mature leaves exposed to sunlight (when the maximum quantity of light, 2500 µmol m^{-2} s^{-1}, was obtained). Prevailing environmental conditions were 22°C and 56% relative humidity. The first CO_2 reading was taken by placing the leaves in the clip of the IRGA under ambient light and measuring CO_2 fixation, maximum base rate; subsequently, shade netting was placed in order to reduce the light reaching the leaf, and CO_2 fixation was measured at each level of PPFD until the light was reduced to a value of approximately 20 µmol m^{-2} s^{-1}. Similarly, photosynthesis curves were also run from the lowest value of PPFD up to 100% light. The readings were performed independently on four leaves per tree.

In addition, light curves were obtained with the Mini-PAM, from leaves selected under similar conditions to those described above. The leaves were placed in the clip and the light curves were carried out with the Mini-PAM program, which applies 8 pulses of actinic light every 2s, increasing light intensity in each pulse until 200 µmol m^{-2} s^{-1} is reached. Five repetitions were performed.

Water use efficiency

Water use efficiency (WUE) was defined as CO_2 absorption/transpiration rate. Ten replicates per tree in each hour of sampling were determined.

Chlorophyll content

During the experiment, chlorophyll content was determined in the same leaves selected for the estimation of gas exchange; ten leaves per tree were measured (n=30): Chlorophyll determination was carried out with the Chlorophyll Meter SPAD-502 (Minolta, Co., Spectrum Technologies, Inc., Japan).

Statistical Analyses

In order to determine the differences between means of the variables analyzed at different times of the day, a repeated measures analysis of variance and Tukey tests were carried out; significance was determined when $p<0.01$. The light curves were adjusted to a logarithmic model. All statistical analyses were performed with the statistical package, Statistica 7.0 (Statsoft Inc.,Tulsa, OK, USA).

Results and Discussion

Relationship between CO_2 fixation, transpiration and stomatal conductance

It has been reported that *B. alicastrum* plantlets present a limited photosynthetic plasticity as well as a low acclimation potential to different light levels (Peters, 1989; Ramos & Grace, 1990; Strauss-Debenedetti, 1989; Strauss-Debenedetti & Bazzas, 1991), however, these evaluations were performed under greenhouse conditions and in growth chambers, with environmental conditions which were quite different from their natural habitat. From the results presented in the present study, it is possible to establish the characteristics of the CO_2 fixation pattern of *B. alicastrum* adult trees in their natural habitat, the tropical dry forest.

The ambient temperature presented a slight variation throughout the day, with an average of 28°C and a maximum value of 30°C. The CO_2 fixation pattern of *B. alicastrum* is shown in Figure 1 (a) where one can observe a very low photosynthetic rate during the early hours and an average maximum CO_2 absorption peak of 4.2 µmol m^{-2} s^{-1} at 12 h. This value is lower than those reported in a study carried out on plantlets of the same species by other authors (Montgomery, 2004; Ramos & Grace, 1990); in all probability, environmental conditions such as the amount of light received and the temperature in the field may have been limiting factors for CO_2 fixation. In addition, CO_2 fixation at 8h and 17 h is minimum, in contrast with the values observed in other tree species such as *Picea mariana* (black spruce) and *Larix laricina* (tamarack larch) in which maximum CO_2 fixation was carried out at eight in the morning but diminished around midday (Dang *et al*., 1991).

The highest CO_2 fixation at midday is an indication that photoinhibition is not present in this species, as was mentioned by Thiele *et al.* (1998) who reported the value of 0.56 in potential photosynthetic efficiency (F_v/F_m) at midday. In the present study, the pre-dawn Fv/F_m, a value that is taken into consideration to determine if a species presents photoinhibition, was 0.71, which is similar to the values reported for Pinus *halapensis* in the summer months and for a number of tree species growing in the Mediterranean regions (Navarro *et al*., 2004) with values below 0.83, which is considered to be optimal for many species (Bjorkman & Demming 1987). Photosynthetic efficiency ($\Delta F/Fm'$) in *B.alicastrum* at maximum light intensity was determined at 0.2 on average and at 0.55 in shade plants (Figure 2), indicating that the variation in photochemical efficiency of the PSII under illuminated conditions is very effective. The electron transport rate (ETR) in plants exposed to 100% light was 80 µmol m^{-2} s^{-1}, while shade plants registered 15 µmol m^{-2} s^{-1} (Figure 3), which would suggest that electron transport in the PSII is very stable.

