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Differential leaf traits of a neotropical tree *Cariniana legalis* (Mart.) Kuntze (Lecythidaceae): comparing saplings and emergent trees

Maria Cristina Sanches · Sérvio Pontes Ribeiro · Valdnéa Casagrande Dalvi · Milton Barbosa da Silva Junior · Hildeberto Caldas de Sousa · José Pires de Lemos-Filho

Abstract *Cariniana legalis* is an emergent tree that reaches the upper canopy in Brazilian Semideciduous Forest. Spatial contrasts in microclimatic conditions between the upper canopy and understorey in a forest may affect morpho-physiological leaf traits. In order to test the hypothesis that the upper canopy is more stressful to leaves than a gap environment we compared emergent trees of *C. legalis*, 28–29 m in height to gap saplings, 6–9 m in height, for the following parameters: leaf area, leaf mass area (LMA the dry weight:leaf area ratio), leaf thickness, leaf anatomical parameters, stomata conductance, and chlorophyll *a* fluorescence. Leaves from emergent trees had smaller leaf areas but greater LMA compared to saplings. Leaf thickness, palisade layer thickness, and stomatal density were higher for emergent trees than for saplings. The opposite pattern was observed for spongy layer thickness and spongy/palisade ratio. Stomatal conductance was also higher for emergent tree leaves than for sapling leaves, but the magnitude of depression on stomatal conductance near midday was more pronounced in emergent trees. The potential quantum yield of photosystem II, as determined by the F_v/F_m ratio was lower for leaves from saplings. The lower values of stomatal conductance, indicating restriction in CO_2 diffusion into the mesophyll can be related to higher photoinhibition observed in the saplings. Leaves from emergent trees and saplings exhibited similar values for apparent electron transport rates and non-photochemical quenching. Our results suggest that changes in leaf traits could be associated to dry conditions at the upper canopy as well as to the ontogenetic transition between sapling/emergent tree life stages.

Keywords Forest canopy · Leaf anatomy · Leaf mass area · Stomatal conductance · Stomatal density

M. C. Sanches (✉)
Instituto de Biologia, Campus Umuarama, Bloco 2D,
Universidade Federal de Uberlândia, Caixa Postal 593,
Uberlândia, MG 38400-902, Brazil
e-mail: sanchesmc@yahoo.com.br

S. P. Ribeiro · V. C. Dalvi · M. Barbosa da Silva Junior ·
H. Caldas de Sousa
Departamento de Biodiversidade, Evolução e Meio Ambiente,
Universidade Federal de Ouro Preto, CEP 35400-000,
Ouro Preto, MG, Brazil
e-mail: spribeiro@iceb.ufop.br

V. C. Dalvi
e-mail: dya_casagrande@yahoo.com.br

M. Barbosa da Silva Junior
e-mail: miltonbsjunior@yahoo.com.br

H. Caldas de Sousa
e-mail: hcdsousa@iceb.ufop.br

J. Pires de Lemos-Filho
Departamento de Botânica, Instituto de Ciências Biológicas
Universidade Federal de Minas Gerais, CEP 3161-970,
Belo Horizonte, Brazil
e-mail: lemos@icb.ufmg.br

Introduction

Leaves from emergent trees developed at the upper canopy in tropical forests face higher irradiances, higher air temperature variance, and higher wind speeds and thus, must cope with microclimatic conditions that potentially can lead to desiccation, while leaves from seedlings and/or saplings growing in lower stratum canopy experience milder conditions, such as lower irradiance and constant temperature, but higher humidity. These contrasts must affect leaf structure and physiology during life and

ontogenetic development of tree (Niinemets 1997; Cavender-Bares and Bazzaz 2000; Ishida et al. 2005).

In general, under higher light conditions plants will produce thicker leaves than in the shade (Boardman 1977). Variation in leaf thickness is largely due to the formation of taller palisade cells and/or an increase in the number of palisade layer cells. These traits are considered as an important mechanism by which plants under high light conditions achieve a high photosynthetic capacity (Lambers et al. 1998; Kenzo et al. 2004). However, high irradiances associated with a high vapour pressure deficit (VPD) may lead to strong midday stomatal closure and depression of photosynthesis in leaves produced in the upper canopy (Roy and Salager 1992; Koch et al. 1994; Zotz and Winter 1996; Nabeshima and Hiura 2008). Leaf physiological traits can be adversely affected by water stress conditions that are also related to hydraulic constraints due to tree height and desiccation in the tree tops (Matsuzaki et al. 2005; Kitahashi et al. 2008). If stomatal closure occurs, it can result in a decrease in the intercellular CO_2 concentration and cause midday depression of photosynthesis. In this situation, the absorption of an excessive amount of photons compared to that used in photosynthesis may cause photoinhibition, as measured by a decline in the potential efficiency of photosystem II (PSII) (Powles 1984; Krause et al. 2001).

