RESEARCH ARTICLE

Growth, photosynthesis and leaf water potential in young plants of *Copaifera langsdorffii* Desf. (Caesalpiniaceae) under contrasting irradiances

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ABSTRACT

Growth and leaf nutrient content were compared in young potted plants of C*opaifera langsdorffii* in sunny and shaded areas without water stress. Besides, carbon assimilation and leaf water relations were evaluated by net photosynthesis, potential photochemical efficiency and leaf water potential during daily courses in dry and rainy periods under natural conditions in both contrasting irradiances. Higher values of total biomass, height and leaf area occurred in sunny than in shaded area. On the other hand, all young plants survived in shade under natural water stress probably by reason of fast and intense biomass accumulation in favor of roots in early development. There was no significant difference about nutrient concentration in leaves between plants growing in sunny and shaded areas. Net photosynthesis in shade increased occasionally when bunches of direct light reached the leaves. Theses sunflecks took place more frequently and at high intensity in dry period but they were more effective for net photosynthesis in rainy period. The ability of young plants to persist under natural conditions in contrasting irradiance up to 1,230 days after sowing could explain the wide distribution of *C. langsdorffii* in Cerrado physiognomies and in different types of forest.

Key words: Cerrado, biomass partitioning, chlorophyll fluorescence, leaf gas exchange, irradiance, sunflecks.

RESUMO

O crescimento e o conteúdo de nutrientes nas folhas foram comparados em plantas jovens envasadas de C*opaifera langsdorffii* crescendo em área sob irradiância total e em área sombreada sem estresse hídrico. A assimilação de carbono e as relações hídricas foram avaliadas por meio da fotossíntese líquida, da eficiência fotoquímica potencial e do potencial hídrico foliar durante cursos diários no período seco e chuvoso nas duas áreas com irradiâncias contrastantes. Maiores valores de biomassa total, altura e área foliar ocorreram na área sob irradiância plena. As plantas jovens sobreviveram sob estresse hídrico em condições naturais na área sombreada provavelmente em função do rápido e intenso acúmulo de biomassa nas raízes logo no início do desenvolvimento. Não houve diferença significativa em relação à concentração de nutrientes entre as plantas crescendo sob diferentes regimes de iluminação. A fotossíntese líquida sob sombreamento aumentou ocasionalmente quando feixes de luz que atravessaram as copas atingiram diretamente as folhas de *C. langsdorffii*. Esses feixes de luz ocorreram com maior intensidade e mais freqüentemente no período seco, mas foram mais efetivos para a assimilação líquida no período chuvoso. A habilidade das plantas jovens persistirem em condições naturais sob os dois regimes contrastantes de irradiância até 1.230 dias após a semeadura pode explicar a vasta distribuição de *C. langsdorffii* nas diferentes fisionomias do Cerrado e em outras formações florestais.

Palavras-chave: Cerrado, feixes de irradiância, fluorescência da clorofila, partição da biomassa, sobrevivência, trocas gasosas foliares.

INTRODUCTION

The Brazilian Cerrado is the greatest region of neo-tropical savannas of the world occupying approximately 2 x 10^6 km^2 in Brazil. Gallery forests, semideciduous forests, and palm swamp co-occur with Brazilian neo-tropical savannas that show different physiognomies (Ratter et al., 2003). There are many environmental factors such as distrophic soils (Haridasan, 2008) and contrasting irradiance patterns (Nardoto et al., 1998; Kanegae et al., 2000) that impose adjustments to Cerrado plants during their life cycle, especially in young plants growing under contrasting irradiance (Ronquim et al., 2003; Prado et al., 2006).

Distinct light regimes in each cerrado physiognomy may promote the establishment of different species assemblage or functional types. Shade tolerant species are found in Cerrado physiognomy with tall trees, where closed canopy decreases the irradiance load on beneath strata (Kanegae et al., 2000). However, species adapted to high irradiance loads should be more easily found in open sites as in cerrado *stricto sensu* or cerrado grassland physiognomy (Hoffman et al., 2004). The great discrepancy of irradiance availability in Cerrado physiognomies could benefit the species with high acclimation capacity. Adjustments in growth and photosynthesis to different light regimes are one of the most important features that remain obscure in Cerrado vegetation (Franco, 2002) especially about the photosynthetic response to light bunches in beneath strata.

The low availability of irradiance under canopy may decrease the biomass accumulation and the tolerance to environmental stresses as water shortage, fire and herbivore (Hoffmann and Franco, 2003; Prado et al., 2006). Sunflecks frequency and the decreased of respiration rate in leaves are important factors for young plant to survive under low irradiance availability, but the combination of sunflecks and water stress may cause photoinhibition (Valladares and Pearcy, 2002). The survival and growth of young plants under low irradiance are also related to carbon acquisition and partitioning. Young tree species of Cerrado show great total biomass under full solar irradiance (Prado et al., 2006) and they allocate biomass in favor of roots but gallery forest species distribute biomass in favor of leaf area and stem (Hoffman and Franco, 2003).

Copaifera langsdorffii is a neo-tropical tree occurring in principal Cerrado physiognomies and in gallery and dry forests (Castro et al., 1999). The wide variation of light environments which *C. langsdorffii* grows indicates great capacity of young plants to survive under contrasting irradiance availability. *C. langsdorffii* should present leaf physiological mechanisms and morphological adaption which allow this species to overcome the spatial variation of irradiance and the seasonal water stress in distinct Cerrado physiognomies and forest types.

The main objective here was to verify the impact of irradiance availability and water stress on carbon assimilation and biomass allocation during juvenile phase of *C. langsdorffii*. Photosynthesis was related to biomass accumulation looking for physiological and morphological adaptations which increase the survivorship of young individuals of *C. langsdorffii* growing under distinct light availability. The ecophysiological features under contrasting irradiances will make possible to explain the broad tolerance to environmental stresses and the wide geographic distribution of *C. langsdorffii* in different kinds of vegetation.

MATERIALS AND METHODS

Growth conditions: Seeds of *Copaifera langsdorffii* Desf. (Caesalpiniaceae) were collected from adult plants growing on cerrado *stricto sensu* reservoir with 86 ha at 850 m a.s.l. in Federal University of São Carlos (UFSCar) in municipality of São Carlos, São Paulo state, Brazil (21º58´-22º00´ S e 47º51´-47º52´ W). All seeds were sowed in black plastic pots with storage capacity of 10 L in august 2000. Only one plant per pot was maintained during all experiment and the soil was irrigated up to field capacity just after the soil surface to be dry.

