

## PHOTOSYNTHETIC TRAITS OF CANOPY LEAVES OF *Dinizia excelsa* (Fabaceae)

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**ABSTRACT** – The response of leaf traits to irradiance and [CO<sub>2</sub>] in canopy leaves of several tall trees remains to be determined under natural conditions. Thus, the objective of this work was to determine gas-exchange parameters in sun and shade leaves of *Dinizia excelsa* Ducke in 35–45 m tall trees of Central Amazonia. We assessed light saturated photosynthesis ( $A_{max}$ ), stomatal conductance ( $g_s$ ), mesophyll conductance ( $g_m$ ), transpiration rates ( $E$ ), water use efficiency (WUE), intrinsic water use efficiency (WUEi), maximum electron transport rate ( $J_{max}$ ), the maximum carboxylation rate of Rubisco ( $V_{cmax}$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ) specific leaf area (SLA) and fresh leaf thickness. We also estimated the CO<sub>2</sub> concentration at the chloroplast level ( $C_c$ ) and determined the light and CO<sub>2</sub> saturated photosynthesis ( $A_{pot}$ ).  $A_{max}$  was obtained at light saturation (1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), whereas  $A_{pot}$ ,  $V_{cmax}$ ,  $J_{max}$  and  $g_m$  were obtained after constructing  $A/C_i$  response curves. There was a significant difference between sun and shade leaves in  $C_i$  and  $C_c$ , but for other parameters no differences were observed.  $A_{max}$  was positively correlated with  $g_s$ ,  $g_m$  and  $E$ , and there was also a significant correlation between  $g_s$  and  $g_m$  ( $p \leq 0.05$ ), as well as between  $J_{max}$  and  $V_{cmax}$ . Thicker leaves had higher values of  $A_{max}$ ,  $g_s$ ,  $C_i$ ,  $C_c$  and  $E$ .  $A_{pot}$  was limited by the electron transport rate and by low  $g_m$ . The canopy of the tree caused a decrease in irradiance (30-40%), but this reduction was not enough to reduce important photosynthetic parameters. Thus, all resources allocated to leaf production led to maximum use of the solar energy received by the leaves, which allowed this species to grow at fairly rapid rates.

**Keywords:** Mesophyll conductance; Maximum carboxylation rates of Rubisco; Central Amazonia.

## CARACTERÍSTICAS FOTOSSINTÉTICAS DE FOLHAS DE DOSSEL DE *Dinizia excelsa* (Fabaceae)

**RESUMO** – A resposta das características foliares à irradiância e concentração de CO<sub>2</sub> em folhas de dossel de várias espécies arbóreas de alto porte ainda permanece para ser determinada em condições naturais. Assim, o objetivo deste trabalho foi determinar parâmetros de troca gasosa em folhas de sol e sombra de *Dinizia excelsa* Ducke, em árvores de 35–45 m de altura, na Amazônia Central. Foram avaliados fotossíntese saturada de luz ( $A_{max}$ ), condutância estomática ( $g_s$ ), condutância do mesófilo ( $g_m$ ), taxa de transpiração ( $E$ ), eficiência de uso de água (WUE), eficiência intrínseca de uso de água (WUEi), taxa máxima de transporte de elétrons ( $J_{max}$ ), taxa máxima de carboxilação da Rubisco ( $V_{cmax}$ ), concentração de CO<sub>2</sub> intercelular ( $C_i$ ), área foliar específica (SLA) e a espessura da folha fresca. Também foi estimada a concentração de CO<sub>2</sub> no nível de cloroplasto ( $C_c$ ) e determinada a fotossíntese saturada de luz e CO<sub>2</sub> ( $A_{pot}$ ). O  $A_{max}$  foi obtido com saturação de luz (1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), enquanto que  $A_{pot}$ ,  $V_{cmax}$ ,  $J_{max}$  e  $g_m$  foram obtidos após a construção de uma curva de resposta  $A/C_i$ . Houve diferença significativa entre as folhas de sol e sombra em  $C_i$  e  $C_c$ , mas nenhuma diferença foi observada para outros parâmetros avaliados.  $A_{max}$  foi positivamente correlacionado com  $g_s$ ,  $g_m$  e  $E$ , e também houve uma correlação significativa entre  $g_s$  e  $g_m$  ( $p \leq 0,05$ ), bem como entre  $J_{max}$  e  $V_{cmax}$ . As folhas mais espessas apresentaram maiores valores de  $A_{max}$ ,  $g_s$ ,  $C_i$ ,  $C_c$  e  $E$ .  $A_{pot}$  foi limitado pela taxa de transporte de elétrons e pelo baixo  $g_m$ . A folhagem da copa da árvore causou diminuição da luminosidade (30-40%), mas essa redução



mostrou-se insuficiente para reduzir parâmetros importantes da fotossíntese. Dessa forma, todo recurso alocado a produção de folhas levou a um máximo aproveitamento da energia solar recebida pelas folhas, possibilitando que esta espécie tenha um crescimento moderadamente rápido.

Palavras-Chave: Condutância do mesófilo; Taxa de carboxilação da Rubisco; Amazônia Central.

## 1. INTRODUCTION

In the last decades the effect of the increase of atmospheric CO<sub>2</sub> concentration on plant physiology has been intensively investigated due to its presumed consequences on climate changes (Manter and Kerrigan, 2004; Knauer et al., 2019). It is well-known that stomata play an important role on carbon uptake, as they impose the first major diffusional limitation to CO<sub>2</sub> diffusion, from the atmosphere to carboxylation sites (Nascimento and Marengo, 2013; Xiong et al., 2018). The photosynthetic capacity of a leaf depends on diffusive (i.e. stomatal conductance –  $g_s$  and mesophyll conductance –  $g_m$ ) and non-diffusive factors (i.e. the maximum carboxylation rate of Rubisco –  $V_{cmax}$  and maximum electron transport rate,  $J_{max}$ ).  $V_{cmax}$  and  $J_{max}$  can be obtained from response curves of photosynthesis to intercellular CO<sub>2</sub> concentration –  $A/C_i$  (Farquhar et al., 1980; Stinziano et al., 2019). Both  $V_{cmax}$  and  $J_{max}$  are important parameters in modeling studies that aim to predict the impacts of climate change on plant functioning (Knauer et al., 2019).