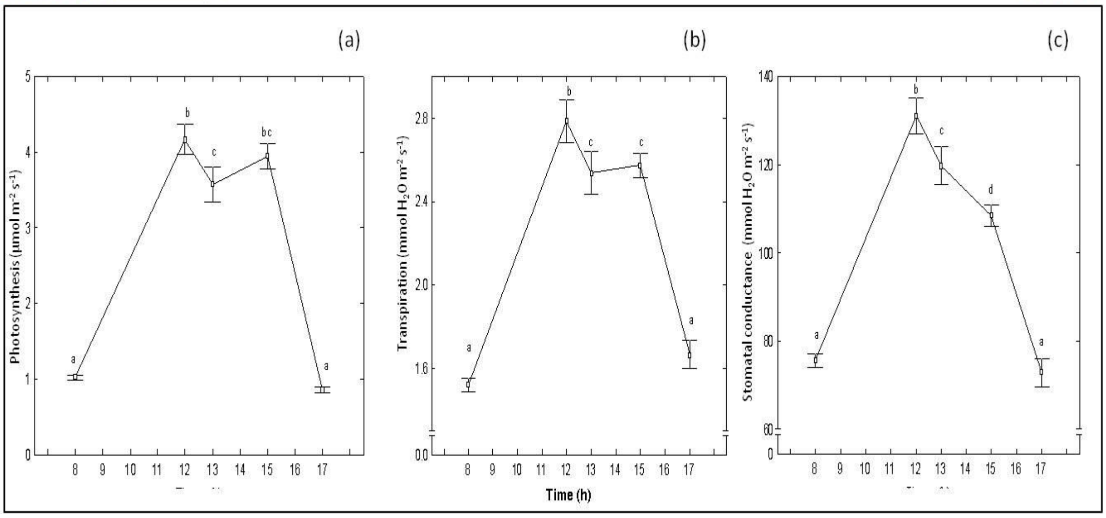

Figure 1. (a) Patterns of photosynthesis of *Brosimum alicastrum*. Each point is the mean of 30 measurements ± standard error. Values with the same letter do not differ significantly (Tukey; $p<0.001$); (b) Transpiration pattern of *Brosimum alicastrum* leaves. Each point is the mean of 30 measurements ± standard error. Values with the same letter do not differ significantly (Tukey; $p<0.001$); Stomatal conductance pattern of *Brosimum alicastrum* leaves. Each point is the mean of 30 measurements ± standard error. Values with the same letter do not differ significantly (Tukey; $p<0.001$).