Studies that focus on physiological and anatomical traits of trees growing under natural conditions are scarce in the tropical forests from the southeast region of Brazil. *Cariniana legalis* (Mart.) Kuntze (Lecythidaceae) locally known as "jequitibá" tree, is a semideciduous species described by Martins and Rodrigues (2002) as a late secondary successional species. This species is typical of the Brazilian Semideciduous Forest. To our knowledge, this paper is the first one that compares eco-physiological parameters on emergent trees (28–31 m height) with saplings (6–9 m) of *Cariniana legalis*, growing in this forest.

We hypothesized that upper canopy conditions are more stressful for tree leaves, resulting in a tendency for xeromorphic leaf traits that are not presented in lower, younger plants of the same species. We predict that leaf area per leaf would be lower and leaf mass per unit area would be higher in adult compared with young trees, hereafter called emergent trees and saplings, respectively. We also predicted that leaves formed at the upper canopy would have an extensive development of palisade parenchyma in the mesophyll with a corresponding decrease in spongy mesophyll and intercellular spaces, resulting in a thicker, denser leaf compared to saplings (Dickson 2000). In addition, due to the environmental conditions at the upper canopy midday stomatal closure and photoinhibition would be more pronounced in adult trees than in saplings. We

investigated several leaf traits, such as leaf area, leaf mass area (the leaf dry weight:leaf area ratio—LMA), leaf thickness, and various anatomical parameters. Also, we investigated stomatal conductance and chlorophyll *a* fluorescence.

Materials and methods

Study site and selected species

The measurements were taken in trees within the largest preserved remnant of Atlantic rainforest in the Minas Gerais State, Brazil, with 36,000 ha (19°29′24″ W–42°28′18″E; 230–515 m altitude). The climate is characterized by a dry season with mild temperatures (12–27°C) from April to September and a wet and warm season (18–34°C) from October to March. Based on the last 20 years, average annual precipitation, relative humidity and temperature at the study site are 1,300 mm, 79% and 23°C, respectively (Lopes et al. 2002). The study site was representative of a typical semideciduous tropical forest with 20 to 50% deciduous trees (Veloso et al. 1991).

Cariniana legalis (Mart.) Kuntze (Lecythidaceae) is a semideciduous tree that is considered shade tolerant and is amongst the tallest tree from the Brazilian flora (Lorenzi 1992). Its geographical distribution encompasses any lowland semideciduous forests from eastern to western South America, around the tropic of Capricorn. According to Martins and Rodrigues (2002) this is a late successional species typical of regions with a well-defined dry season. Seedlings and saplings are scarce at the floor forest, but sapling trees were observed growing in small gaps in our study site. Mature adult trees can reach the upper canopy and some emergent individuals can be up to 50-m tall, making them an important model species for studying morphological and physiological responses to the upper canopy habitat.

We selected three sapling individuals of *Cariniana legalis* with height ranging from 6 to 9 m growing in a small gap (about 50 m^2), and 3 emergent trees of the same species with height ranging from 28 to 30 m. Leaves in the upper portion at the canopy of emergent trees and saplings were selected for all measurements. The gap was located in a downhill portion of the forest, which influenced the light angle that penetrated into gap. Mean stem diameter at ground level was 142.7 cm and 20.5 cm for emergent trees and saplings, respectively. The study was carried out at the end of dry season from 3 to 11 October 2005, when the leaves were exposed to potentially more stressful environmental conditions for their physiological performance, such as high temperatures, high irradiances, and high water deficit compared to the wet season.

Canopy access

The access to leaves was done by using climbing techniques, plus safety crown movement techniques. We used leaves localized at the periphery of the tree crowns, which, for the emergent trees, required the development of a safety procedure beyond the usual trunk-based climbing methods. However, as these trees were emergent, and therefore had their crowns opened up above most of the other upper canopy crowns, the branches expanded further from the trunk in an almost horizontal position, with three layers of very large branches (above 1.5 m circumference at the base). By taking advantage of this crown architecture, a trapezium rope system was set from the trunk up to an upper branch that was then sampled, thus compensating the climber weight and providing a balance while allowing access to the branch extremity. We sampled healthy and totally expanded leaves in the crown using this method. The leaves measured were positioned at a height of 25 m and total height of the tree was about 30 m.

Environmental variables

The daily photosynthetic photon flux density (PPFD: 400–700 nm) was recorded from the leaf level and full sun with a LI-190SA quantum sensor (Li-Cor, Inc., Lincoln, NE). Dry and moist bulb thermometers (Incoterm, Brazil 5195) were installed at the upper canopy of *C. legalis*, and the forest floor to measure relative humidity (RH) and vapour pressure deficit (VPD). The same measurement was made in the gap, around the saplings trees.