The soil utilized was collected at the cerrado *stricto sensu* reservoir in an area of 15 m^2 from soil surface until 20 cm of depth. The main chemical traits of the distrophic oxisol collected were determined on the laboratory of soil fertility in the College of Agrarian and Veterinarian Sciences in Jaboticabal city, state of São Paulo, in the São Paulo State University (UNESP). The chemical soil features were: organic content = 26 g dm^{-3}, pH in $CaCl_2$ = 3.9, P = 4 mg dm^{-3}, K = 1.3 $mmol_c$ dm^{-3}, Ca = 3 $mmol_c$ dm^{-3}, Mg = 1 $mmol_c$ dm^{-3}, H + Al = 58 $mmol_c$ dm^{-3}, sum of bases = 5 $mmol_c$ dm^{-3}, cation exchange capacity = 63.3 $mmol_c$ dm^{-3} and the index of base saturation = 8.

The climate in study area is Cwa according to Köppen's classification with dry winter (from June to September) and wet summer (from October to May). Historical annual average ± standard deviation values of rainfall, relative humidity and vapor pressure deficit are, respectively, 1506 ± 26 mm, 71 ± 5%, 21.0 ± 0.5 and 0.72 ± 0.13 kPa (Damascos et al., 2005). The meteorological data in 2002 and 2003 were registered by the meteorological station number 83726 of the Meteorological National Institute located 1.0 km far from the study area. In July 2003 and in January 2003 were accomplished the daily courses of leaf gas exchange on young plants transplanted to soil in March 2002 at 570 days after sowing.

Irradiance availability during the growth: Young plants of *C. langsdorffii* grew in open area with 50 m^2 free of shade at the experimental garden of the Department of Botany of UFSCar, and in an adjacent forest fragment with several species of woody Cerrado physiognomy named *cerradão*. Shaded plants received in sunny days approximately 11% and 5% of full solar irradiance in dry and rainy periods, respectively. The income irradiance increased 100% in dry period because of leaf fall in upper strata. The daily course of irradiance in shaded area was similar to that below the canopy of cerradão physiognomy as described by Kanegae et al. (2000).

Biomass, biometry and survivorship: Fifty individuals of *C. langsdorffii* were maintained in each light regime. Biomass, height, leaf area ratio (LAR, total foliage surface per total biomass) and the specific leaf mass (SLM, dry leaf mass by corresponding leaf area) were sampled on 240, 360 and 570 days after sowing (DAS) between august 2000 and March 2002. Ten individuals of *C. langsdorffii* were used in each treatment and in every age. Biomass determinations were performed removing the individuals from the soil in plastic bags by water spurt working at low intensity. Individuals were separated in root, stem and leaves. These plants were placed immediately in stove at 70ºC during 48 hours. Dry mass was measured using a digital analytical balance model AE260, Mettler Instrument AG, Greifense, Switzerland.

Plant height (cm) was determined with milimetric ruler since the soil surface up to the last leaf insertion. The foliage image was captured by a scanner and the leaf area was measured by the software Image-Pro 4.0 (American Media Cybernetics, Silverspring, Maryland, United States). SLM determinations were performed through leaf discs with 5.0 mm of diameter detaching one disc per leaflet. Fifty discs were collected in ten individuals in every light treatment at each age of measurement (240, 360 and 570 DAS). The leaf discs were dried in stove at 70ºC during 48 hours. The average values of SLM were obtained by the ratio of dry mass per disc area (Prado and Moraes, 1997). The mortality of young plants in both light regimes was recorded monthly up to 1,230 DAS.

Gas exchange measurements up to 570 DAS: An infra red gas analyzer (IRGA, ADC, LCA-4, Hoddesdon, UK) was used to obtain the curves of net photosynthesis (P, μmol CO_2 m^{-2} s^{-1}) as a function of photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}). The narrow Parkinson Leaf Chamber (PLCN-4, ADC) was connected to light cannon PLU-002, ADC. The leaf temperature was maintained between 25-27ºC by Peltier system (ADC) attached to PLCN-4. The variation of PPFD in P-PPFD curves was obtained by the voltage controller in intensities between 2000-800 mol m^{-2} s^{-1} and by neutral glass filters (Comar Instruments, Cambridge, UK) positioned between light source and leaflet in intensities between 800-10 mol m^{-2} s^{-1}.

P-PPFD curves on 240, 360 and 570 DAS were obtained from a leaflet selected in previous P determinations in three leaflets on three different individuals. The leaflet which presented the highest P was that chosen for accomplishing the P-PPFD curve. The leaflets selected were completely expanded, without signals of herbivory, infection or senescence. P-PPFD curve were obtained in the morning (07:00 and 09:00 h) and the equation used to adjust the pairs of points on the P-PPFD curve that described by Prado and Moraes (1997):

$$P = P_{max.} (1 - e^{-k.(PPFD - LC)}) \qquad (I)$$

where:
P = Net photosynthesis, μmol CO_2 m^{-2} s^{-1}
P_{max} = Maximum net photosynthesis, μmol CO_2 m^{-2} s^{-1}
e = Euler constant
k = Constant of proportionality
PPFD = Photosynthetic photon flux density, μmol photons m^{-2} s^{-1}
LC = Light compensation point, μmol photons m^{-2} s^{-1}

The respiration in dark (RD, μmol CO_2 m^{-2} s^{-1}) and the light saturation point of photosynthesis by PPFD (LS, μmol m^{-2} s^{-1}) were also determined using equation (I). RD was calculated attributing in equation I the zero value for PPFD. LS was found at 90% of P_{max} in P-PPFD curve adjusted using the equation I. The P-CO_2 curves were obtained utilizing a gas mixer (GD-602, ADC, Hoddesdon, UK). A cylinder with 1100 vpm of CO_2 was connected to GD-602 and the mixer to IRGA. This set was organized as a semi-open system. The air volume of the mixer was first analyzed on the IRGA. This air achieved the leaflet inside the PLCN-4 and returned to the IRGA where it was analyzed again and set free to atmosphere. Each P-CO_2 curve was obtained registering P during every variation of external CO_2 concentration from 1100 to 20 vpm. The initial CO_2 concentration was 1100 vpm and each dilution resulted in 90% of the previous concentration. The PPFD values used in P-CO_2 curves were 1800 μmol m^{-2} s^{-1} on individuals in open area and 900 μmol m^{-2} s^{-1} on plants grown in shade. During P-CO_2 curves the IRGA LCA-4 calculated the values of substomatal concentration of CO_2 (Ci) for each P value. P-Ci curves were adjusted utilizing the equation (I) exchanging the independent variable (PPFD) by substomatal CO_2 concentration (Ci) as described by Monteiro and Prado (2006).