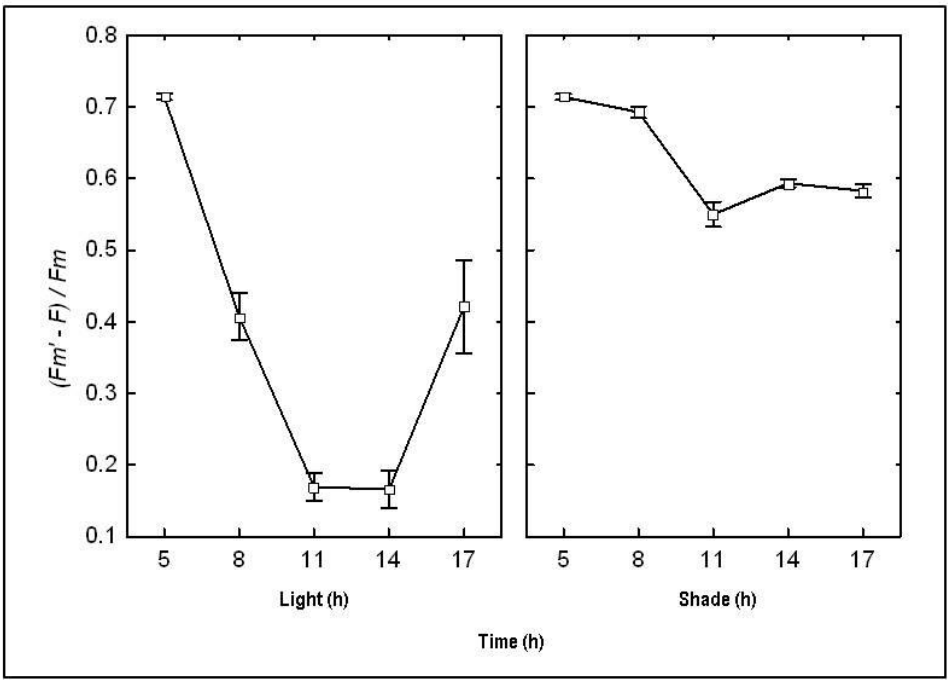

Figure 2. Photosynthetic efficiency pattern of *Brosimum alicastrum* under conditions of light and shade. Each point is the mean of 10 measurements ± standard error.

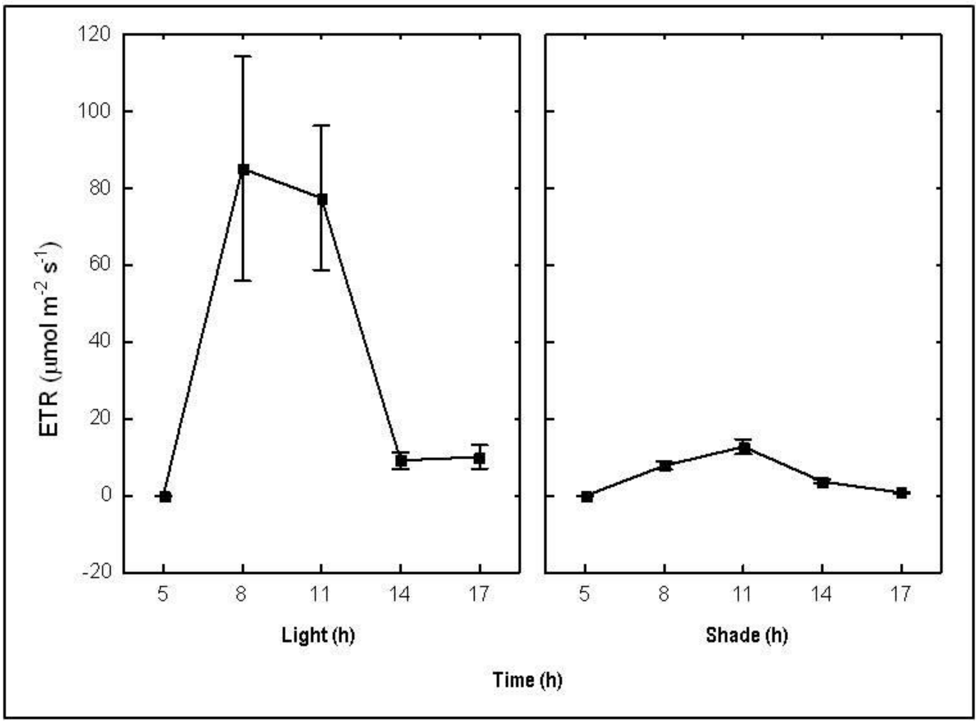

Figure 3. Electron transport rate (ETR) of *Brosimum alicastrum* leaves under conditions of light and shade. Each point is the mean of 10 measurements ± standard error.

