RESEARCH ARTICLE

Light as an indicator of ecological succession in brazilwood (*Caesalpinia echinata* Lam.)

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ABSTRACT

The ecophysiological behavior of brazilwood (*Caesalpinia echinata* Lam.) plants was evaluated as affected by light quantity and quality. Nine-month-old plants were cultivated under 0%, 50%, and 80% artificial shading, and natural shading imposed by a closed canopy for a period of 392 days. At the end of that period growth parameters were measured, including relative growth rate (*RGR*), net assimilation rate (*NAR*), leaf area ratio (*LAR*), specific leaf mass (*SLM*) and root:shoot ratio (*R:S*). Higher leaf area, dry mass and *RGR* were obtained under 50% artificial shade. Under full sunlight (0% shade), growth was satisfactory showing higher *R:S* and *SLM*. Growth was inhibited under 80% artificial and natural shading indicating that *C. echinata* is not pioneer-succession or climax specie. Simulating a gap, plants cultivated under artificial shade (50%) were transferred to full sunlight for an interval of 192 h, during which net photosynthesis (*A*), transpiration (*E*), stomatic conductance (*gs*), deficit of pressure vapor (*VPDleaf-to-air*) and the efficiency quantum potential of photosystem II (*Fv*/*Fm*) were analyzed. Plants transferred to full sunlight presented severe burning and abscission of the leaflets, as well as inhibition of *A* and of the photochemical efficiency of PSII (*Fv*/*Fm*). This observation associated with the reduced growth under full sunlight and better performance under moderate shade suggests characteristics of intermediate species.

Key words: chlorophyll fluorescence, gas exchange, growth, light stress, shade, tropical tree.

INTRODUCTION

The use of tropical native tree species has increased aiming reforestation and this activity depends on data about species regeneration as related to the light gradient. Such information is of great value in defining the position of the species on the forest succession and is essential for the artificial regeneration and management of forests (Kitajima, 1996; Koerselman and Meuleman, 1996; Duz et al., 2004).

Such studies allow the evaluation of physiological plasticity in plants due to environmental factors (Zamith and Scarano, 2004; Lima et al., 2008) and this plasticity is of great importance for conferring stress resistance to plants. This ensures survival and the adequate development of plants in field conditions where environmental factors are adverse.

The knowledge of plant responses to fluctuations in environmental factors is important to determine the best possible strategy for restoration of green cover, which is based on the capacity of plants to express their physiological mechanisms with maximum efficiency (Musil et al., 2002; Machado et al., 2005). Therefore, ecophysiological studies can generate important information for conservation and forest restoration, as well as the establishment of *in situ* and *ex situ* germoplasm collections, including endangered species as Brazilwood.

Brazilwood (*Caesalpinia echinata* Lam.) is a tree native to the Brazilian Atlantic Forest with important historical and economical value in Brazil. However, the species is under serious risk of genetic erosion due to intensive exploration associated with the lack of information about its physiological characteristics. The density, flexibility and acoustics of its wood make brazilwood internationally valuable, since it is the ideal raw material for the manufacture of arches for string musical instruments (Franco and Yojo, 2008). Despite its commercial importance, historical and cultural value, and the threat of extinction, little is known about the ecophysiology of this species, a symbol of Brazil.

Current information about the ecological characteristics of brazilwood is quite contradictory. Its classification fits species of rain and semidecidual forests (Lima, 1992; Lorenzi, 2002). There are indications that the species can be an example of tree that needs moderate shade in the juvenile phase, while full development is achieved under full sun when gaps are formed (Carvalho, 1994).

There is a consensus that the light is one of the most important environmental factors for forest establishment (Souza and Válio, 2003a; Duz et al., 2004; Gonçalves et al., 2005). Sunlight irradiance is absorbed by the canopy and just less than 3% of the light reaches the understorey (Clark et al., 1996). This limits the growth of many plants in the understory due to the low radiation with wave length predominant at 730 nm (Souza and Válio, 2003a).

The movement of canopies due to winds, the abscission of leaves in during dry seasons, and the opening of gaps alter the incidence of radiation and the light quality within the forest (Kitajima, 1996; Minotta and Pinzauti, 1996).

Those alterations in light quality and intensity modify the floristic composition and the morphology of the population of seedlings. They also influence the allocation pattern and biomass partition between root and shoot simultaneously with the net assimilation rate of CO_2, relative growth rate and the leaf area ratio (Lambers and Poorter, 1992; Osunkoya et al., 1994; Souza and Válio, 2003a,b). The growth responses due to light can indicate the adaptation ability of species in maximizing the efficient use of nutrients and water in environments with variation of light radiation (Gonçalves et al., 2005).