Among the factors that affect photosynthesis, the response of stomatal functioning to environmental and endogenous factors has been the subject of much research (Terashima et al., 2011; Mendes and Marengo, 2014; Marengo et al., 2017). Although  $g_m$  is also quite relevant to CO<sub>2</sub> diffusion and photosynthesis, it attracted less attention in the past decades, but in recent studies, the importance of  $g_m$  to carbon assimilation has been highlighted (Flexas et al., 2016; Knauer et al., 2019). Nevertheless, research is still needed to assess the relevance of  $g_m$  in tropical species, particularly the Amazonian species.

The strata of tropical forests, as well as gap opening and closing lead to the formation of a light gradient in the canopy of a tree. This gradient can lead to changes in the strategy of the leaf for light capture and light use efficiency, and thereby to variations in leaf structure and physiology (Givnish, 1984; Clark and Clark, 1992; Marengo et al., 2017). In fact, light is probably the

most important environmental factor affecting plant establishment, growth and survival (Niinemets et al., 2015; Gitelson et al., 2017).

The angelim (*Dinizia excelsa* Ducke, Fabaceae) is an emerging tree that can reach 50–60 m in height and 1.0 to 1.8 m in diameter (Lorenzi, 1992). It has a dense wood –0.91 g cm<sup>-3</sup> (Fearnside, 1997), and trees over 20 cm in diameter have a growth rate of 5.30 mm per year (Schwartz et al., 2016). In the emergent phase, the angelim canopy stands out in the forest landscape; hence it is not shaded by neighboring trees. It has wide distribution in the Brazilian Amazon, can occur in density of one individual per 6 ha, and the trees have good silvicultural performance (Dick, 2001; Ferreira et al., 2004). Angelim is economically important in silvicultural systems and in the timber industry. The wood of angelim is resistant to the attack of fungi and termites, and it is used in the manufacture of decorative plates, construction, shipbuilding, woodworking, and carpentry (Melo and Varela, 2006; Oliveira et al., 2008). In addition, the angelim can also be used in garden and urban afforestation (Lorenzi, 1992).

Gas exchange studies of Amazonian tree species under natural conditions are relevant due to current trends in global warming and atmospheric CO<sub>2</sub> enrichment (Magrin et al., 2014). Thus, measurements of gas exchange parameters (e.g.  $g_s$ ,  $A_{max}$ ,  $V_{cmax}$  and  $J_{max}$ ) in Amazonian species is of paramount importance for the construction of models aimed at predicting the performance of Amazonian ecosystems. Thus, the objective of this work was to determine the effect of leaf position in the canopy stratum on leaf traits of angelim trees in the Central Amazon. Leaf trait measurements included light saturated photosynthesis ( $A_{max}$ ),  $g_s$ ,  $g_m$ , transpiration ( $E$ ), water use efficiency (WUE), intrinsic water use efficiency (WUEi),  $J_{max}$ ,  $V_{cmax}$ , intercellular CO<sub>2</sub> concentration ( $C_i$ ), specific leaf area (SLA) and fresh leaf thickness (FLT). It was also estimated the concentration of CO<sub>2</sub> at chloroplast level ( $C_c$ ) and determined light and CO<sub>2</sub> saturated photosynthesis ( $A_{pot}$ ).

## 2. MATERIALS AND METHODS

The study was conducted at a 10-ha plot of a dense terra-firme forest fragment at the Colosso Reserve (02° 24' 13.2"S, 59°51' 54"W). The region has a humid equatorial climate, with annual precipitation of 2240 mm, distributed over a rainy season from November to May (> 180 mm per month) and a mild dry season from June to September (≤ 100 mm per month). October is a transitional month. The average annual air temperature is 26.7°C, and the average relative humidity is 84%.

In this study, six trees (35–45 m in height and 1.30–2.00 m in diameter) of angelim (*Dinizia excelsa* Ducke, Fabaceae) were used. In the experiment we used a completely randomized design with two treatments and six replications (trees). The treatments were the leaves from two positions in the canopy: upper part of the tree (hereinafter referred to as sun leaves, which were under direct solar radiation) and lower part of the canopy (shade leaves), which received about 60–70% of total solar radiation. From these canopy strata branches were detached for data collection. They were about 7 to 12 cm in diameter and approximately 12 m in length. All branches were above the canopy of neighboring trees and the lower branches of a tree were shaded only by the upper ones. We used detached branches because of the impossibility of accessing the canopy of the tree for data collection.

To assess the effect of branch detachment on stomatal conductance ( $g_s$ ), a previous study was carried out using two gas exchange systems (Li-6400, Li-Cor, Lincoln, USA), collecting data simultaneously from two small branches (about 4 cm in diameter) from the same tree, which was accessible from a 40-m observation tower. After a stabilization period of 15 minutes, one branch was randomly selected and kept intact while the other branch was severed from the tree by a rapid cut, without interrupting gas-exchange measurements. The measurement process on both leaves (i.e. from the undisturbed branch and the severed one) was uninterrupted for 60 min, and during this time stomatal conductance remained similar in both branches. Thus, in the current experiment gas exchange and fluorescence data were measured within that time interval, and for further precaution data were collected on branches thicker than 4 cm in diameter.