$$P = P_{pot.} (1 - e^{-k.(Ci - \Gamma)}) \qquad \text{(II)}$$

where:

P = Net photosynthesis, μmol CO_2 m^{-2} s^{-1}
P_{pot} = Potential photosynthetic capacity, μmol CO_2 m^{-2} s^{-1}
e = Euler constant
k = Constant of proportionality
Ci = substomatal CO_2 concentration, vpm
Γ = CO_2 compensation point, vpm

From P-Ci curves was possible to calculate the apparent carboxylation efficiency (ε, mol CO_2 m^{-2} s^{-1}) utilizing the first linear phase of P-Ci curve represented by the first derivation of equation II (Monteiro and Prado, 2006):

$$\varepsilon = k\, P_{pot}\, e^{k\,\Gamma} \qquad \text{(III)}$$

where:

ε = apparent carboxylation efficiency
k = Constant of proportionality
P_{pot} = Potential photosynthetic capacity, μmol CO_2 m^{-2} s^{-1}
e = Euler constant
Γ = CO_2 compensation point, vpm

Chlorophyll content index on 570 days after sowing (DAS): The chlorophyll content index (CCI) was obtained by means of the Chlorophyll Content Meter model CCM-200, Optic-Science, USA. CCM-200 estimates the amount of chlorophyll in circular samples of intact leaflet expressing as CCI index. CCI was determined in January 2002 in two points at the middle of the leaflet in four expanded leaflets being each one from different individuals in ten young plants resulting 80 measurements of CCI in each irradiance condition.

Leaf nutrient concentration on 240, 360 and 570 days after sowing (DAS): The foliage collected in biomass measurements on 240, 360 and 570 DAS were used to determine nutrient concentration (N, P, K, Ca and Mg) in each treatment, being two determinations at each age in every light regime resulting in a total of six samples after 570 DAS. Samples with 5 g of dry leaflets from ten distinct individuals in every age and in each irradiance condition were used to determine nutrient concentration according to Silva (1999). The N concentration was determined by titration (Kjeldahl) after digestion by nitro-percloric acid. The K concentration was determined by flame spectrophotometer and the Ca and Mg concentrations by atomic absorption. These surveys were performed in the Soil Analyzes Laboratory in Minas Gerais State University, in state of Minas Gerais, Brazil.

Daily course of leaf water potential, gas exchange and chlorophyll fluorescence: On March 2002 on 570 DAS twenty individuals were transplanted directly on soil in both corresponding irradiance regime. On these two groups were determined the survivorship and the daily course of leaf water potential (Ψ), net photosynthesis (P) and potential photochemical efficiency. The ration between variable (Fv) per maximum fluorescence (Fm) was used to calculate the potential photochemical efficiency (Mattos et al., 2002). Five young plants on open and shaded areas were chosen for daily course determinations. In each light regime two leaflets per plant completely expanded from five selected individuals (n= 10 leaflets) were used in every daytime for obtaining the average ± standard deviation of □Y, P and Fv/Fm. The measurements were performed in sunny days in dry (July 17, 2002) and rainy (February 02, 2003) periods in both treatments. □Y (MPa) were determined by a pressure chamber model 3000, Santa Barbara Soil Moisture, Santa Barbara, CA, USA. The infrared gas analyzer (IRGA, ADC, LCA-4, Hoddesdon, UK) previously described was used to obtain P. The PPFD in open and shaded areas was determined by the sensor on the head of PLC-N. The leaf temperature was maintained by Peltier system (ADC) tracking the ambient air temperature during the day. The air temperature was measured by a regular thermometer in shade.

Fv/Fm was obtained immediately after gas exchange measurements on leaflets completely expanded by means of a portable fluorimeter model PAM-2000 (Heinz-Walz, Effeltrich, Germany) connected to pocket PC (200cx model, HP, Corvallis, USA). The leaflets used were chosen following same criteria utilized in gas exchange being the leaflets adapted previously to darkness during 30 minutes. The pre-adaptation to darkness was accomplished using clips which blocked the income irradiance. Each leaflet was exposed to light saturation pulse (2,250 μmol photons m^{-2} s^{-1}) during five seconds to determine Fv/Fm.

Integrated values of the PPFD and P: The integrated values of the P during daily course (IP) were obtained with the equation described by Ronquim et al. (2003)

$$IP = \int \grave{o}\ f(x).\ d(x) \qquad (IV)$$

where:

IP = Integrated value of net photosynthesis (mmol CO_2 m^{-2} s^{-1})

x = Time interval during daily course

f(x) = Net photosynthesis (μmmol CO_2 m^{-2} s^{-1})

d(x) = Time interval

The integrated values of PPFD and P in the daily course (day^{-1}) were calculated by the equation IV exchanging the variable P (net photosynthesis) by PPFD (mol m^{-2} day^{-1}).

Net photosynthesis (P) during incident sunflecks: P values under sunflecks were accomplished in four times during the day: 08:00, 11:00, 12:00 and 13:00 h, when direct irradiance took place on transplanted young plants. The P values under sunflecks were determined on the same days of the measurements of the daily courses of leaf gas exchange. Sunfleck can be defined with the irradiance beam which crosses the canopy of woody strata and attain directly the leaflet under the canopy. The sunflecks were determined by means of portable infrared gas analyzer model LCA-4 previously described. The measurements were acquired in six leaflets from three distinct individuals being two leaflets per individual under direct irradiance resulting in fifteen P values at each every time of the day.

Statistical analyzes: The values of total dry mass, height, root/aerial biomass ratio, total leaf area, specific leaf mass (SLM), leaf area ratio (LAR), leaf nutrients concentration in each treatment on 240, 360 and 570 DAS and the chlorophyll content index (CCI) at 570 DAS were tested to verify normal distribution of the data set through the GraphPad InSTAT version 3.0 (GraphPad software, San Diego, USA). As the data set shows normal distribution the mean values in each light regime were compared by Student t test ($p > 0.05$). The mean values and the standard deviation of the P_{max}, LC, LS, and RD, were obtained by adjusting the curves of net photosynthesis as a function of PPFD and P_{pot} and ε by adjusting the curves of P as a function of Ci on 240, 360 and 570 DAS.