Leaf morphology and anatomical traits

Twelve leaves from each individual were harvested, sealed in plastic bags, and immediately stored in a cold box to prevent water loss. Afterwards, the leaves were brought to the laboratory for morphological measurements. Leaves were scanned and the area calculated through the program AREA 2.1 C (Caldas et al. 1992). The leaves were then dried at 70°C for 48 h before the leaf dry mass measurements. From these data the leaf mass area (LMA) was determined. Another set of healthy leaves ($n = 15$) was used for histological analyses. Slices from leaves were cut with a razor and fixed in a solution of 50% FAA (form-aldehyde:ethanol:[50%]:acetic acid 18:1:1). Hand-cut transverse sections were taken from the mid-lamina region of the leaf to avoid variation in thickness along the blade. Leaf thickness (LT), palisade layer thickness (PLT), and spongy layer thickness (SLT) were measured with a compound microscope equipped with a $40\times$ objective and calibrated with an ocular micrometer attached to a LEICA DMLS 30 microscope. In addition the spongy/palisade ratio and palisade/total layer thickness ratio were calculated. Stomatal density and cell guard length were determined from the epidermis dissociation method by Jeffrey (Johansen 1940).

Stomatal conductance and chlorophyll *a* fluorescence

The daily course of stomatal conductance to water vapour (g_s—mmol m^{-2} s^{-1}) was determined with an AP4 (Delta-T, Cambridge, UK) porometer. We sampled four fully expanded and healthy leaves from each individual. Leaf temperature (T_{leaf}) at the lower surface of the enclosed leaf was measured with a thermocouple from the porometer holder. Also diurnal patterns of chlorophyll *a* fluorescence were performed with a pulse-amplitude modulated photosynthesis yield analyser (MINI-PAM, Heinz Walz, Effeltrich, Germany) equipped with a leaf clip holder (Model 2030-B Walz) on the same leaves that were used for the g_s measurements. In order to obtain the values of the potential quantum yield of PSII ($(F_m - F_o)/F_m = F_v/F_m$), the maximum fluorescence (F_m) and minimum fluorescence (F_o) were measured in darkness before dawn and after 30 min of dark adaptation during the day. The maximum fluorescence (F_m') and steady-state fluorescence (F) in the light-adapted state were measured three times during the day, following the methods described by Bilger et al. (1995). Saturated light pulses of 700 ms duration were applied through a fibre-optic cable oriented at 60° to the leaf surface at the prevailing irradiance. The angle and distance between the leaf and the fibre-optic cable were manually adjusted and maintained with the leaf clip holder. For measurements of the light-adapted state, care was taken not to shade the leaves by the fibre-optic probe and the leaf clip holder. The effective quantum yield of PSII was calculated according to Genty et al. (1989) as $(F_m' - F)/F_m' = \Delta F/F_m'$. Non-photochemical quenching (NPQ) was calculated according to Maxwell and Johnson (2000) as NPQ $= (F_m - F_m')/F_m'$. The pre-dawn measurement values of maximum fluorescence (F_m) were used to calculate this quenching parameter. The apparent electron transport rate through PSII (ETR) was calculated as ETR $= \Delta F/F_m' \times 0.5 \times$ PPFD where, $\Delta F/F_m'$ is the photochemical yield of the light; PPFD corresponds to radiation incident on the leaf surface in μmol m^{-2} s^{-1}; the 0.5 is a multiplication factor which assumes that the transport of a single electron requires the absorption of 2 quanta (White and Critchley 1999).

Statistical analysis

The daily courses of stomatal conductance and chlorophyll fluorescence were evaluated by parametric statistics with average and standard error. The comparison between emergent trees and saplings for physiological and anatomical leaf traits was carried out by the Student's *t*-test at

the 5% level. Linear regressions were performed to investigate whether differences in leaf physiology between emergent trees and saplings were related to differences in microclimate, particularly VPD.

Results

Environmental variables

In the upper canopy the maximum T_{air} and VPD were 33°C and 2.75 kPa, respectively while in the gap, maximum air temperature was 28°C and the vapour pressure deficit was 1.32 kPa (Fig. 1). The maximum PPFD values reached in the upper canopy and in the small gap are presented in Table 1. The light intensity varied in terms of the time of the day when the maximum of PPFD values was achieved in each environment. In the morning (0800–09:00 h) the PPFD at full sun and leaf level on emergent trees was 1,476 and 836 µmol m^{-2} s^{-1}, respectively (Table 1). At about midday, the PPFD in the upper canopy remained high at 1,320 µmol m^{-2} s^{-1}, but the light intensity that reached the leaf level was only 78 µmol m^{-2} s^{-1}. The sampled leaves in emergent trees were overlap by more external branches, which explain the low values of incident light measurements. In the small gap the maximum PPFD value attained during midday was 1,237 µmol m^{-2} s^{-1} while the value reached at leaf level was 243 µmol m^{-2} s^{-1}. In general drastic reductions of PPFD were observed during the afternoon (between 14:00 and 15:00 h) for the saplings. The decrease of the PPFD in the gap was due to shading by the surrounding forest.