Gas exchange and fluorescence measurements were made with a gas exchange system (Li-6400, Li-

Cor, Lincoln, NE) with a 2-cm<sup>2</sup> integrated fluorescence chamber head (Li-6400-40, Li-Cor). Just after detachment, the branch was taken to the gas exchange instrument for data collection. The time lag from branch detachment up to the leaflet insertion in the leaf chamber of the gas exchange system was about 10 minutes. After a stabilization period (5–10 min) at ambient CO<sub>2</sub> (380 μmol mol<sup>-1</sup>), photosynthetically active radiation (PAR) of 1200 μmol m<sup>-2</sup> s<sup>-1</sup> (light saturation) and ambient temperature (27 °C), photosynthetic rates ( $A$ ) as a function of the intercellular CO<sub>2</sub> concentration –  $C_i$  ( $A/C_i$  response curves) were measured. The  $A/C_i$  response curves were generated by increasing the reference CO<sub>2</sub> concentration from 50 to 2000 μmol mol<sup>-1</sup> in nine steps, i.e. 380, 250, 100, 50, 380, 550, 1000, 1,500 and 2,000 μmol mol<sup>-1</sup> (Long and Bernacchi, 2003). Light saturated photosynthesis ( $A_{max}$ ) was determined at light saturation (a value determined in a previous experiment) and a CO<sub>2</sub> concentration of 380 μmol mol<sup>-1</sup>. Whereas the light and CO<sub>2</sub> saturated photosynthesis (hereinafter termed potential photosynthesis –  $A_{pot}$ ) was measured at light saturation and a reference CO<sub>2</sub> concentration of 2000 μmol mol<sup>-1</sup>. The maximum carboxylation rate of Rubisco ( $V_{cmax}$ ) and maximum electron transport rate ( $J_{max}$ ) were calculated according to Farquhar et al. (1980).

$$A_c = [V_{cmax} (C_i - \Gamma^*)] / [C_i + K_c(1 + O/K_o)]$$

$$A_j = [J_{max} (C_i - \Gamma^*)] / ([4C_i + 8\Gamma^*])$$

Where  $A_c$  and  $A_j$  denote the net photosynthetic rates limited by Rubisco activity and electron transport rate ( $A_j$ ), respectively.  $\Gamma^*$  represents the CO<sub>2</sub> compensation point in the absence of respiration in the light;  $C_i$  is the intercellular CO<sub>2</sub> concentration,  $O$  represents the oxygen concentration in the intercellular spaces;  $K_c$  and  $K_o$  represent the Michaelis-Menten constant of Rubisco for carboxylation and oxygenation, respectively.  $V_{cmax}$  values were standardized to 25 °C (Medlyn et al., 1999).

The quantum yield of the photosystem II system ( $\Phi_{psII}$ ) was calculated as previously described (Nascimento and Marengo, 2013).  $C_c$  values were determined according to Epron et al. (1995), whereas  $g_m$  was obtained as follows:  $g_m = A/(C_i - C_o)$ . We also calculated, water use efficiency (WUE) the photosynthesis to transpiration ratio ( $A/E$ ) and the intrinsic water use efficiency (WUEi) the  $A/g_s$  ratio. Data were collected

from September to November 2010 from two healthy and physiologically mature leaflets per stratum, and from six trees and two canopy strata per tree.

To assess the effect of treatments on leaf morphology, fresh leaflet thickness (FLT) was measured in two leaflets from each canopy stratum on each tree with digital calipers (0.01 mm accuracy). The leaflet

thickness was determined in the middle portion of the leaves, avoiding major veins. The leaf area was determined with an area meter (Li-3000, Li-Cor), and the dry matter was determined after oven-drying at 72°C until constant mass. Specific leaf area (SLA) was determined as the leaf area to leaf mass ratio. For further information we also measured the chlo-

**Table 1** – Light saturated photosynthesis ( $A_{max}$ ), light and CO<sub>2</sub> saturated photosynthesis ( $A_{pot}$ ), stomatal conductance ( $g_s$ ), mesophyll conductance ( $g_m$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ), chloroplast CO<sub>2</sub> concentration ( $C_c$ ), vapor pressure deficit (VPD), leaf temperature ( $T_{leaf}$ ), water use efficiency (WUE), intrinsic water use efficiency ( $WUE_i$ ), the maximum carboxylation rate of Rubisco ( $V_{cmax}$ ), electron transport rate ( $J_{max}$ ),  $F_v/F_m$  ratio, specific leaf area (SLA), chlorophyll content, SPAD values, fresh leaf thickness (FLT), and absolute chlorophyll content ( $a$ ,  $b$  and  $a + b$ ) in sun and shade leaflets of *Dinizia excelsa*. Different capital letters denote significant differences ( $p = 0.05$ , t-test) between the canopy strata (sun and shade leaflet). Each value represents the mean ( $\pm$  SE) of six trees and two leaflets per tree.

**Tabella 1** – Fotossíntese saturada por luz ( $A_{max}$ ), fotossíntese saturada por luz e CO<sub>2</sub> ( $A_{pot}$ ), condutância estomática ( $g_s$ ), condutância do mesófilo ( $g_m$ ), concentração intercelular de CO<sub>2</sub> ( $C_i$ ), concentração de CO<sub>2</sub> de cloroplasto ( $C_c$ ), déficit de pressão de vapor (VPD), temperatura da folha ( $T_{leaf}$ ), eficiência no uso da água (WUE), eficiência intrínseca no uso da água ( $WUE_i$ ), taxa máxima de carboxilação de Rubisco ( $V_{cmax}$ ), taxa de transporte de elétrons ( $J_{max}$ ), relação  $F_v/F_m$ , área foliar específica (SLA), conteúdo de clorofila, valores SPAD, espessura foliar fresca (FLT) e teor absoluto de clorofila ( $a$ ,  $b$  e  $a + b$ ) em folíolos de sol e sombra de *Dinizia excelsa*. Letras maiúsculas diferentes indicam diferenças significativas ( $p = 0,05$ , teste t) entre os estratos do dossel (folhas do sol e folhas de sombra). Cada valor representa a média ( $\pm$ SE) de seis árvores e dois folhetos (de duas folhas) por árvore.