Light has great effect in gas exchange in plants, as indicated by chlorophyll fluorescence. The chlorophyll *a* fluorescence of FSII allows a feasible analysis of the quantum potential of photosynthesis efficiency in plants developing under different light conditions (Gonçalves and Santos Jr., 2005). Pigment content and fluorescence chlorophyll *a* analyses are common stress indicators for excess irradiance (Hendry and Price, 1993). High radiation causes chlorophyll photo-oxidation. In general, trees tend to present better photochemical efficiency (*Fv*/*Fm*) and high chlorophyll concentrations in moderate shade and could present photoprotection mechanisms when cultivated in the full sunlight (Gonçalves et al., 2005).

The lack of information about the position of brazilwood in the ecological succession, its economical importance and its threatened status provide the rationale for further studies. The present study aimed at assessing the effect of light on the growth and photosynthesis in brazilwood. The study of light effects in brazilwood's juvenile phase may provide valuable information to support reforestation programs.

MATERIAL AND METHODS

***Study area and experiment installation*:** Brazilwood seeds were obtained from FUNBRASIL (Fundação Nacional do Pau Brasil) located in Pernambuco state, Brazil. Seeds were germinated in Petri dishes at 25ºC (Mello and Barbedo, 2007) in continuous light. On the fifth day after germination, seedlings were transferred to black polyethylene pots (5 L) containing a mix of dirt and sand (1:1, w/w), with no fertilization added. Plants were maintained under local temperature and photoperiod in a greenhouse located at the Department of Biological Sciences, Espírito Santo University, Vitória city (20º18'52'' S and 40º19'06'' W) where the experiments were carried out. The region's climate is tropical humid with an average annual precipitation of 900 mm, average temperature of 24±2ºC and photoperiod of 13 h. in the summer and 11 h. in the winter.

After three months of acclimation period, 9-month-old plants were divided into 4 groups of fourteen plants. The first 3 groups were submitted to different shading conditions: 0, 50 and 80% artificial shade provided by neutral screens. The fourth group was placed natural shading provided by a closed canopy near the greenhouse. To avoid heating and loss of water due to soil evaporation, pots under full sunlight (0% shade) were wrapped in aluminum foil and a vermiculite layer was added to the surface of the soil. The photosynthetic active radiation in the different shading conditions and the relationship of red:far-red light ratio (*R:Fr*) was determined at noon on a sunny day using a spectroradiometer (Spectrum Technologies, Inc., Illinois, USA). The *R:Fr* was calculated based on the values of irradiance spectrum in the wavelengths of 656-664 nm and 726-734 nm, respectively, with the sensor placed above the plants. During the experiments, plants were watered daily as needed.

Growth analyses: At the beginning of the experiment and 392 days later, brazilwood plants were randomly selected and harvested for initial and final growth evaluations, respectively, including height, number of leaves, leaf area, and dry mass after oven-drying samples at 60°C until constant mass. Brazilwood is a species with bipinnate leaves and it has many primary and secondary leaflets. The leaf area total was made by using scanner-generated images (LI-3100C Area Meter, Nebraska, USA). Absolute growth rate ($AGR=(M_2-M_1)/(t_2-t_1)$), relative growth rate ($RGR=(LnM_2-LnM_1)/(t_2-t_1)$), net assimilation rate ($NAR=[(DM_2–DM_1)/(t_2–t_1)]\times[(1nA_2–1nA_1)/(A_2–A_1)]$), leaf area ratio ($LAR=AL/TDM$), root:shoot ratio (*R:S*), specific leaf mass ($SLM=LM/LA$), leaf mass ratio ($LMR=LM/TM$), stem mass ratio ($SMR=SM/TDM$), and root mass ratio ($RMR=RM/DMT$) were calculated as described by Hunt (1982), where, M_1=initial mass; M_2=final mass; t =initial time; t_2=final time; A_1=final leaf area; A_2=initial leaf area; *Ln*= natural log; *LM*=leaf mass, *SM*=stem mass, *RM*=root mass, *LA*=leaf area; *TDM*=total dry mass.

***Gas exchange measurements*:** Plants cultivated in 50% shading were transferred to full sun. At 0, 3, 14, 48 and 192 h. after transfer, gas exchange and chlorophyll fluorescence analyses of were performed using the first mid-apical leaves that were completely expanded and the secondary leaflets. Photosynthesis (*A*), stomatal conductance (g_S), transpiration (*E*) and vapor pressure deficit (*VPDleaf-to-air*) were determined using a portable, closed system, infrared gas analyzer (IRGA, model LI-COR 6200, Nebraska, USA). Measurements were performed between 9:00 and 11:00 h. a.m., using the atmospheric CO_2 concentration of 1,500 μmol m^{-2} s^{-1} as a preliminary value for the curve of CO_2. Light curves were obtained for the photosynthetic photon flux density (*PPFD*) between 0 and 3,000 μmol m^{-2} s^{-1}.