RESULTS AND DISCUSSION

Survival, growth and biomass allocation

All potted young plants survived in both light regimes until the last harvesting of biomass on 570 DAS and when they were transplanted under natural conditions from March 2002 to January 2004 up to 1,230 DAS. Young plants of *C. langsdorffii* survived under herbivory growing under forest canopy more closed than that utilized here (Leite and Salomão, 1992). On the other hand, young plants growing in sunny area showed higher values of height, leaf area and total biomass on 570 DAS (Figure 1). The growth of *C. langsdorffii* was very slow in shade. In fact, the height after two years on 1,230 DAS was 28.3±12.7 cm which represents an increase of 29% in relation to height showed before the transplanting on 570 DAS. Contrastingly, transplanted individuals to open area showed 134.8±56.7 cm in height on 1,230 DAS, an increase of 435% in relation to potted plants on 570 DAS. Reduced growth rates and the ability to resist to pathogens and herbivores are crucial for growing in shade (Kitajima, 1994) besides low respiration and photorespiration rates (Valladares and Pearcy, 2002; Prado et al., 2006). Such morphological and physiological features in shade permit a more favorable carbon balance when net photosynthesis rate is reduced under low light availability.

Only leaf area ratio (LAR) was higher in young plants in shaded than in sunny area on 570 DAS (Figure 1). An increase in LAR in nine Cerrado species in response to shading offset a decrease in net assimilation per unit of leaf area (Hoffmann and Franco, 2003). After 360 DAS there was a shift about biomass allocation in favor of foliage instead of roots in plants grown in shade. Therefore, after to assure sufficient biomass in roots to overcome the first dry season, the young plants turned their resources to foliage to prevail over low light availability.

Young plants that develop adaptive traits to water stress as high biomass allocation to the roots show great success for growing under dry forest canopy (Khurana and Singh, 2001). Therefore, the behavior of *C. langsdorffii* on early stage of development in shade was quickly to accumulate resources in roots when water acquisition will be soon more restrict. This capacity to alter the biomass allocation between aerial and underground parts according to water stress and light availability explains in part why *C. langsdorffii* is one of the most important species in Cerrado domain occurring in more than 50% of the 376 studied areas (Ratter et al., 2003) and in gallery forest in more than 50% of the 43 extra-amazonian surveys (Rodrigues and Naves, 2000).

Net photosynthesis (P) as function of photosynthetic photon flux density (PPFD) or internal CO_2 concentration (Ci) and the contents of chlorophyll and nutrients

There are pronounced effects of irradiance regimes on P-PPFD and P-Ci curves (Figure 2) on 240, 360 and 570 DAS. Higher values of maximum net photosynthetic rate (P_{max} and P_{pot}), light compensation and saturation points (LC and LS, respectively), respiration in dark (RD) and carboxylation efficiency (ε) were reached in sunny than in shaded areas (Figure 2 and Table 1). Higher P_{max} and ε per unit of leaf area and higher leaf area (Figure 1) allowed a much more favorable carbon balance in sunny than in shaded sites resulting in total biomass 2.5 times higher on 570 DAS. In addition, lower values of ε in shaded than in sunny area indicate low concentration of ribulose-1,5-bisphosphate carboxylase oxygenase in shaded leaflets (Kozlowski and Pallardy, 1996). On the other hand, the carbon use efficiency defined as the ration of net photosynthesis per dark respiration (Larcher, 2000) was equivalent between light regimes with 9.0 and 9.3 μmol m^{-2} s^{-1} in sunny and shaded sites, respectively. Therefore, low rates of respiration in dark offset reduced net photosynthetic and low photosynthetic capacity keeping carbon balance as positive as possible in shade. In addition, higher chlorophyll content index in leaflets in shaded (57.2 ± 6.4) than in sunny (41.3 ± 8.2) sites could be considered one physiological adjustment for increasing the efficiency of irradiance capture (Larcher, 2000).

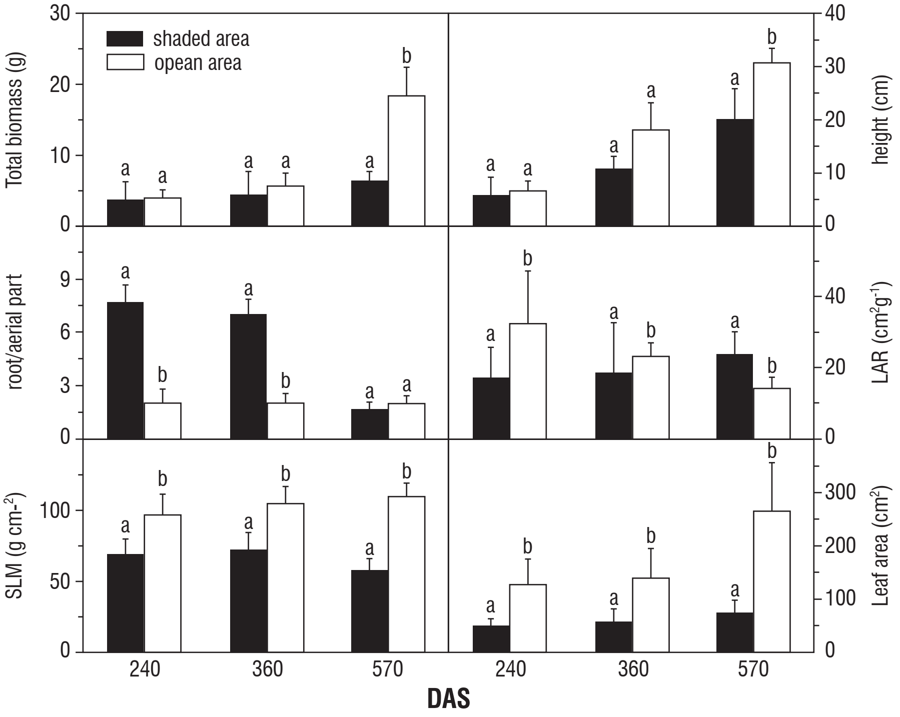

Figure 1. Mean values (column) and standard deviation (lines above columns) of total biomass, height, root/aerial biomass ratio, leaf area ratio (LAR), specific leaf mass (SLM) and total leaf area of *Copaifera langsdorffii* growing in sunny (white columns) and shaded areas (black columns) on 240, 360 and 570 days after sowing (DAS). Mean values followed by different lower case letters indicate significant differences (Student t test, $p<0.05$, n=10) between irradiance regimes in corresponding trait and age.