PARAMETER	SUN LEAFLET	SHADE LEAFLET
$A_{max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	8.7 $\pm$ 1.3 A	5.9 $\pm$ 0.7 A
$A_{pot}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	24.1 $\pm$ 4.0 A	19.3 $\pm$ 2.1 A
$g_s$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )	0.13 $\pm$ 0.03 A	0.07 $\pm$ 0.02 A
$g_m$ ( $\text{mol m}^{-2} \text{s}^{-1} \text{bar}^{-1}$ )	0.07 $\pm$ 0.009 A	0.05 $\pm$ 0.005 A
$C_i$ ( $\mu\text{mol mol}^{-1}$ )	232.6 $\pm$ 11.4 A	201.8 $\pm$ 11.6 B
$C_c$ ( $\mu\text{mol mol}^{-1}$ )	107.9 $\pm$ 5.3 A	93.8 $\pm$ 5.4 B
$V_{cmax}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	41.1 $\pm$ 4.9 A	38.4 $\pm$ 3.3 A
$J_{max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	101.2 $\pm$ 14.6 A	80.6 $\pm$ 8.3 A
VPD (kPa)	1.8 $\pm$ 0.2 A	1.9 $\pm$ 0.2 A
$T_{leaf}$ (°C)	33.4 $\pm$ 0.8 A	33.6 $\pm$ 0.8 A
WUE ( $\text{mmol mol}^{-1}$ )	4.4 $\pm$ 0.6 A	5.2 $\pm$ 0.2 A
$WUE_i$ ( $\mu\text{mol mol}^{-1}$ )	76.4 $\pm$ 9.5 A	108.3 $\pm$ 13.3 A
$F_v/F_m$ (unitless)	0.78 $\pm$ 0.005 A	0.78 $\pm$ 0.005 A
SLA ( $\text{m}^2 \text{kg}^{-1}$ )	7.8 $\pm$ 0.5 A	7.5 $\pm$ 0.4 A
FLT (mm)	0.20 $\pm$ 0.003 A	0.16 $\pm$ B
SPAD	68.1 $\pm$ 4.4 A	57.2 $\pm$ 3.7 A
Chlorophyll $a$ ( $\mu\text{mol m}^{-2}$ )	362.1 $\pm$ 42.7 A	317.6 $\pm$ 19.4 A
Chlorophyll $b$ ( $\mu\text{mol m}^{-2}$ )	93.6 $\pm$ 26.0 A	98.1 $\pm$ 19.3 A
Chlorophyll $a + b$ ( $\mu\text{mol m}^{-2}$ )	470.8 $\pm$ 39.9 A	415.7 $\pm$ 27.9 A

rophyll content of leaf pigment in 80% acetone and measured the SPAD values with a chlorophyll meter (SPAD-502-Minolta, Japan), as previously described (Mendes et al., 2013; Gouvêa and Marengo, 2018).

Data were subjected to analysis of variance (ANOVA) and the t test was used to determine significant differences between sun and shade leaflets. The relationships between quantitative variables (e.g.  $A_{\max}$  versus  $V_{\text{cmax}}$ ,  $A_{\text{pot}}$  versus  $J_{\max}$ ) were examined by regression analysis. Pearson's correlation coefficient was used to evaluate the relationships between gas exchange variables and morphological traits (SLA and leaflet thickness). Statistical analyzes were performed using Statistica 10.0 software (StatSoft, Tulsa, OK, USA).

### 3.RESULTS

There was no significant difference between sun and shade leaflets in  $A_{\max}$ ,  $A_{\text{pot}}$ ,  $g_s$ ,  $g_m$ ,  $V_{\text{cmax}}$ ,  $J_{\max}$ , VPD,  $T_{\text{leaf}}$ , WUE, WUEi, SPAD values, and chlorophyll contents ( $p \geq 0.05$ ; Table 1). Only  $C_i$  and  $C_c$  showed differences between canopy strata, i.e. sun versus shade leaflet ( $p \leq 0.05$ ; Table 1). On average, the  $A_{\max}$  and  $A_{\text{pot}}$  values were 8.7 and 24.1  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the sun leaflets, whereas shade leaflets had  $A_{\max}$  and  $A_{\text{pot}}$  values of 5.9 and 19.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Table 1). In ambient  $[\text{CO}_2]$ , the  $g_m$  values were 46% (sun leaflets) and 29% (shade leaflets) lower than those recorded for  $g_s$  (Table 1). The maximum quantum efficiency of PS II ( $F_v/F_m$  ratio) did not vary between sun and shade environment. There was a significant effect of the ambient condition on FLT ( $p \leq 0.05$ ; Table 1). Mean FLT values were 0.20 and 0.16 mm for the sunny and shade conditions, respectively. However, SLA showed no significant differences between the sun and shade environment ( $p \geq 0.05$ ; Table 1). As the ambient condition had no effect on gas exchange parameters ( $A_{\max}$ ,  $A_{\text{pot}}$ ,  $g_s$  and  $g_m$ ), data from both environments were pooled for correlation analysis.

$A_{\max}$  was positively correlated with  $g_s$  ( $r = 0.95$ , Table 2),  $g_m$  ( $r = 0.96$ ), and  $E$  ( $r = 0.92$ ). However,  $A_{\max}$  negatively correlated with VPD ( $r = -0.52$ ),  $T_{\text{leaf}}$  ( $r = -0.32$ ) and WUE ( $r = -0.28$ ). There was a positive correlation between  $g_s$  and  $g_m$  ( $r = 0.83$ , Table 2), and, it was also found that  $g_s$  decreased with increasing in VPD and  $T_{\text{leaf}}$  ( $r = -0.65$  and  $r = -0.46$ ; respectively). However, there was a weak correlation between  $g_m$  and VPD ( $r = -0.37$ ) and  $T_{\text{leaf}}$  ( $r = -0.20$ ), and between

WUE and  $g_s$  ( $r = -0.30$ ) and  $g_m$  ( $r = -0.155$ ). WUE and WUEi were positively correlated with the  $g_m/g_s$  ratio ( $r = 0.99$ ; Fig. 1).