Leaf morphology and anatomical traits

Leaves from emergent trees showed higher LMA, a higher stomatal density and had shorter guard cells in relation to saplings (Table 2). Also, leaves from emergent trees showed significantly higher PLT and higher palisade/total layer thickness while SLT and spongy/palisade ratio were higher for saplings (Table 2). Leaf cross-sections indicated that leaves from emergent trees were isobilateral and had one to two well-developed palisade layers, whereas the contribution of the spongy layer to total leaf thickness was higher in saplings (Fig. 2). The variation in leaf mass per unit area was positively associated with variation in leaf thickness (Fig. 3).

Stomatal conductance, leaf temperature, and chlorophyll *a* fluorescence

Leaves from both emergent trees and saplings exhibited midday decreases in g_s. However, the proportion of this
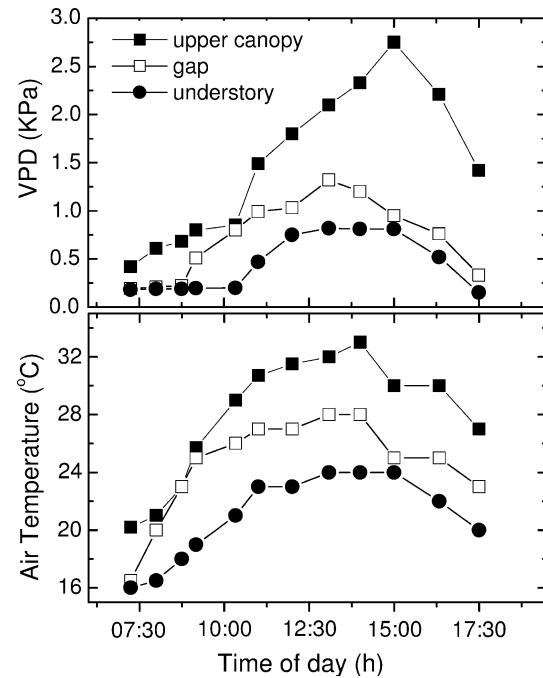

Fig. 1 Vapour pressure deficit (VPD) and air temperature in a Brazilian semideciduous tropical forest measured on 8 October at the upper canopy and the understory and 9 October 2005 at a small gap

decrease differed between the habitats (Fig. 4). The maximum g_s value observed for emergent trees was 283 mmol m^{-2} s^{-1} around 08:00 h, which decreased to 123 mmol m^{-2} s^{-1} near midday, a decrease of 56%. Emergent trees also showed a tendency for recovery of g_s in the afternoon near 14:00 h. Leaves from saplings exhibited a maximum g_s value of 126 mmol m^{-2} s^{-1} around 10:30 h and 76 mmol m^{-2} s^{-1} around midday, a decrease of 44%. The lowest g_s values for saplings were measured during the afternoon. Leaf temperature in emergent trees changed from 23.4 to 31.7°C while T_{leaf} in saplings varied from 25.8 to 32.2°C. In the first case T_{leaf} in emergent trees did not exceed air temperature, but T_{leaf} in saplings was 4°C higher than T_{air} (Fig. 4). For both, emergent trees and saplings, g_s was negatively correlated with VPD (Fig. 5).

Leaves of both emergent trees and saplings exhibited decreases in F_v/F_m values during the day. The minimum values of F_v/F_m for emergent trees were obtained around 10:00 h and the minimum values for saplings were found near midday (Fig. 6). The minimum values of F_v/F_m for emergent tree leaves and saplings were 0.786 and 0.749, respectively, indicating a lack of strong photoinhibition. Higher values of effective quantum efficiency of PSII ($\Delta F/F_m'$) of both emergent and sapling leaves were recorded in the morning. The $\Delta F/F_m'$ values were influenced by PPFD mainly in saplings. The maximum ETR values were observed during morning for emergent trees and at midday for saplings. This difference of when ETR values peaked

Table 1 Mean photosynthetic photon flux density (PPFD, $\mu mol\ m^{-2}\ s^{-1}$) $\pm$ SE in a semideciduous tropical forest in the upper canopy (UC) and in a small gap (SG), $n = 12$