***Fluorescence measurements*:** Chlorophyll fluorescence analysis was performed using a portable fluorometer (Plant Efficiency Analyzer-PEA, Hanstech, King's Lynn, UK). After 30 min. of conditioning in the dark, leaves were exposed to a high intensity saturated light (1,680 μmol.m^{-2}.s^{-1}) pulse for 5 sec. at 8:00 h. a.m. From the induction of fluorescence of fast kinetics, the photochemical efficiency of PSII (F_v/F_m) was obtained. Water-use efficiency (*WUE* = [mmol CO_2.mol^{-1}(H_2O)]) was calculated by photosynthesis (*A*) / transpiration (*E*) ratio and intrinsic water-use efficiency (*IWUE* = [μmol CO_2.mol^{-1} H_2O]) was calculated by the photosynthesis / stomatic conductance (*gs*) ratio.

***Experimental design and statistical analysis*:** Both experiments used a completely randomized design (CRD) with 7 plants per replication (7 plants harvested at the beginning of the experiment and 7 plants harvested at the end of the experiment, 392 days later). For the second experiment that simulated a gap, 5 plants were used for the gas exchange and chlorophyll fluorescence analyses. Analysis of variance (ANOVA) was used for statistical analysis and means were separated using the Tukey test at 5% probability level using the software ASSISTAT version 7.4 (Silva and Azevedo, 2007).

RESULTS AND DISCUSSION

Growth analyses: The different shade conditions did not influence stem length, *LN*, *PLN* and *SLN*, but *TLA* and *ULA* were highest under 50% shade (Table 1). Such results affected *LM*, *SDM*, and *TDM* production followed by intermediate values under full sunlight and lower *LM*, *SDM* and *TDM* production under 80% artificial and natural shade. Root mass and *SM* were promoted under 50% artificial shade (moderate shade) and full sunlight (0% shade). These parameters were most affected under artificial shade (80%) and natural shade (closed canopy). Consequently, *TDM* production was promoted by moderate shade as compared to full sunlight and densely artificial shade. As for *LDM* and *SDM* the values were higher than those measured under densely artificial shade and natural shade (Table 1). Similar results, such as height and number of leaves and leaflets indicate the high plasticity of brazilwood in adapting to different light conditions.

Table 1 - Growth parameters for *C. echinata* after 392 days under different light regimes: height; leaf numbers (*LN*); primary leaflets numbers (*PLN*); secondary leaflet number (*SLN*); total leaf area (*TLA*); unitary leaf area (*ULA*); root dry mass (*RDM*); stem dry mass (*SDM*); leaf dry mass (*LDM*); shoot dry mass (*ShootDM*) e total dry mass (*TDM*). Numbers represent means ± standard error (*n*=*7*). Different letters indicate significant differences at 0.05 by Tukey's test.

Shade	Height (cm)	*LN*	*PLN*	*SLN*	*TLA*	*ULA*	*RDM*	*SDM*	*LDM*	*ShootDM*	*TDM*
					(cm^2)				(g)		
0%	50 ±3,8 a	12 ±0,9 a	83 ±6,4 a	846 ±82 a	5522 ±260 b	06,5 ±0,51 b	22 ±2,87 a	11,7 ±0,93 a	14 ±0,87 b	26 ±1,73 b	47 ±4,13 b
50%	68 ±1,7 a	11 ±0,9 a	75 ±4,3 a	833 ±48 a	9876 ±286 a	11,8 ±0,64 a	22 ±1,28 a	13,5 ±0,63 a	18 ±0,96 a	32 ±1,52 a	54 ±2,35 a
80%	51 ±1,5 a	15 ±0,8 a	112 ±9,3 a	1199 ±125 a	5514 ±580 b	04,6 ±0,87 b	04,2 ±0,36 b	04,1 ±0,18 b	10 ±0,62 c	14 ±0,74 c	18 ±1,05 c
Natural	44 ±4,2 a	11 ±1,4 a	67 ±8,2 a	626 ±88 a	4674 ±610 b	07,5 ±0,55 b	04,4 ±0,33 b	04,4 ±0,44 b	09 ±0,90 c	13 ±1,26 c	18 ±1,53 c

The lower *LA* and *ULA* under 80% shade and natural shade might have been influenced by low light and also by the lower *R:Fr*, particularly under natural shade where the ratio was 24% smaller as compared to full sunlight. Reduced leaf area is one of the responses evoked by phytochromes under low *R:Fr* ratio (Smith and Whitelam, 1990; Souza and Válio, 2003a).