At ambient  $\text{CO}_2$  concentration,  $C_i$  and  $C_c$  were positively correlated with  $g_s$  ( $r = 0.78$ ) and  $g_m$  ( $r = 0.42$ , Table 2). There was also a positive correlation between  $J_{\max}$  and  $V_{\text{cmax}}$  ( $r = 0.81$ ), and between  $V_{\text{cmax}}$  (and  $J_{\max}$ ) and  $A_{\max}$  and  $A_{\text{pot}}$  (Table 2).

$V_{\text{cmax}}$  and  $J_{\max}$  were positively correlated with  $g_s$  ( $r \cong 0.6$ ) and  $g_m$  ( $r \cong 0.9$ , Table 2). Gas exchange parameters were correlated with SLA and FLT (Table 2). Thicker leaves had higher values of  $A_{\max}$  ( $r = 0.34$ ),  $g_s$  ( $r = 0.46$ ),  $C_i$  ( $r = 0.52$ ),  $C_c$  ( $r = 0.52$ ) and  $E$  ( $r = 0.38$ ). SLA was negatively correlated with VPD,  $T_{\text{leaf}}$ ,  $V_{\text{cmax}}$  and  $J_{\max}$  ( $p \leq 0.05$ ). On the other hand, FLT had no effect on  $g_m$ ,  $V_{\text{cmax}}$  and  $J_{\max}$  ( $p \geq 0.05$ ). Finally, SLA increased with increasing LFT, which was not expected ( $r = 0.58$ ,  $p \leq 0.001$ ). In Figure 2, irrespective of the environment (sun – shade), the initial portion of the curve showed a linear association between photosynthesis ( $A$ ) and  $C_i$  and  $C_c$  indicating a limitation imposed by Rubisco carboxylation rate. The increase of both  $C_i$  and  $C_c$  led  $A$  to increase to the point of photosynthesis limitation by electron transport rate (dashed line in Figure 2). Below the colimitation point ( $C_i$  values of 433–544  $\mu\text{mol mol}^{-1}$ , indicated by the vertical line in Figure 2 A, B),  $A$  was limited by Rubisco carboxylation rates. The  $C_c$  values at the colimitation point were 201–252  $\mu\text{mol mol}^{-1}$  (indicated by the vertical line in Figure 2 C, D). On the other hand, above the colimitation point (high  $\text{CO}_2$  concentration)  $A$  was limited by electron transport rates (dashed lines in Figure 2).

The  $g_m$  values varied as a function of  $C_i$  or  $C_c$  (Fig. 3). At low  $\text{CO}_2$  concentrations ( $C_i \leq 400 \mu\text{mol mol}^{-1}$ ),  $g_m$  increased linearly with increasing  $C_i$  or  $C_c$  (Fig. 3). On the contrary,  $g_m$  decreased at high  $\text{CO}_2$  concentrations (Fig. 3). In comparison with  $g_m$  values recorded at ambient  $[\text{CO}_2]$ , ( $C_i$  of 200–300  $\mu\text{mol mol}^{-1}$ ),  $g_m$  decreased 70% at high  $\text{CO}_2$  concentration (Fig. 3).

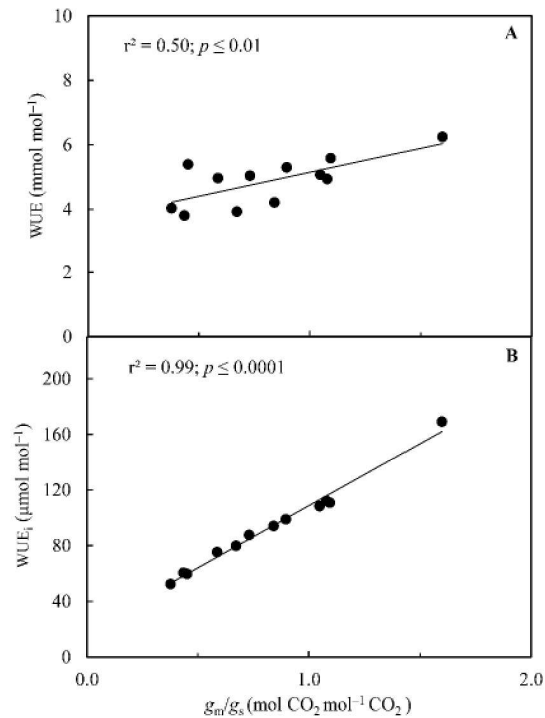
### 4.DISCUSSION

The mean  $F_v/F_m$  value recorded in this study (0.78; Table 1) is within the range of values for non-stressed leaves, i.e.  $F_v/F_m$  of about 0.80 (Björkman

**Table 2** – Pearson's correlation matrix between light saturated photosynthesis ( $A_{\max}$ ), light and  $\text{CO}_2$  saturated photosynthesis ( $A_{\text{pot}}$ ), stomatal conductance ( $g_s$ ), mesophyll conductance ( $g_m$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), chloroplast  $\text{CO}_2$  concentration ( $C_c$ ), transpiration ( $E$ ), vapor pressure deficit (VPD), leaf temperature ( $T_{\text{leaf}}$ ), water use efficiency (WUE), intrinsic water use efficiency (WUEi), the maximum carboxylation rate of Rubisco ( $V_{\text{max}}$ ), electron transport rate ( $J_{\text{leaf}}$ ), specific leaf area (SLA), fresh leaf thickness (FLT) in *Dinizia excelsa*. \*: significant ( $p \leq 0.05$ ); \*\*: significant ( $p \leq 0.01$ ); ns: not significant ( $p > 0.05$ ).