The flow of water through the plant is necessary in order to transport nutrients and carry out CO_2 fixation. The transpiration rate (Figure 1(b)) and CO_2 fixation in *B. alicastrum* have a similar diurnal pattern; they lose the largest amount of water through transpiration (2.6 mmol H_20 m^{-2} s^{-1}) during the interval from 12 h to 15 h with the highest temperature and greatest incident light. Oberbauer (1985) has reported that perennial evergreen species close the stomas when conditions are most stressful because they have leaves all year round; however, the diurnal courses of stomatal conductance in *B. alicastrum* indicate that stomatal aperture was greatest at midday. It is important to note that stomatal conductance (or stomatal aperture) has a pattern similar to that of the photosynthetic rate and transpiration rate in the early hours of the day, with the highest value (132 mmol m^{-2} s^{-1}) observed at midday; however, this diminishes by 20% at 15 h **(**Figure 1 (c)).

The results indicate a gradual stomatal closure as the vapor pressure deficit (VPD) increases (Table 1); nevertheless, although the stomas partially close, CO_2 fixation remains high up to 15h. Therefore, this could be a response indicating that *B. alicastrum* tolerates environmental water stress without its photosynthesis being affected. This may be due to its efficiency in extracting water from bedrock strata, as it is known to have deep roots. (Querejeta *et al*., 2006). The internal CO_2 concentration of the mesophyll remains high at 15 h, allowing the plant to continue carrying out photosynthesis, as has been reported for other perennial species (Niinemets *et al*., 2005).

Table 1. Water use efficiency (WUE), vapor pressure deficit (VPD) and chlorophyll content at different times of the day in *Brosimum alicastrum* plants grown in their natural habitat.

Time (h)	WUE† (µmol $mmol^{-1}$)	VPD† (kPa)	Chlorophyll (SPAD unit)

8	0.68 ± 0.026^{a}	2.036 ± 0.007^{a}	51.6
12	1.49 ± 0.037^{bc}	2.160 ± 0.025^{b}	50.8
13	1.40 ± 0.078^{b}	2.151 ± 0.023^{b}	52
15	1.57 ± 0.076^{c}	2.419 ± 0.040^{c}	52.8
17	0.55 ± 0.031^{d}	2.310 ± 0.008^{d}	52.8

† Each value is the mean of 30 replicates ± standard error. Values with the same letter do not differ significantly (Tukey; $p<0.001$).

From the results obtained in the present study, this species can be considered shade tolerant and capable of maintaining an efficient photosynthetic rate despite receiving a light intensity of at least 1200 µmol m^{-2} s^{-1}. CO_2 fixation and high electron transport within the leaf, even with partial stoma closure, results in high water use efficiency (WUE). The presence of abaxial papillae in *B. alicastrum* leaves has been reported, which could explain, to some extent, the values obtained in stomatal conductance, transpiration and CO_2 fixation. Strauss-Debenedetti (1989) has reported that the presence of papillae can be seen as an evolutionary adaptation to the environmental conditions of light and limited water availability in which this species grows.

Light response curves

In the curve of CO_2 fixation in response to different light intensities for *B. alicastrum* (Figure 4 (a)), CO2 absorption can be seen to increase rapidly with the increment of incident light on the leaf, until the light level required to saturate the photosystem II is reached, approximately 500 µmol m^{-2} s^{-1} of photon flux with a maximum photosynthetic rate of 5 µmol m^{-2} s^{-1}. The CO_2 assimilation rate with different amounts of light and its value of saturation to the light was similar to those reported by Peters (1989) for plantlets of this species and other perennial evergreen species growing in tropical forests (Langenheim *et al*., 1984), which usually present low values in their photosynthetic rates in comparison with herbaceous plants and shrubs. Similarly, Mooney & Gulman (1982) have reported that the low CO_2 assimilation values of perennial evergreen species, such as *B. alicastrum*, may be related to their photosynthesis throughout the year and the longevity of their leaves.