The values of leaf nutrient content in *C. langsdorffii* showed that P appeared in inferior concentration in both areas in relation to the average requirement of terrestrial plants (Table 2). Indeed, phosphorus is one of the most nutrients with low availability in soil for biomass production in Cerrado (Furley, 1999; Haridasan, 2008). Aerts and Chapin (2000) suggested that N/P ratios above 16 indicate P deficiencies and ratios below 14 indicate N deficiencies. The N/P ratios from Table 1 are 19.4 and 20.5 in sunny and shaded areas, respectively, indicating P deficiency in leaves in both light regimes. On the other hand, nutrient concentrations were similar in leaflets in sunny and shaded sites (Table 2) probably by means of low foliage growth rate in shaded area (Figure 1).

Table 1. Mean± standard deviation on 240, 360 and 570 days after sowing of maximum photosynthetic rate (photosynthetic capacity, P_{max}), light compensation of photosynthesis (LC), light saturation of photosynthesis (LS), respiration in dark (RD), maximum photosynthetic rate under saturated concentration of CO_2 (potential photosynthetic capacity, P_{pot}) and apparent carboxylation efficiency (ε) in leaflets of *C. langsdorffii* growing in sunny and shaded areas.

Variables	Sunny	Shaded
Pmax (μmol m-2 s-1)	9.9 ± 1.1	5.6 ± 1.4
LC (μmol m-2 s-1)	59.0 ± 20.8	45.7 ± 18.2
LS (μmol m-2 s-1)	1318 ± 259	961 ± 237
RD (μmol m-2 s-1)	1.1 ± 0.1	0.6 ± 0.1
Ppot (μmol CO2 m-2 s-1)	21.9 ± 0.8	12.5 ± 3.5
ε (mol CO2 m-2 s-1)	0.077± 0.02	0.029± 0.01

Table 2. Mean values ± standard deviation of leaf nutrient concentration (g kg^{-1}) in young plants of *C. langsdorffii* growing in sunny and shaded areas at 240, 360 and 570 days after sowing. The last column presents the average necessity of nutrient concentration for terrestrial plants according to Larcher (2000) based on several authors. There is not significant difference (Student t test, $p<0.05$) between mean values in line.

Leaf nutrient concentration (n=6)	Sunny	Shaded	Average mean of the terrestrial plants
Nitrogen (N)	19.4 ± 0.6	22.6 ± 0.8	15-25
Phosphorus (P)	1.0 ± 0.2	1.1 ± 0.2	1.5-3
Potassium (K)	7.9 ± 2.2	9.2 ± 1.2	5-20
Calcium (Ca)	6.6 ± 2.9	6.8 ± 1.6	3-15
Magnesium (Mg)	1.5 ± 0.9	1.7 ± 0.3	1-3

Daily course of net photosynthesis (P), leaf water potential (Ψ) and potential photochemical efficiency (Fv/Fm) of transplanted young plants under natural conditions

Figure 3 shows the seasonality in study area with dry period at the middle of the year. Therefore, the months free of rain were April and June in 2002 and July in 2003. Fortunately, the dry period takes place when air temperature decreases in study area attenuating the evapotranspiration and water stress. In 2002 the dry period was similar to that in core area of Cerrado nearby the center of Brazil, where dry period usually shows two or three months without precipitation (Franco, 2002). In the central plains of Brazil there is a decrease of soil water potential up to -1.8 MPa at 0.60 m depth on Cerradão physiognomy (Kanegae, 2000). When precipitation decreased during dry period the income irradiance on beneath strata was more intense due to typical leaf fall in cerradão physiognomy (Ressel et al., 2004). Therefore, the values of PPFD were higher around midday and the frequency of sunflecks above 600 μmol m^{-2} s^{-1} was greater in dry than in rainy period (Figures 4 and 5). In addition, the integrated values of photosynthetic photon flux density (IPPFD, mol photons m^{-2} day^{-1}) in shaded area showed a decreased of 49% in rainy period (Table 3).

The combined events of high PPFD and P in dry and rainy periods (Figure 4 and Table 3) on a large leaf area with high photosynthetic capacity certainly were responsible for the higher total biomass accumulation in plants grown in sunny than in shaded area on 570 DAS (Figure 1). The leaf water potential (Ψ) was more negative during whole daily course in both sites in dry period with Ψ even more negative in young plants in shaded than in sunny area (Figure 4). Despite higher irradiance availability in dry than rainy period, young plants in shaded area showed greater values of integrated net photosynthesis (IP) during the daily course in rainy period (Table 3). Therefore, the low water potential indicated that the tension in xylem water column and the leaf water status in dry season was enough to impose significant restriction to net photosynthesis in dry season despite high PPFD availability. On the other hand, lower values of Ψ in shaded than in sunny area in dry season reveal that water availability probably is more limited than that expected in soil under closed canopy as in Cerradão physiognomy. Indeed, the minimum soil water potential at the depths 5.0 and 60.0 cm in Cerradão were -2.8 and -1.8 MPa at the peak of dry period (Kanegae et al., 2000).

Fv/Fm ratio is the variable largely utilized to detect the photoinhibition of photosynthesis and values around 0.83 indicate that leaf is free of stress as occurred during the whole daily course in shaded leaves in rainy period (Figure 3). On the other hand, Fv/Fm ratio was lower than 0.76 and reached 0.7 around midday in shaded area (Figure 4) revealing that significant stress took place in photochemical apparatus on photosystem II (Mattos et al., 2002). Therefore, photoinhibition occurred during dry period even in shade and it was combined to leaf water stress indicated by low Ψ values, especially around midday, resulting in decreasing of IP in dry period (Table 3). Young individuals growing in sunny area was able to keep P higher than those in shaded area during the whole daily course in dry season. Higher values of Ψ in leaves of sunny area during the daily course in dry season indicated that the root system was capable to supply the foliage water demand more efficiently than in shaded plants. Indeed, the root system in plants under full solar irradiance was greater in size and/or in efficiency for absorbing water because of root/aerial biomass ratio did not differ between plants in distinct light regimes but LAR was lower in sunny than shaded plants despite their leaf area to be 3 times higher on 570 DAS.