Time	08:00–09:00	12:00–13:00	14:00–15:00	16:00–17:00
UC				
Full sun	1,476.24 ± 103.31	1,320.42 ± 161.44	1,010.08 ± 168.32	48.15 ± 12.04
Leaf level	836.40 ± 128.72	78.75 ± 20.25	70.61 ± 13.91	23.90 ± 4.99
SG				
Full sun	1,125.75 ± 113.21	1,237.18 ± 96.11	412.55 ± 110.9	22.90 ± 3.17
Leaf level	231.10 ± 71.85	243.28 ± 29.04	21.66 ± 2.85	16.02 ± 4.83

Table 2 Leaf characteristics of *Cariniana legalis* (Mart.) Kuntze from Southeast Brazilian Tropical Forest

Leaf characteristics	Emergent trees	Saplings
Leaf area (cm^2)	12.43 ± 0.74	19.72 ± 0.82
Leaf mass area ($g\ m^{-2}$)	97.0 ± 5.6	57.5 ± 1.4
LT (μm)	212.17 ± 11.52	129.51 ± 7.26
PLT (μm)	140.31 ± 6.59	39.42 ± 1.77
SLT (μm)	44.4 ± 4.4	69.89 ± 5.63
Spongy/palisade ratio	0.38 ± 0.02	1.77 ± 0.38
Palisade/total layer thickness	0.67 ± 0.009	0.31 ± 0.002
SD (number/mm^2)	322.51 ± 9.49	153.16 ± 5.62
Cell guard length (μm)	21.10 ± 0.15	22.65 ± 0.18

The data represent mean (±SE). All means in each column differed at $P < 0.05$ (*t* Student test)

LT leaf thickness; *PLT* palisade layer thickness, *SLT* spongy layer thickness, and *SD* stomatal density

was probably occasioned by actual PPFD values that reached the leaves. Adult tree leaves showed NPQ values around 1.6, while the maximum value for the leaves of the saplings was 1.3.

Discussion

Tropical forests are characterized by great variations in their environment, both in time and space (Chazdon et al. 1996; Lüttge 1997). In our study, a vertical gradient in environmental conditions among understorey, gap, and upper canopy locations were observed. In the morning, PPFD were higher on the upper canopy leaves compared with the leaves of the saplings. However, at midday a reduction in light intensity was detected at leaf level, even in the upper canopy. The presence of clouds was not responsible for this reduction since that the day of the measurements was clear. Hence, the distribution and proportion of overlapping leaves as well as the crown architecture that produces a complex topography, and thus an extreme variation in light over short distances are related with the light reduction (see Mulkey et al. 1996). Although the leaves of adult plants were not fully

sun-exposed, they grew in a higher height than leaves from saplings. Under this condition the leaves from emergent trees had higher T_{air} and higher VPD in relation to saplings, and were exposed to drier conditions. It appears that these microclimatic variations influenced several morphological and physiological leaf responses. In general, the leaf area was lower in emergent trees, but the LMA was higher in leaves from emergent trees when compared to saplings, which corroborates our hypothesis of a more stressful habitat in the canopy. Indeed the LMA is a leaf trait subjected to strong environmental influences, being the variations between the same species are affected not only by light conditions but also by water availability (Lusk et al. 2008). Similarly high LMA, an indicator of xeromorphic leaves, in the canopy compared to the understorey was also found by Ribeiro and Basset (2007) for 73 species of trees and lianas in Panama.

Lower values of leaf area in emergent trees exposed to drier conditions can be an important mechanism by which plants regulate their T_{leaf}. In the morning, leaves from emergent trees exhibited lower T_{leaf} than saplings and, at midday its T_{leaf} was slightly below T_{air}. Although smaller leaves show a reduced area for transpiration, they have an improved efficiency for heat exchange due to their lower boundary layer resistance (England and Attiwill 2006). Thus, smaller and narrower leaves tend to have temperatures closer to T_{air} than large leaves (Lambers et al. 1998). On the other hand, the higher T_{leaf} observed in saplings can be related to their lower stomatal density and consequently lower g_s as compared with leaves from emergent trees. Moreover, the higher leaf area as observed in leaves from saplings (and lower wind speeds too) also contribute to increases in T_{leaf} because they result in a larger boundary layer.

Differences in LMA must be due to variations in leaf thickness, density, or both (Witkowski and Lamont 1991). We found a significant correlation between leaf thickness and LMA. As in other studies (Rozema et al. 1997; Rôças et al. 1997, 2001; Ishida et al. 2005) the mesophyll of leaves from the upper canopy had a well-developed palisade layer, while those, which developed in a small gap, were thinner and had a smaller palisade layer. Leaves from