Stem elongation under natural shade, as expected, did not occur because of the low source-sink interactions under restricted photosynthetic photon flux under natural shade. Under similar conditions, reduced photosynthesic rate can limit the capacity of stem elongation, regulated by the low *R:Fr* ratio (Bartlett and Remphrey, 1998).

Reports on the ecological characteristics of brazilwood as related to light are controversial (Carvalho, 1994; Lorenzi, 2002). For example, 8-year-old trees grown in the city of Domingos Martins, Espírito Santo state, Brazil (20°21'49" S and 40°39'33" W), in a slope receiving direct sunlight in the morning, averaged 6.60 m in height while another lot of the same age in the same location, but grown inside the forest did not exceed 1.50 m (Mengarda, personal observation). The limited growth inside the forest associated with the low performance of brazilwood grown under 80% artificial shade and natural shade (Table 1) confirms that this tropical tree cannot be considered an early or late-successional species. In fact brazilwood has characteristics of intermediate species and present better growth under moderate shade (50% shade). Chazdon (1992) indicates that plants in this succession category have high growth potential and can express maximum growth potential under moderate irradiance.

Furthermore, besides light, climate and topography are additional environmental factors of great importance for plant growth and cell metabolism. Brazilwood may respond differently to light variation according to the particular environmental conditions under which it develops. While the climate of Vitória city varies from tropical dry to tropical humid, the city of Domingos Martins is situated at altitude of 400 m, and has a tropical mountain climate.

Brazilwood under full sunlight showed similar pattern to that observed under 50% artificial shade, such as higher values of *DMR* which reflected in the highest *R:S* ratio on conditions of higher light intensity (Table 2). Tropical trees under shade tend to allocate more biomass for the root system in response to increase in irradiance intensity to the detriment of shoots (Osunkoya et al., 1994; Souza and Válio, 2003a; Duz et al., 2004; Gonçalves et al., 2005). This adaptive strategy reduces the loss of water through leaf transpiration, thus helping maintenance of the metabolic processes at high temperature and irradiance. In fact, higher *RMR* and smaller *LMR* were verified in brazilwood plants under 0 and 50% shade (Table 2), thus supporting this premise. However, one should take into account that most luminous radiation is associated with higher temperatures and which together may affect the growth in intermediate species as observed by Rondon et al. (2006) in brazilwood of plants grown in elevated temperatures.

Leaf analyses are considered a key factor for ecophysiological studies because the effects of environmental disturbances reflect on the morphological and physiological characteristics of leaves (Lambers and Poorter, 1992). Plants that grow under low light incidence usually show higher specific leaf area (*SLA*), and succulent and thin leaves (Evans and Poorter, 2001; Souza and Válio, 2003a,b), while those that grow under high irradiance present smaller leaf area and higher *SLM*, with thick leaves and sclerophylly. Thus, the most sensitive measures to the environmental variations are *SLA* and *SLM*.

Table 2 - Ratio and growth rate of *C. echinata* after 392 days under different light regimes: Root:shoot ratio (*R:S*); specific leaf mass (*SLM*); specific leaf area (*SLA*); root mass ratio (*RMR*); stem mass ratio (*SMR*); leaf mass ratio (*LMR*); leaf area ratio (*LAR*); absolute growth rate (*AGR*); relative growth rate (*RGR*) e net assimilatory rate (*NAR*). Numbers represent means ± standard error (*n*=*7*). Different letters indicate significant differences at 0.05 by Tukey's test.

Shade	**R:S**	***SLM*** **(mg cm-^{2})**	***SLA*** **(cm^{2} mg^{-1})**	***RMR*** **(g g^{-1})**	***SMR*** **(g g^{-1})**	***LMR*** **(g g^{-1})**	***LAR*** **(cm^{2} d^{-1})**	***AGR*** **(g d^{-1})**	***RGR*** **(mg g^{-1} d^{-1})**	***NAR*** **(mg cm^{-2} dia^{-1})**
0%	0,83 ±0,08 a	2,57 ±1,0 a	0,39 ±0,02 b	0,45 ±0,02 a	0,24 ±0,01 a	0,30 ±0,02 b	116 ±0 d	0,11 ±0,01 a	6,21 ±0 a	0,033 ±0 a
50%	0,69 ±0,04 a	1,86 ±0,7 b	0,54 ±0,04 a	0,41 ±0,01 a	0,25 ±0,01 a	0,34 ±0,01 b	182 ±0 c	0,13 ±0,01 a	6,54 ±0 a	0,036 ±0 a
80%	0,30 ±0,02 b	1,81 ±1,0 b	0,55 ±0,05 a	0,23 ±0,01 b	0,22 ±0,01 a	0,54 ±0,01 a	301 ±0 a	0,03 ±0,00 b	3,78 ±0 b	0,013 ±0 b
Natural	0,33 ±0,02 b	1,90 ±1,0 b	0,53 ±0,06 a	0,25 ±0,01 b	0,25 ±0,01 a	0,50 ±0,02 a	264 ±0 b	0,03 ±0,00 b	3,70 ±0 b	0,014 ±0 b