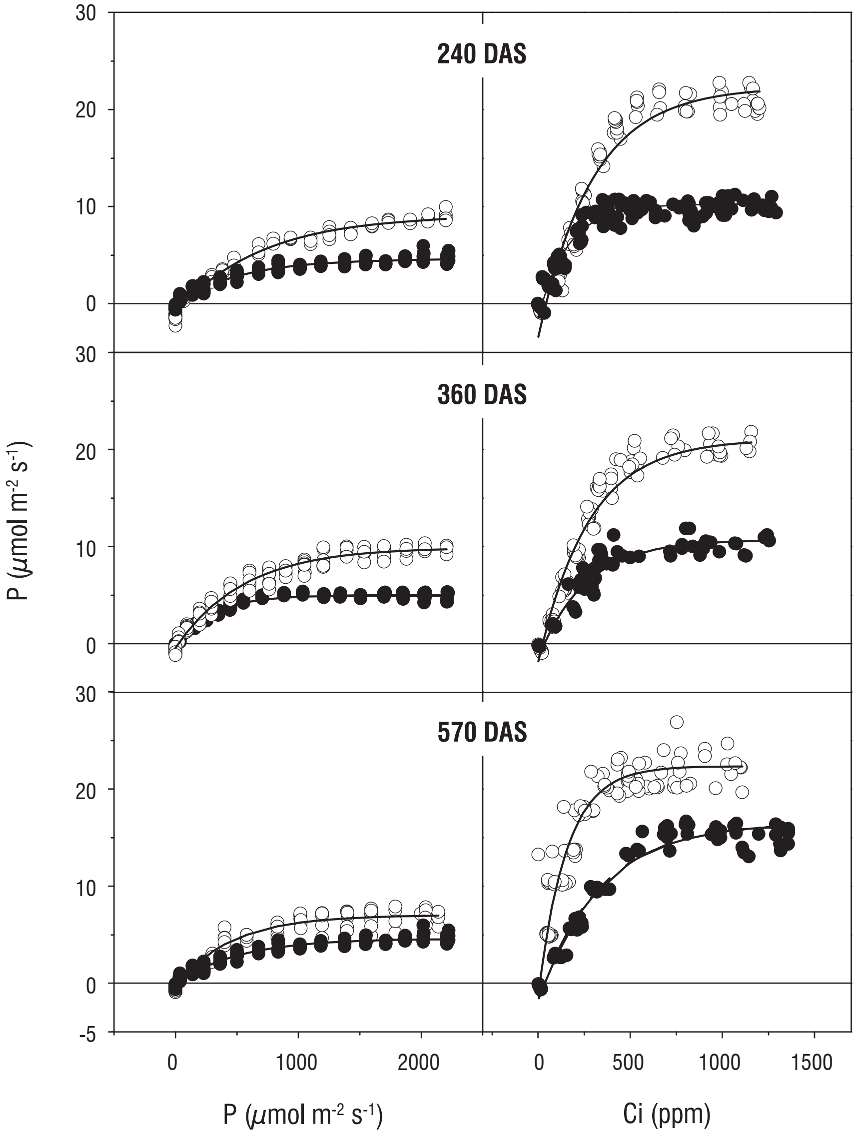

Figure 2. Net photosynthesis (P, μmol CO_2 m^{-2} s^{-1}) as a function of photosynthetic photon flux density (PPFD, μmol photons m^{-2} s^{-1}) and internal concentration of CO_2 (Ci). Values were obtained in expanded leaflets of *Copaifera langsdorffii* in sunny (open symbols) and shaded (solid symbols) areas at 240, 360 and 570 days after sowing (DAS).

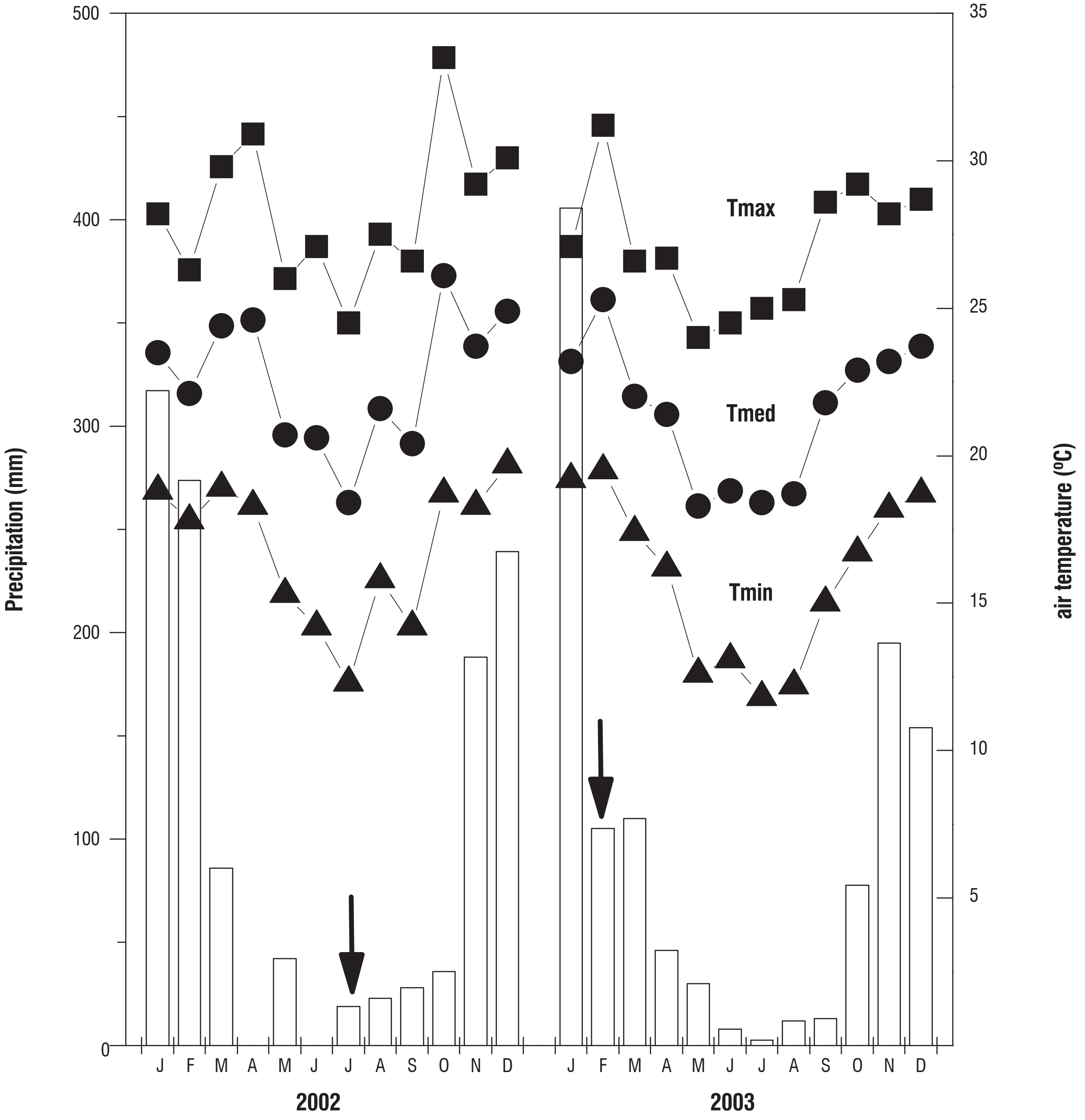

Figure 3. Monthly values of total precipitation (bars) and mean values of maximum (Tmax), average (Tmean) and minimum (Tmin) air temperature at the months that the daily courses of net photosynthesis, leaf water potential and chlorophyll fluorescence were obtained in young plants of *Copaifera langsdorffii* growing under natural conditions in shaded area.