Fig. 2 Leaf cross-section of mature leaves from saplings (**a**) and emergent trees (**b**) of *Cariniana legalis*. *Scale bars* 100 μm
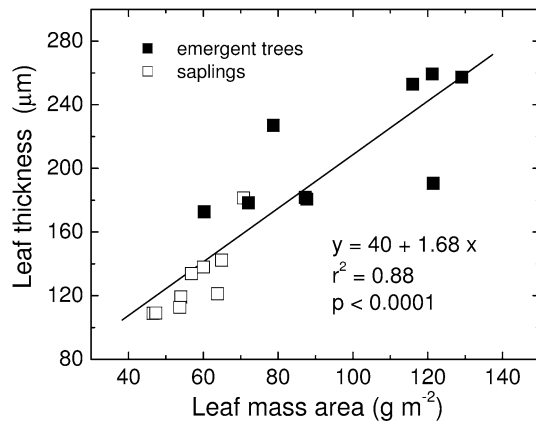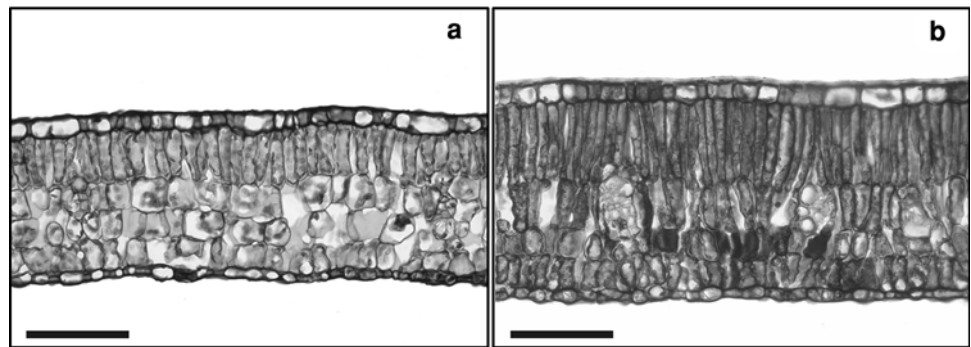

Fig. 3 The relationship between leaf thickness and leaf mass area for leaves from saplings (*open square*) and emergent trees (*filled square*) of *Cariniana legalis* from a Brazilian semideciduous tropical forest

emergent trees with high LMA were thicker and had shorter cell guards and higher stomatal densities. Such traits are a consequence of the more stressful environment in the upper canopy, further corroborating our hypothesis, i.e., for a tendency for xeromorphic traits in emergent tree leaves compared to leaves from saplings. Higher light, temperature, and VPD must play a role in these responses, but the adult tree stature must also be taken into account. Moreover, the different leaf traits that we found could be related to the size of the saplings and emergent trees. Tall trees have an increased hydraulic and gravitational resistance to water flow in the xylem, as water has to travel a longer distance from the soil to a leaf (Ryan and Yoder 1997; Ryan et al. 2006). As a result, the turgor pressure caused by water movement into the cells would remain impaired, resulting in reduced leaf growth (Woodward 2004; England and Attiwill 2006). This hypothesis was proposed by Thomas and Winner (2002) to explain increases in LMA as a function of height in all tree species in their meta-analysis study from conifers, temperate deciduous, and tropical tree species. Indeed, other studies have also shown a positive relationship between LMA and tree height (Poorter et al. 1995; Niinemets 1997; Rijkers
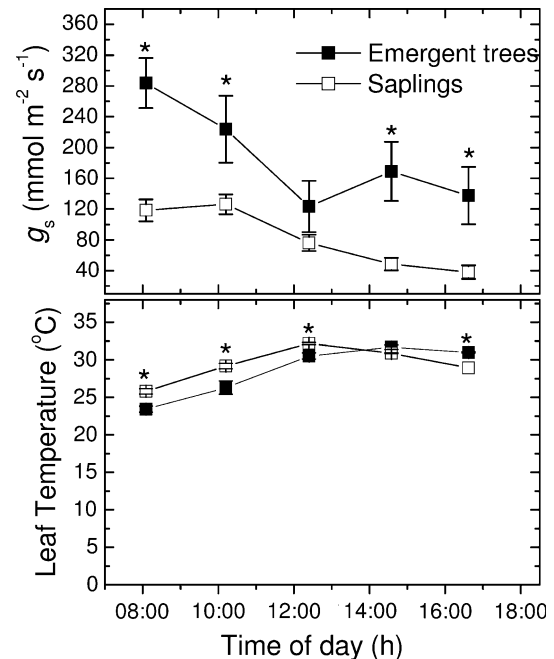

Fig. 4 Diurnal changes in stomatal conductance (g_s—mmol m^{-2} s^{-1}) and leaf temperature for leaves of *Cariniana legalis* growing at upper canopy, 25 m and at gap, 9 m. Each point is the average of four leaves from three individuals ± SE. *Asterisk* indicates a significant difference ($P < 0.05$) between emergent trees and saplings. The data were obtained on October 2005

et al. 2000; Martínez-Garza and Howe 2005; Ribeiro and Basset 2007).