Brazilwood plants cultivated under full sunlight presented higher *SLM* and, consequently, smaller *SLA* (Table 2), suggesting that this species fits well under high sun radiation, thus developing thickening of the leaf mesophyll evidenced by higher *SLM* values. This reduces the damages in leaves by exposure to high radiation and reduces the loss of water, thus ensuring good photosynthetic performance (Nakazono et al., 2001). High *SLA* values under low radiation are an indicative of better use of the low radiation reaching the plant (Files et al., 2008) confirming that brazilwood has physiological characteristics of intermediate species in contrast to early or late-successional species.

In addition to the low *SLM* and smaller *R:S* ratio under low irradiation, plants tend to present higher *LAR* under similar conditions (Poorter, 1999). Brazilwood plants cultivated under 80% shade presented higher *LAR* followed by intermediate values under natural shade, and smaller value under full sunlight (Table 2). It has been shown that many tropical trees grown under shade demonstrate higher *LAR* (Souza et al., 2003a; Lima et al., 2008).

Although *LAR* can be directly related to relative growth rate (*RGR*) (Poorter and Nagel, 2000; Souza and Válio, 2003a), highest *LAR* in brazilwood plants under 80% artificial and natural shade was not associated to *RGR* (Table 2). Also, a positive correlation exists between *SLM* and the photosynthetic rate besides being directly related to *RGR*, (Poorter and Nagel, 2000).

Brazilwood plants under 80% and natural shade presented smaller *LDM* due to lower *NAR*. The highest *RGR* and *NAR* of brazilwood under moderate shade (50%) and full sunlight suggests that this species has characteristics of intermediate or early-successional. However, the smaller leaf area production and *TDM* under full sunlight contrasts this hypothesis. To verify this, the *LA*, *SLM*, and *TDM* as well as *AGR*, *RGR* and *NAR* analyses (Table 2) showed moderate shade to be ideal for the initial growth of brazilwood.

Based on the lowest values of shoot mass under density shade (80% and natural shade), brazilwood cannot be classified as late-successional or climax species. The ability to tolerate moderate shade during the juvenile phase indicates that some species, such as the brazilwood could complete their development when the opening of gap happens (Whitmore, 1990). Low growth rate under high levels of shade (80% and natural shade) demonstrate lower capacity of brazilwood in developing under density shade and this can be associated to lowest photosynthetic efficiency under conditions of low or intensive photon rate.

Photosynthesis: In the experiment that simulates an artificial gap, plants that were transferred to full sunlight and those that remained under moderate shade showed a decrease in CO_2 assimilation at 48 h after the beginning of the treatment. However, the values of *A* were always higher in the plants under moderate shade. This reduction of *A* during the first 48 h may be due to environmental variations, such as temperature, irradiance, and relative humidity (Oliveira et al., 2002; Costa and Marenco, 2007; Dias and Marenco, 2007). In plants transferred to full sunlight, the photosynthesis rate decreased progressively until 192 h, a time in which the shaded plants are recovering the photosynthetic capacity (Figure 1A).