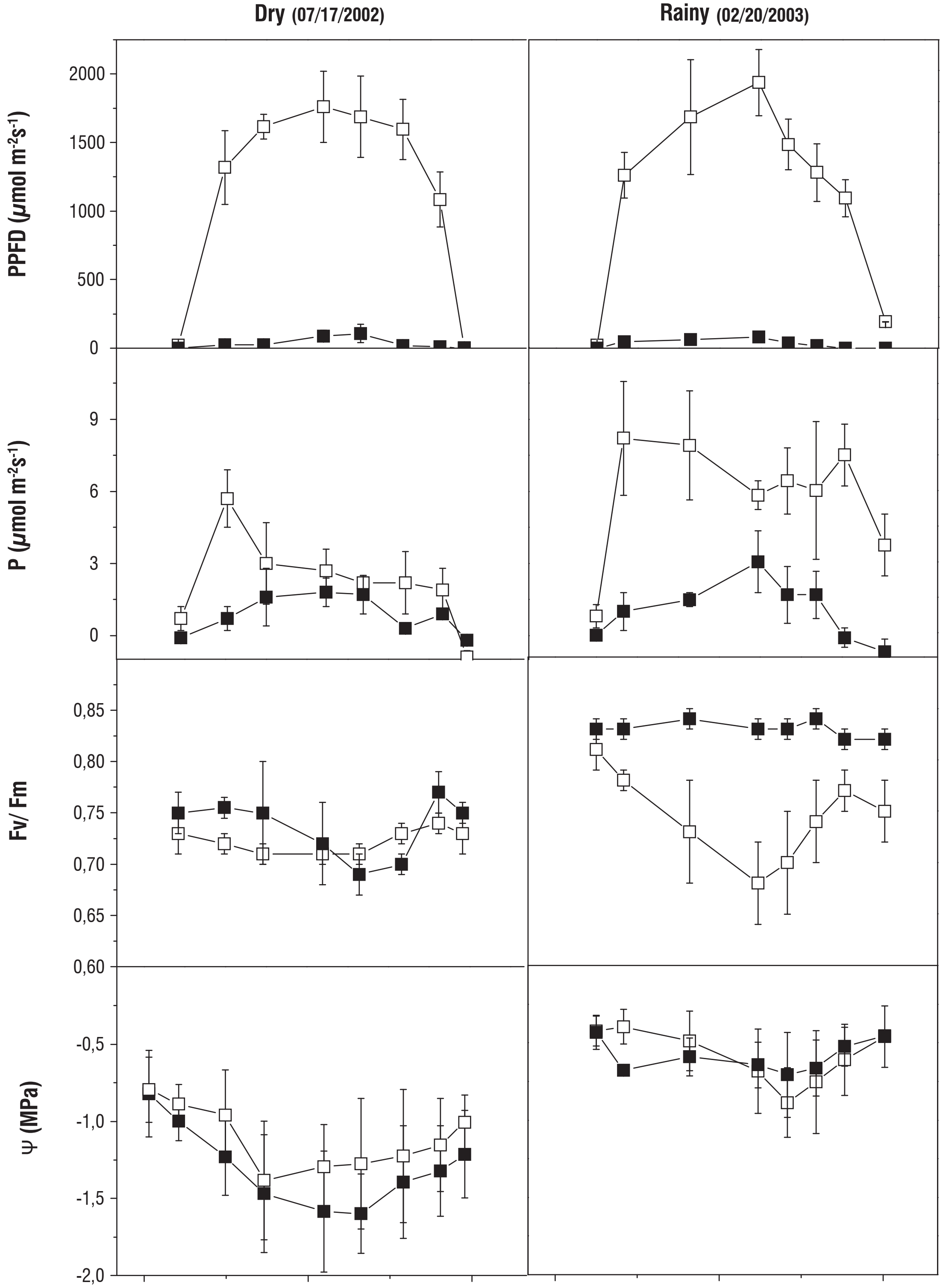

Figure 4. Mean values (symbols) ± standard deviation (bars) during the daily course of photosynthetic photon flux density (PPFD), net photosynthesis (P), potential photochemical efficiency (Fv/Fm) and water potential (Ψ) obtained on leaflets of young plants of *Copaifera langsdorffii* growing in sunny (open symbols) and shaded (solid symbols) areas during dry and rainy periods.

Table 3. Integrated values (day^{-1}) of photosynthetic photon flux density (IPPFD) and net photosynthesis (IP) during daily course of leaf gas exchange in young plants of *C. langsdorffii* growing in sunny and shaded areas in dry and rainy periods.

Variables	Dry		Rainy	
	Sun	Shade	Sun	Shade
IPPFD (mol photons m^{-2} day^{-1})	50.98	5.49	58.74	2.77
IP (mmol CO$_2$ m^{-2} day^{-1})	101.56	36.12	285.40	62.43

Figure 5 shows P during sunflecks on shaded plants at 08:00, 11:00, 12:00 and 13:00 hs during the daily course in dry and rainy periods. In spite of high frequency of PPFD above 600 μmol m^{-2} s^{-1} in dry period, P was usually larger in rainy period. For instance, there were not values of P higher than 4 μmol m^{-2} s^{-1} in dry period but P was higher than this benchmark in many times in rainy period. The precarious leaf water status and the intense photoinhibition in shaded area, especially around midday, limited the photosynthetic capacity to around a half of that measured in rainy period. Therefore, shaded plants were not able to use sunflecks with high PPFD around midday in dry season for starting net CO$_2$ assimilation. However, even under this worse condition, the bunches of PPFD should be imperative to carbon balance of *C. langsdorffii* growing under closed canopy because of PPFD usually did not reach shaded leaves at intensities higher than 100 μmol m^{-2} s^{-1} during the day in dry period.