Emergent trees exhibited a twofold difference in maximum g_s in relation to saplings. The greater g_s values could be related to higher stomatal density in adult trees, as demonstrated by Pearce et al. (2005). Both emergent trees and saplings showed a decline of g_s near midday and were sensitive to increases to VPD and temperature. However, the magnitude of depression and the slope of the relationship between g_s and VPD were more pronounced in emergent trees when comparing to saplings. Furthermore, emergent trees showed higher g_s at low VPD resulting in higher g_s maximum values in relation to saplings. This result is in agreement with the general tendency that
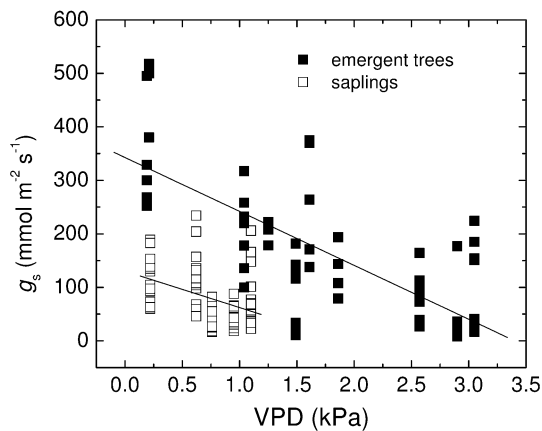
Fig. 5 The relationship between stomatal conductance (g_s) and vapour pressure deficit (VPD) in emergent trees (*filled square*) and saplings (*open square*) of *Cariniana legalis* from a Brazilian semideciduous tropical forest. The *lines* are regressions fitted to the data. For emergent trees: y = 342.49 − 100.69x, R^2 = 0.71, $P < 0.0001$, for saplings: y = 130.35 − 68.18x, R^2 = 0.40, $P = 0.0002$

species with high g_s under low VPD tend to be more sensitive to increases in VPD (Oren et al. 1999; Addington et al. 2004). The daily course of g_s revealed this tendency, where reductions of 56% of g_s for leaves from emergent trees were observed near midday.

The marked control of stomatal aperture can be considered crucial for leaves that are developed in the upper canopy. The regulation of stomatal aperture is the main mechanism used by plants to prevent water loss and has been considered as an important leaf response to water stress and high T_{air}, such as those found in the upper

canopy (Matsuzaki et al. 2005). Midday reductions in g_s are frequently observed on bright days as a consequence of the increase of VPD (Zotz and Winter 1996; Meinzer et al. 1999). This phenomenon is widely known for plants in arid regions (Tenhunen et al. 1984) and savannas (Franco and Lüttge 2002; de Mattos et al. 2002). For moist tropical forest, where climatic conditions are milder, such a mechanism has also been described for canopy species (Aylett 1985; Roy and Salager 1992; Zotz and Winter 1996; Kitahashi et al. 2008).

However, stomatal closure may lead to increased susceptibility to photodamage resulting in the reduction of quantum yield, which can be determined by the ratio between F_v/F_m (Björkman 1981; Powles 1984; Muraoka et al. 2000). Values of F_v/F_m around 0.8 indicate healthy and not photoinhibited leaves (Lüttge et al. 1998), and thus our results from the daily course of F_v/F_m indicated photoinhibition, since F_v/F_m values were lower than 0.8 at some hour of the day for both emergent trees and saplings. In spite of marked stomatal control near midday, fast recovery of F_v/F_m values was found for emergent trees. This recovery may be related to light variation that reaches leaves in the upper canopy, or to a leaf anatomy better acclimated to the stress condition. Moreover, F_v/F_m values from emergent trees were higher when compared to those from saplings. This indicates absence of photoinhibitory damage to PS II, even at the upper canopy, where microclimatic conditions are propitious for photoinhibition occurrence. The same result was found for leaves from the upper canopy in the temperate species *Fagus crenata* (Iio et al. 2004) and the tropical tree *Macaranga conifera* (Ishida et al. 1999). Thus, in *C. legalis*, as leaf structure