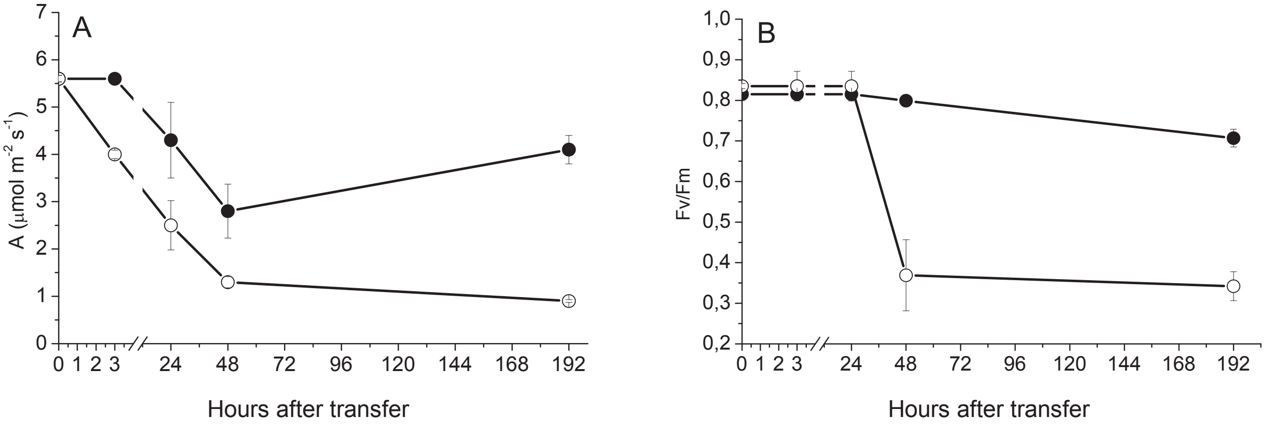

Figure 1. (A) Photosynthetic carbon assimilation (A) and (B) photosynthetic potential efficiency (Fv/Fm) of C. echinata plants subjected to constant artificial shade of 50% (●) and transferred from shade to full sunlight (○) at 0, 3, 24, 48 and 192 h. after the start of the experiment. Vertical bars indicate standard error (n=5).

Although the carbon assimilation rate usually presents a lineal increase as a function of the light intensity, high irradiancy can limit the photosynthetic assimilation of CO_2 due to dissipation energy mechanisms, such as photorespiration and photoinhibition (Dias and Marenco, 2007; Costa and Marenco, 2007). Photoinhibition and oxidation of chlorophyll in brazilwood exposed to full sunlight could be evidenced by burning and chlorosis of leaflets after 72 h of exposure to the direct sun radiation as shows the physiognomic leaflets aspects of the *C. echinata* (Figure 4).

The stomatic conductance (*gs*) followed a similar pattern as for *A*, however, the damage from exposure to full sunlight started dropping quickly by the third hour, reaching the lowest values at 192 h (Figure 2A). Under moderate shade, the *gs* followed the behavior observed for the photosynthesis rate. The transpiration rate (*E*) suffered a fast reduction under full sunlight already at the third hour of exposure and it increased simultaneously with the plants on moderate shade, however, without significant difference (Figure 2B). Afterwards, plants from both treatments showed progressive reduction in *A* until the end of the experiment. Plants showed increase in *VPDleaf-to-air*, at 24 h followed by a decrease until 192 h, with higher values for the plants that were transferred to direct sun radiation (Figure 2C).

The reduction in photosynthetic rate associated to the increase in *VPDleaf-to-air* and reduction of *gs* has been shown to occur possibly due to the increase of time of exposure to the light and temperature (Dias and Marenco, 2007). High *VPDleaf-to-air* can cause closing of the stomata, decrease of the internal carbon concentration and, consequently, reduction in photosynthesis. For brazilwood plants transferred to direct sun radiation it was observed an increase in *VPDleaf-to-air* and gradual reduction of *A* during the 192 h of direct sun exposure.