B. alicastrum is very sensitive to incident light, and a light intensity of 24 µmol m^{-2} s^{-1} (value corresponding to the light compensation point) is enough for the metabolic system of this tree's leaves to initiate CO_2 fixation (Figure 4 (b)). The light compensation point of this species was very low and is similar to those reported for other late successional species (Bazzaz & Carlson, 1982), thus allowing it to be included in this group of species. However, it is important to note that B. *alicastrum* has the capacity to maintain its photosynthetic rate of 5 µmol m^{-2} s^{-1}, which is a value similar to that reported by Walters & Reich (1999), even though the amount of light received is increased to 2500 µmol m^{-2} s^{-1} and no apparent photoinhibition is presented; which is confirmed with the pre-dawn values of *Fv/Fm* and the values of *ΔF/Fm'* recovery throughout the day. The CO_2 fixation rate in adult plants of this tree is similar to those reported in plantlets during experiments carried out under greenhouse conditions (Peters, 1989; Strauss-Debenedetti, 1989) and in experiments performed in growth chambers (Ramos & Grace, 1990); thus we can appreciate the extensive photosynthetic plasticity of this species since it presents the same response under such different environmental conditions. Although reports have confirmed its classification as a shade tolerant species (Montgomery & Chazdon, 2002; Peters, 1989; Walters

& Reich, 1999) it is also capable of tolerating high variations of light without affecting its diurnal pattern of CO_2 fixation, and can therefore be considered a climax species, as indicated by Ramos & Grace (1990).

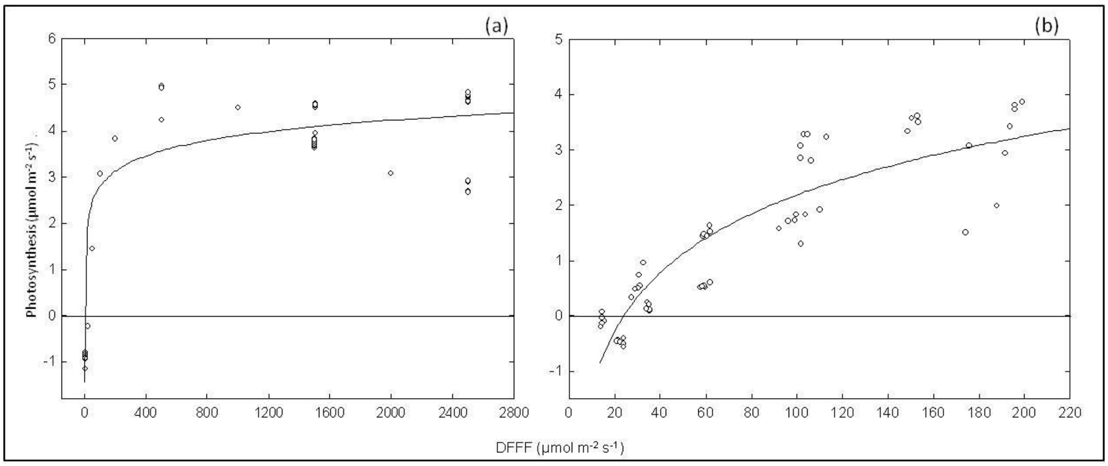

Figure 4. (a) Photosynthetic response curve at different intensities of ambient light for *Brosimum alicastrum* leaves; (b) Photosynthetic response curve with low-light intensity for *Brosimum alicastrum* leaves. Maximum light level was 200 µmol m^{-2} s^{-1}.

Under natural conditions, *in vivo*, fluorescence emission of the photosystems PSI and PSII changes constantly as it continues to adapt to the environmental conditions in which the plants grow. Plants have to face frequent fluctuations of intensity and spectral quality of their primary source of life-light, whose energy is needed to drive the processes of photosynthesis (Ruban, 2009). In this study, the light curves of chlorophyll fluorescence and the changes presented by $\Delta F/Fm'$ as the light received by the *B. alicastrum* leaves increases (Figure 5) indicate that, as the fluorescence diminishes with the continuous increase in light, photochemical efficiency also rises due to the fact that the centers of PSII reaction are receiving a greater proportion of electrons, therefore, photochemical efficiency and the resulting photosynthesis are very efficient in the summertime, when this work was carried out.