Fig. 6 Diurnal changes in F_v/F_m ratio, actual PSII efficiency ($\Delta F/F_m'$), apparent electron transport rate (ETR), and non-photochemical quenching (NPQ) in emergent trees and saplings of *Cariniana legalis*. Each point is the average of four leaves from three individuals ± SE. *Asterisk* indicates a significant difference ($P < 0.05$) between emergent trees and saplings. The data were obtained on October 2005
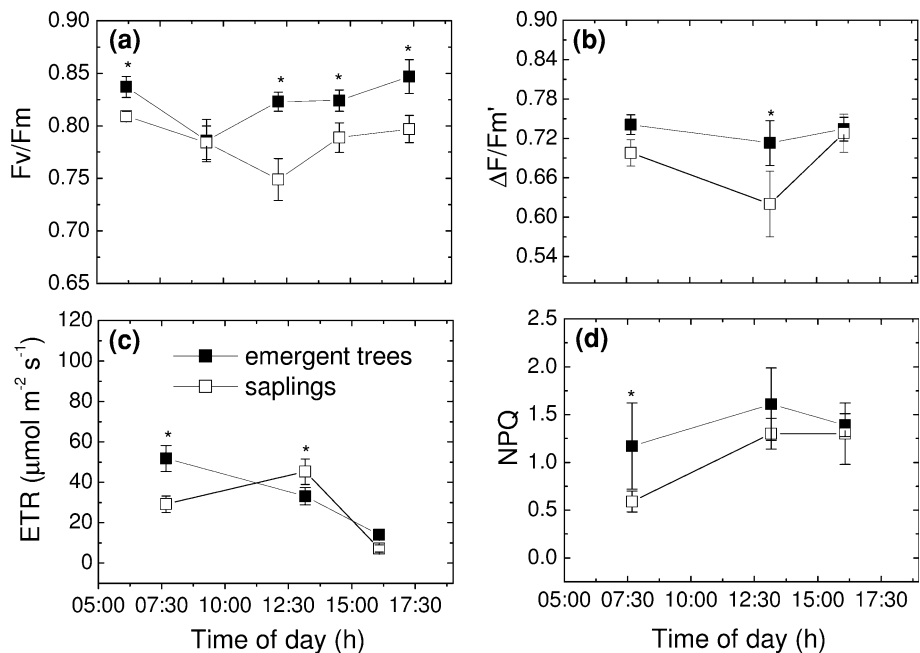
changes, and so does physiology, both structure and physiology change in a coordinated manner to adapt these leaves to the drier environment of the upper canopy.

On the other hand, leaves from saplings were characterized by a more pronounced decline in F_v/F_m and their values were lower in relation to emergent trees. Values of F_v/F_m lower than 0.8 indicate occurrence of plant stress. Susceptibility of photoinhibition may be determined by several structural and physiological leaf traits (Araus and Hogan 1994; Krause et al. 1995, 2001). Ribeiro et al. (2005) working with several tropical tree seedlings under controlled light conditions, showed that *C. legalis* was the only species that suffered photoinhibition and this species was not able to recover F_v/F_m values by the end of the day. One explanation for this difference is the fact that the work of Ribeiro et al (2005) was carried out with potted seedlings, which could limit the root growth and lead to some nutrition stress. But, the size pot used by them was high enough to allow root growth. In this case, the results from Ribeiro et al. (2005) and ours suggest that the ontogenetic transition between seedlings, saplings, and emergent trees can be related to differences in physiological leaf traits, such as susceptibility to photoinhibition.

Moreover, the ETR and NPQ values were low for both adult and saplings. Ribeiro et al. (2005) found similar results in a study of photosynthetic responses conducted under controlled light conditions. In their study, the ETR values for *C. legalis* under full sunlight and 34% of sunlight were 50 and 35 μmol m^{-2} s^{-1}, respectively, and no significant differences were observed in these two treatments. Low ETR values can be a specific trait of this species, indicating a low concentration of the thylakoid proteins responsible for electron flow. Besides, in saplings the reduction of $\Delta F/F_m'$, i.e., the quantum yield in light, was followed by maintenance of ETR. This suggests a detour of electron flux to another process not directly involved in carbon assimilation, such as photorespiration (Lambers et al. 1998). In the saplings T_{leaf} was higher than that for adult trees. The solubility of CO_2 declines faster than O_2 with increasing temperature, favouring the oxygenating reaction of Rubisco (Muraoka et al. 2000; Franco and Lüttge 2002). The low values of NPQ, which is related to thermal dissipation of excess light, suggest that an efficient process of light utilization may be operating in these species.

In response to a drier environment at the upper canopy, emergent trees of *C. legalis* showed leaves with lower values of leaf area and higher LMA, even though marked control of stomatal aperture was observed in both emergent trees and saplings. However, due to their stomatal in higher density and reduced size, the magnitude of depression on g_s was higher in leaves from the emergent trees. Our results suggest that the differences in physiological and morphological leaf traits may be related not only to the micro-climatic conditions associated to the upper canopy, but also to greater distance between soil and leaf of emergent trees which may induce hydraulic constraints and also to ontogenetic transition between saplings to emergent trees (Rijkers et al. 2000; Thomas and Winner 2002; Martínez-Garza and Howe 2005; Ishida et al. 2005; Rozendaal et al. 2006). These differences in leaf traits, i.e., its phenotypic plasticity can be important for *C. legalis* to exploit distinct environments in the Brazilian Tropical Forest (Lusk et al. 2008).

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