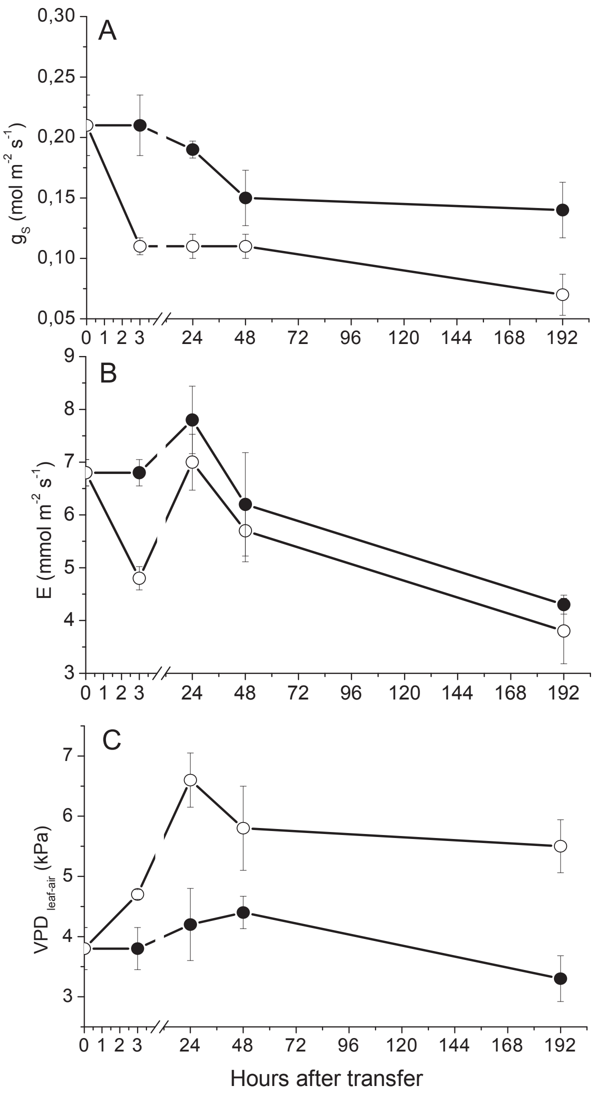

Figure 2. (A) Stomatic conductance (g_S), (B) transpiration (*E*), and (C) vapor pressure deficit (*$VPD_{leaf\text{-}air}$*), of *C. echinata* plants subjected to constant artificial shade of 50% (●) and transferred from shade to full sunlight (○) at 0, 3, 24, 48 and 192 h. after the start of the experiment. Vertical bars indicate standard error (*n=5*).

The fast reduction of *E* in the first 3 h of exposure to sunlight as related to net photosynthetic rate is an indicative of an efficient mechanism of adaptation aiming to control the reduction of loss of water (Oliveira et al., 2002; Cavalcante and Conforto, 2006). This can be confirmed by the reduction of *WUE* associated to the decreasing values of *A* during the first 48 h after transfer to sunlight, after which differences intensified up to 192 h (Figure 3A). Some tropical tree species, such as *Aniba roseadora* has shown a relative decrease in *WUE* when subjected to high light intensity (Gonçalves et al., 2005). Similar results were also observed for *IWUE* (Figure 3B) demonstrating that brazilwood limits water-use when exposed to direct sun radiation due to the partial closing of the stomata (Figure 2A).

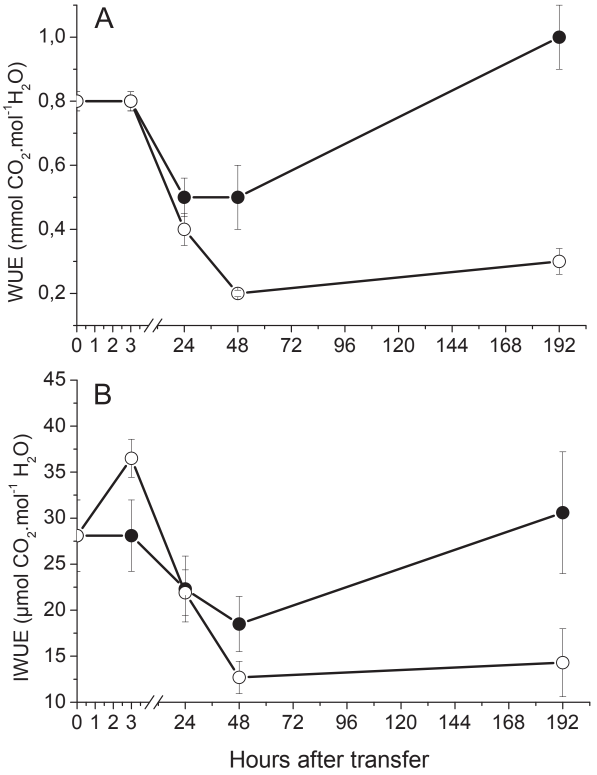

Figure 3. (A) Water-use efficiency (*WUE*) and (B) intrinsic water-use efficiency (*IWUE*) of *C. echinata* plants subjected to constant artificial shade of 50% (●) and transferred from shade to full sunlight (○) at 0, 3, 24, 48 and 192 h. after the start of the experiment. Vertical bars indicate standard error (*n=5*).

2,5cm

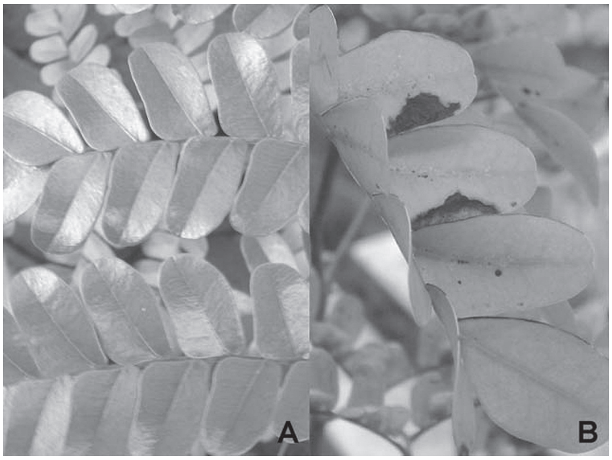

Figure 4. Physiognomic leaflets aspects of the *C. echinata* plants subjected to 50% of artificial shade constant (A) and after the transfer to full sunlight (B). Leaflets of all plants show burn and marginal chlorosis at 72 h after the transfer to full sunlight (*n=5*).