**Tabela 2** – Matriz de correlação de Pearson entre fotossíntese saturada por luz ( $A_{\text{pot}}$ ), fotossíntese saturada por luz e  $\text{CO}_2$  ( $A_{\max}$ ), condutância estomática ( $g_s$ ), condutância do mesófilo ( $g_m$ ), concentração intercelular de  $\text{CO}_2$  ( $C_i$ ), concentração de  $\text{CO}_2$  de cloroplastos ( $C_c$ ), transpiração ( $E$ ), déficit de pressão de vapor (VPD), temperatura da folha ( $T_{\text{leaf}}$ ), eficiência no uso da água (WUE), eficiência intrínseca no uso da água (WUEi), taxa máxima de carboxilação de Rubisco ( $V_{\text{max}}$ ), taxa de transporte de elétrons ( $J_{\text{leaf}}$ ), área foliar específica (SLA), espessura da folha fresca (FLT) em *Dinizia excelsa*. \*: significativo ( $p \leq 0,05$ ); \*\*: significativo ( $p \leq 0,01$ ), ns: não significativo ( $p > 0,05$ ).

Parameter	$A_{\max}$	$A_{\text{pot}}$	$g_s$	$g_m$	$C_i$	$C_c$	$E$	VPD	$T_{\text{leaf}}$	WUE	WUEi	$V_{\text{max}}$	$J_{\text{leaf}}$	SLA	FLT
$A_{\max}$	—														
$A_{\text{pot}}$	0.75**	—													
$g_s$	0.95**	0.60**	—												
$g_m$	0.96**	0.82**	—	—											
$C_i$	0.66**	0.30*	0.78**	—	—	—									
$C_c$	0.92**	0.74**	0.90**	0.85**	—	—									
$E$	0.92**	0.74**	0.90**	0.85**	0.74**	—									
VPD	-0.52**	-0.12ns	-0.65**	-0.37*	-0.60**	-0.60**	—								
$T_{\text{leaf}}$	-0.32*	-0.03ns	-0.46*	-0.20ns	-0.38*	-0.38*	-0.08ns	—							
WUE	-0.28*	-0.21ns	-0.30*	-0.15ns	-0.59**	-0.59**	-0.57**	-0.29*	—						
WUEi	-0.7**	-0.35*	-0.77**	-0.49*	-0.96**	-0.96**	-0.79**	-0.42*	0.18ns	—					
$V_{\text{max}}$	0.82**	0.73**	0.65**	0.92**	0.21ns	0.21ns	0.67**	0.42*	0.18ns	0.73**	—				
$J_{\text{leaf}}$	0.81**	0.97**	0.63**	0.89**	0.32*	0.32*	0.79**	0.42*	0.18ns	0.73**	0.21ns	—			
SLA	-0.05ns	-0.30*	0.10ns	-0.18ns	0.24ns	0.24ns	-0.19ns	-0.63**	-0.08ns	-0.31*	0.81**	0.81**	—		
FLT	0.34*	0.08ns	0.46*	0.20ns	0.52**	0.52**	-0.43*	-0.40*	-0.40*	-0.19ns	0.09ns	0.09ns	0.09ns	—	
															0.58**

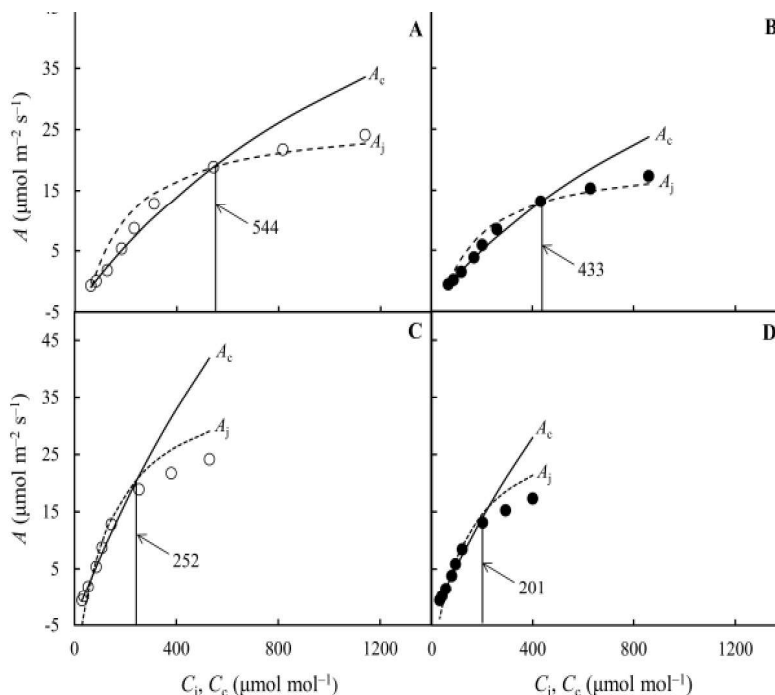


**Figure 1** – Water use efficiency (WUE; A) and intrinsic water use efficiency (WUEi; B) as a function of the  $g_m/g_s$  ratio in *Dinizia excelsa*. Each symbol corresponds to the mean of two leaflets per tree.

**Figura 1** – Eficiência no uso da água (WUE; A) e eficiência intrínseca no uso da água (WUEi; B) em função da relação  $g_m/g_s$  em *Dinizia excelsa*. Cada símbolo corresponde à média de dois folíolos (de duas folhas) por árvore.

and Demmig, 1987). Very low  $F_v/F_m$  values, e.g. 0.60 or lower (Magalhães et al., 2009) often indicate the occurrence of photoinhibition. Therefore, it is concluded that in this study there was no photoinhibition of photosynthesis in *D. excelsa*.