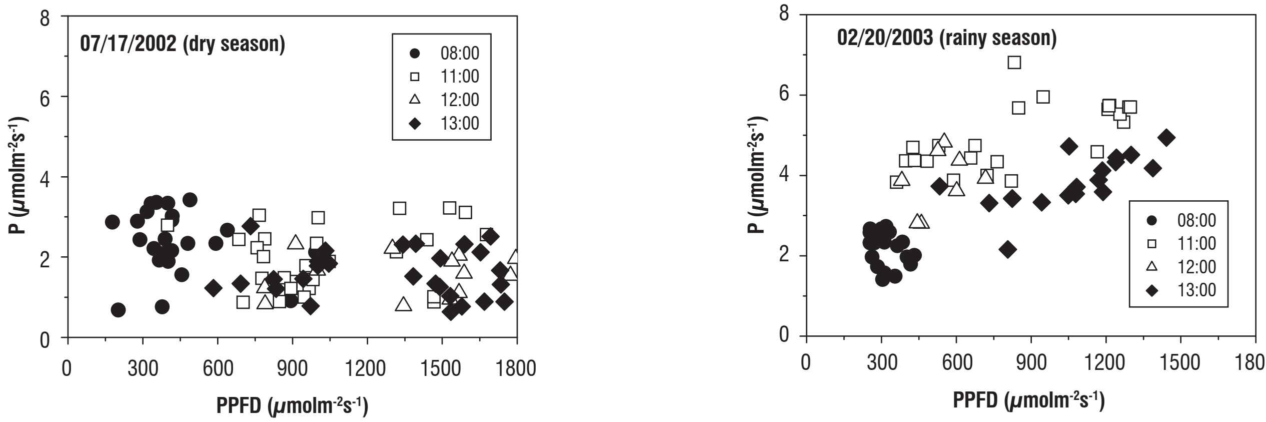

Figure 5. Net photosynthesis (P) under bunches (sunflecks) of direct photosynthetic photon flux density (PPFD) at 08:00, 11:00, 12:00 and 13:00 h in young plants of *Copaifera langsdorffii* growing under forest canopy in dry and rainy periods.

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REFERENCES

Aerts R, Chapin FS (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30:1-67.

Castro AAJF, Martins FR, Tamashiro JY, Sheperd GJ (1999) How rich is the flora of Brazilian Cerrados? Ann. Missouri Bot. Gard. 86:192–224.

Damascos MA, Prado CHBA, Ronquim CC (2005) Bud composition, branching patterns and leaf phenology in Cerrado woody species. Ann. Bot. 96:1075-1084.

Hoffmann WA, Orthen B, Franco AC (2004) Constraints to seedling success of savanna and forest trees across the savanna-forest boundary. Oecologia 140:252-260.

Hoffmann WA, Franco AC (2003) Comparative growth analysis of tropical forest and savanna woody plants using phylogenetically independent contrasts. J. Ecol. 91:475-484.

Franco AC (2002) Ecophysiology of woody plants. In: Oliveira PS, Marquis RJ, (eds), The Cerrados of Brazil: Ecology and natural history of a neotropical savanna, pp. 178-197. Columbia University Press, Irvington.

Furley PA (1999) The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian Cerrados. Global Ecol. Biogeogr. 8:223-241.

Haridasan M (2008) Nutritional adaptations of native plants of the cerrado biome in acid soils. Braz. J Plant Physiol. 20:183-195.

Kanegae MF, Braz VS, Franco AC (2000) Efeitos da disponibilidade sazonal de água e luz na sobrevivência de *Bowdichia virgilioides* em duas fitofisionomias típicas dos Cerrados do Brasil Central. Rev. Bras. Bot. 23:459-468.

Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. Oecologia 8:419-428.

Kozlowski TT, Pallardy SG (1996) Physiology of wood plants. Academic Press, San Diego.

Khurana E, Singh JS (2001) Ecology of seed and seedling growth for conservation and restoration of tropical dry forest: a review. Environ. Conserv. 28:39–52.

Larcher W (2000) Ecofisiologia Vegetal. Rima, São Carlos.

Leite AMC, Salomão AN (1992) Estrutura populacional de regenerantes de Copaíba (*Copaifera langsdorffii* Desf.) em mata ciliar do Distrito Federal. Acta Bot. Bras. 6:123-133.

Mattos EA, Lobo PC, Joly CA (2002) Overnight rainfall inducing rapid changes in photosynthetic behaviour in a Cerrado woody species during a dry spell amidst the rainy season. Aust. J. Bot. 50:241-246.

Monteiro JA, Prado CHBA (2006) Apparent carboxylation efficiency and relative stomatal and mesophyll limitations of photosynthesis in an evergreenh cerrado species during water stress. Photosynthetica 44:39-45.

Prado CHBA, Moraes JAPV (1997) Photosynthetic capacity and specific leaf mass in twenty woody species of Cerrado vegetation under field conditions. Photosynthetica 33:103-112.

Prado CHBA, Ronquim CC, Peron MC (2006) Balanço de carbono em duas espécies lenhosas de Cerrado cultivadas sob irradiação solar plena e sombreadas. In: Scariot A, Sousa-Silva JC, Felfili JM, (org), Cerrado: Biodiversidade, Ecologia e Conservação, pp 189-206. Ministério do Meio Ambiente, Brasília.

Ratter JA, Bridgewater S, Ribeiro JF (2003) Analysis of the floristic composition of the brazilian Cerrado vegetation III: comparison of the woody vegetation of 376 areas. Edinburgh J. Bot. 60:57–109.

Ressel K, Guilherme FAG, Schiavini I, Oliveira PE (2004) Ecologia morfofuncional de plântulas de espécies arbóreas da Estação Ecológica do Panga, Uberlândia, Minas Gerais. Rev. Bras. Bot. 27:311-323.

Rodrigues RR, Nave AG (2000) Heterogeneidade florística de matas ciliares. In: Rodrigues RR, Leitão-Filho HF (Eds), Matas ciliares: conservação e recuperação, pp. 45-72. EDUSP, FAPESP, São Paulo.

Ronquim CC, Prado CHBA, Paula NF (2003) Growth and photosynthetic capacity in two woody species of cerrado vegetation under different radiation availability. Braz. Arch. Biol. Technol. 46:243-252.

Sarmiento G (1984) The ecology of neotropical savannas. Harvard University Press, Cambridge.

Silva FC (1999) Manual de análises químicas de solos, plantas e fertilizantes. EMBRAPA, p. 370.

Valladares F, Pearcy RW (2002) Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. Plant Cell Environ. 25:749-759.