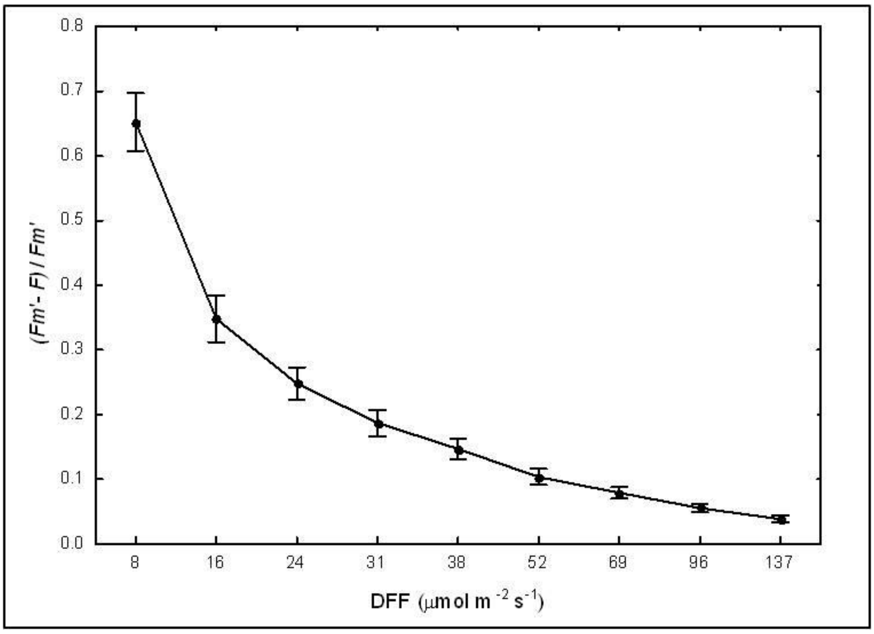

Figure 5. Photosynthetic efficiency curve of *Brosimum alicastrum*. Each point is the mean of 10 measurements ± standard error.

Water use efficiency

Water use efficiency (WUE) increases gradually throughout the day, with the highest value of 1.57 µmol mmol^{-1} registered at 15 h. significant differences were observed as the day advanced and the lowest value was found at 17 h (Table 1). WUE may be related to the capacity displayed by *B. alicastrum* to absorb water from bedrock strata, due to the fact that this species has developed roots extending deep into the earth. (Calderón, 1975; Querejeta *et al*., 2006). Chlorophyll content presents high values (52 SPAD units), which is similar to other perennial evergreen species and remains constant throughout the day (Table 1), contrasting with other species where the chlorophyll value diminishes as the leaves receive more light (Hoel & Solhaug, 1998 Montgomery, 2004). These chlorophyll values also facilitate a high CO_2 fixation during the day.

WUE indicates the water use strategy of a species in its different life stages or between species (Donovan & Ehleringer, 1991). In order to predict the changes associated with plant productivity, it is essential to understand how WUE changes with the climate. In *B. alicastrum*, a change is observed in WUE when the environmental conditions are different. At a higher temperature (at 15 h), water use efficiency was greater (Table 1), in contrast with reports on other perennial evergreen species (Dang *et al*., 1991). WUE also reflects an exchange between water loss and carbon gain in the carbon assimilation process through photosynthesis. In the case of *B. alicastrum*, under more extreme conditions (higher temperature and greater light incidence), a higher carbon absorption with less water loss was observed, which may contribute to maintaining biomass increase even under conditions which could be stressful for other perennial evergreen species.

Conclusions

Although *Brosimum alicastrum* has been classified as a late successional, shade tolerant species, it was found to have the capacity, as well as the physiological and photosynthetic plasticity, to adapt to high-light conditions, even in the dry season, while presenting high water use efficiency. This species, therefore, is capable of thriving under a variety of natural conditions with limited water availability, conferring an adaptive advantage of great ecophysiological relevance.

Acknowledgements

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