The  $A_{\max}$  values observed in this study are similar to those found in other studies, such as *D. excelsa* (Miranda et al., 2005), *Minuartia guianensis*, *Coussapoa orthoneura* and *Protium opacum* (Magalhães et al., 2009; Marenco et al., 2014). The chloroplast  $\text{CO}_2$  concentration ( $C_c$ ) was around 50% lower than the concentration observed in the intercellular spaces (Table 1). Taking  $C_i$  as the base line, the decline in  $C_c$  is due to the barriers against  $\text{CO}_2$  diffusion, from the intercellular space to the carboxylation site in the chloroplast (Niinemets et al., 2015; Tosens and Laa-



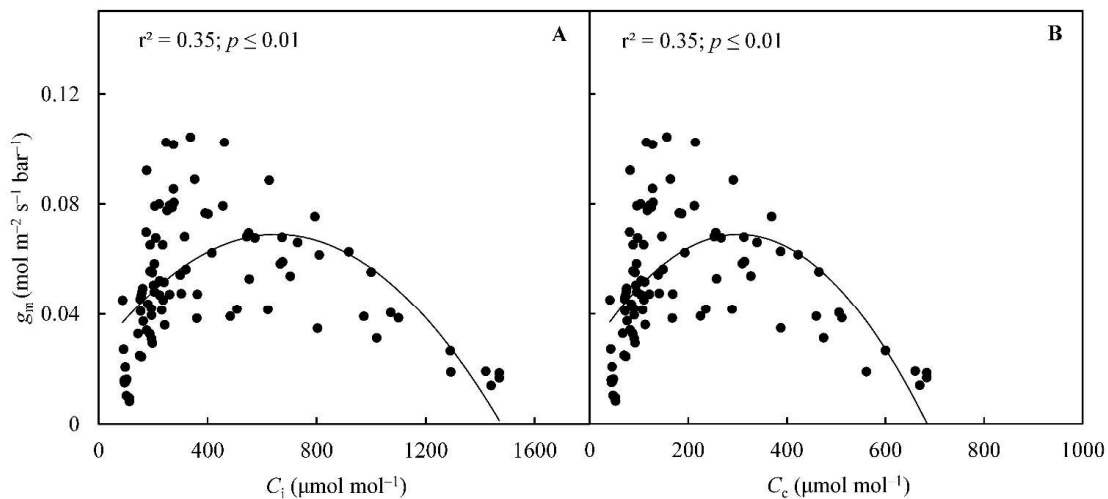
**Figure 2** – Photosynthesis as a function of the intercellular  $[CO_2]$ ,  $C_i$  (A, B) and chloroplast  $[CO_2]$ ,  $C_c$  (C, D) in sun leaflet (open circle,  $\circ$ ) and shade leaflet (closed circle,  $\bullet$ ). The solid line shows the photosynthesis limitation imposed by Rubisco carboxylation rate ( $A_c$ ), and the dashed line indicates the photosynthesis limitation imposed by electron transport rate ( $A_j$ ). The vertical solid line shows the value of  $C_i$  and  $C_c$  at the colimitation point of  $A_c$  and  $A_j$ . The numerals (in the panels) show the values for  $C_i$  and  $C_c$  in  $\mu\text{mol mol}^{-1}$  at the colimitation point. Each symbol represents the mean of six trees and two leaflets per tree.

**Figura 2** – Fotossíntese em função da concentração intercelular de  $[CO_2]$ ,  $C_i$  (A, B) e da  $[CO_2]$  no cloroplasto  $C_c$  (C, D) no folíolo de sol (círculo aberto,  $\circ$ ) e folíolo de sombra (círculo fechado,  $\bullet$ ). A linha sólida mostra a limitação da fotossíntese imposta pela taxa de carboxilação de Rubisco ( $A_c$ ), e a linha tracejada indica a limitação da fotossíntese imposta pela taxa de transporte de elétrons ( $A_j$ ). A linha vertical sólida mostra o valor de  $C_i$  e  $C_c$  no ponto de colimitação de  $A_c$  e  $A_j$ . Os numerais (nos painéis) mostram os valores para  $C_i$  e  $C_c$  em  $\mu\text{mol mol}^{-1}$  no ponto de colimitação. Cada símbolo representa a média de seis árvores e dois folhetos (de duas folhas) por árvore.

nisto, 2018). The greatest resistance to internal diffusion of  $CO_2$  seems to be related to plasma membranes and chloroplast membranes (Warren, 2009; Peguero-Pina et al., 2017).

At the ambient  $CO_2$  concentration ( $380 \mu\text{mol mol}^{-1}$ ) there was a close relationship between  $A_{\text{max}}$  and  $g_s$  and  $g_m$  (Table 2). This occurs because both conductances determine the  $CO_2$  flux to the carboxylation site in the chloroplast (Flexas et al., 2013). Stomatal and mesophyll resistance to  $CO_2$  flux account for about 40% of photosynthesis limitation in well-irrigated plants (Yamori et al., 2006; Tosens et al., 2016). Thus, the strong correlation between  $g_s$  and  $g_m$  indi-

cates a coupling between these parameters. The high correlation between  $A_{\text{max}}$  and  $g_s$  (and  $g_m$ ) suggests that diffusive factors were of paramount importance for determining carbon assimilation in *D. excelsa*. The decline of  $g_s$  under high VPD conditions corroborates that some ambient conditions can lead to a reduction in photosynthetic rates. Park and Furukawa (1999) showed that photosynthesis and stomatal conductance measured in tropical trees decreased due to increased VPD. Stomatal response to air humidity, temperature and VPD has been studied for decades, and it is known that stomata respond to changes in leaf tissue water content or to variations in guard cell water potential (Buckley, 2019).



**Figure 3** – Mesophyll conductance ( $g_m$ ) in response to the intercellular  $\text{CO}_2$  concentration ( $C_i$  – A) and chloroplast  $[\text{CO}_2]$  ( $C_c$  – B) in *Dinizia excelsa*. Each symbol corresponds to a value recorded on one leaflet.

**Figura 3** – Condutância do mesofilo ( $g_m$ ) em resposta à concentração intercelular de  $\text{CO}_2$  ( $C_i$  - A) e cloroplasto  $[\text{CO}_2]$  ( $C_c$  - B) em *Dinizia excelsa*. Cada símbolo corresponde a um valor observado em um folíolo.

Transpiration ( $E$ ) was strongly correlated with  $g_s$ , which is expected as the stomata play a key role in the control of leaf transpiration. Although  $g_m$  is expected to have little impact on leaf transpiration (Ouyang et al., 2017), in this study we found a positive correlation between  $g_s$  and  $g_m$ , which helps to explain the positive correlation between  $g_m$  and WUEi, as reported by Jahan et al. (2014). A high  $g_m/g_s$  ratio contributes to increased water use efficiency, particularly under water stress. Thus, it has been suggested that plants with potential for acclimation to drought have a high  $g_m/g_s$  ratio (Giuliani et al., 2013). The strong relationship observed between the  $g_m/g_s$  ratio and WUE and WUEi found in this study supports the hypothesis of coupling between  $g_s$  and  $g_m$ . These results suggest that in *Dinizia excelsa* water efficiency can be increased by improving  $g_m$ .

Besides  $g_s$ ,  $g_m$  also affects the photosynthetic capacity of the leaf, as shown by the close correlation between  $V_{cmax}$ ,  $J_{max}$ , and  $g_m$  (Table 2). Indeed,  $V_{cmax}$  and  $J_{max}$  were more influenced by  $g_m$  than by  $g_s$ . In Figure 2, one can see that Rubisco carboxylation rate was the most limiting factor for photosynthesis (up to the  $\text{CO}_2$  concentration indicated by the arrows), which is in agreement with Sage and Kubien (2007) and Mendes et al. (2017). At higher  $\text{CO}_2$  concentrations, however,

the electron transport rate became the most limiting factor a closer correlation between  $A_{pot}$  and  $J_{max}$  was observed, which is in agreement with Mendes et al., (2017).

It was observed that  $g_m$  showed a rapid response (minutes) to changes in  $\text{CO}_2$  concentration. However, it is unknown whether this response is a pattern also shared by other Amazonian tree species. At  $C_i$  values of 0–400  $\mu\text{mol mol}^{-1}$  ( $C_c < 200 \mu\text{mol mol}^{-1}$ ),  $g_m$  increased linearly with increasing  $\text{CO}_2$  until it reached a maximum value (at  $C_c \approx 300 \mu\text{mol mol}^{-1}$ ). On the other hand, at high  $C_i$  values  $g_m$  decreased with increasing  $\text{CO}_2$  concentration, which suggests that at that  $\text{CO}_2$  condition photosynthesis is no longer limited by the availability of  $\text{CO}_2$  at the intercellular spaces but by mesophyll resistance and electron transport rates.

Leaf thickness is one of the key leaf traits that affect  $g_m$  (Terashima et al., 2011). However, the significance of this effect was not detected in *D. excelsa* probably because there was little variation in FLT (0.16–0.21 mm). However, it is worth noting that FLT positively affected  $g_s$ ,  $C_i$  and  $C_c$ . This suggests that in thicker leaves there was an increase in the volume of intercellular space, perhaps as a mechanism to maximize mesophyll  $\text{CO}_2$  concentration. A large intercellular space can contribute to reduce the effect  $\text{CO}_2$



limitation under partial stomatal closure (Shao et al., 2008). SLA values recorded at the lower part of the canopy (shade leaflets, Table 2) were lower than those reported for understory trees (12 and 22 m<sup>2</sup> kg<sup>-1</sup>) in the central Amazon (Mendes et al., 2013), which indicates that even leaves of the innermost part of the canopy were receiving relatively high levels of solar radiation. An increase in SLA is often related to a decrease in leaf thickness. Thus, the results presented in this study differ from the classical pattern that shows a negative relationship between SLA and leaf thickness (Niinemets, 1999). This discrepancy can be attributed to the fact that in this study leaf thickness was measured in fresh leaves, and variation in leaf water contents may lead to divergence with the most common SLA-leaf thickness relationship.

### 5. CONCLUSIONS

The canopy leaves of angelim causes a decrease in irradiance (30-40%), which does not appear to be high enough to negatively affect important gas exchange parameters, such as  $V_{\text{cmax}}$  and  $J_{\text{max}}$ . This is quite important for the carbon economy of the tree, as it allows a maximum use of the solar energy received by the leaves. This ultimately contributes to enhance carbon uptake. As a result this species has a fairly fast growth rate, even when it produces wood of high density. On the other hand, an open canopy can lead to high transpiration rate, which might have a negative impact on photosynthesis if drought periods become longer as predicted by climate models.

In the study it is shown that the mesophyll resistance plays an important role in CO<sub>2</sub> diffusion. Therefore, it is recommended whenever possible to include  $g_m$  values in gas exchange calculations to obtain more accurate values of photosynthetic parameters. At low [CO<sub>2</sub>] the photosynthetic rates were limited by Rubisco carboxylation rate, but at high [CO<sub>2</sub>] photosynthesis was limited by both  $g_m$  and the electron transport rate. Changes in CO<sub>2</sub> concentrations have an effect on  $g_m$ , which has its maximum at  $C_c$  values of about 300 μmol mol<sup>-1</sup>. However, a substantial increase in atmospheric CO<sub>2</sub> concentration may lead to an increase (> 50%) of the mesophyll resistance. The results presented in this study may be useful in the construction of climate models that aim to predict the effects of global climate change on the Amazon ecosystem.

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