The exposure of young plants to direct light can intensify the photoinhibition effects caused mainly by the increase in leaf temperature and drastic decrease in leaf hydric potential, which directly affects *gs* and *E* (Valladares and Pearcy, 1997). Photoinhibition can be evidenced from measures of chlorophyll fluorescence, which expresses the maximum quantum efficiency of *PSII* or the *Fv/Fm* ratio (Butler, 1978; Björkman, 1987).

Plants exposed to full sunlight as well as that under moderate shade showed no variation for *Fv/Fm* for the first 24 h of the experiment. The ratio varied from 0.700 to 0.815 (Figure 1B), similar to values found by Gebler et al. (2005) in adult plants of *C. echinata*. After 24 h we noticed a sharp drop in the quantum efficiency of *PSII* in plants under full sunlight; 50% lower than the value found in plants subjected to moderate shade at 192 h. Reductions in the *Fv/Fm* ratio have been related to photoinhibition induction as reported for tropical trees subjected to irradiancy increases (Dias and Marenco, 2007) and plants exposed to several environmental stresses that affect the *PSII* functionality (Long et al., 1994; Rohacek and Bartak, 1999). Indeed, exposure of leaves to intense light can damage the *D1* protein of *PSII* interfering in the electron transport, and thus can reduce the effectiveness of the photochemistry phase of the photosynthesis (Gonçalves and Santos Jr., 2005).

The succession stage can influence photosynthesis and photochemistry efficiency potential of plants when exposed to direct light or in a gap. In fact, some tropical tree has shown reduction in *Fv/Fm* in gaps present in semi-deciduous forests (Brook et al., 2004). However, species that are sun tolerant make better use of the light energy and are capable of dissipating the excess of energy as compared to shade tolerant species. Consequently, we can assume that gas exchange and fluorescence of chlorophyll *a* of *C. echinata* are less efficient in use of the energy under direct light. This can result in structural and physiologic damages causing the reduction of *A*, *gs*, *E* and increase in *VPDleaf-to-air*.

Given the short evaluation period, it was possible to identify the signaling phase of the light stress of *A* and *gs* already in the first hour, which continued up to 192 h of exposure to direct sun radiation. Based on the results with young plant, brazilwood appears to be an intermediate species because it establishes better under conditions of moderate shade (50% artificial shade). This species cannot be considered early or late-successional because it shows to be not density shade-tolerant (80% artificial shade and natural shade) neither full sunlight-tolerant. The classification of young brazilwood plants as intermediate can be confirmed by inhibition of photosynthesis under the gap simulated full sunlight (Figure 1A,B). Furthermore, late-successional species comprise the species usually found in the density shaded understory, whereas early-successional species comprise the species that are absent from large gaps or forest edges (Souza and Válio, 2003a). Therefore, the results confirm that young brazilwood plants could be intermediate species because they occur under conditions of moderate shade, such as in small gaps, shaded forest edges or moderately shaded understory, as observed in its area of natural occurrence. To further confirm these observations, the natural shade simulated under close canopy caused reduction in both *PPFD* and *R/Fr* ratio, where by some of the brazilwood was influenced by light quality and quantity.

In contrast, plants under simulated gaps were affected by full sunlight (Tables 1, 2). Plant growth under natural shade was more affected than under artificial density shade (80% shade). This might reflects a high sensitivity of *C. echinata* to light quality and quantity. When full sunlight reaches the canopy it is filtered reducing both *PPFD* and *R/Fr* (Souza and Válio, 2001, 2003). Only early-successional species can tolerate these conditions. The intolerance of brazilwood to the density shaded understory and the inhibitory effects on photosynthesis (Figures 1A, B) associated to reduced growth (Tables 2, 3) allows us to classify this species as intermediate. This suggestion is supported by its natural habitat of seasonal semideciduous forests and slow growth occurring in soils of Brazilian Atlantic Forest between Rio Grande do Norte and Rio de Janeiro State (Gebler et al. 2005).

The reduced *LN* might be a phytochrome response under conditions of reduced *R:Fr* ratio (Smith and Whitelam, 1990). However, the effects on *LN* and on stem height under natural simulation of closed canopy show that brazilwood does not have a typical morphogenetic response of late-successional species. Additional evidence is the higher *RGR* and *NAR* on lower shade (Table 2).

Possibly brazilwood plants developing under a natural gap can find the satisfactory conditions for rustification. This can ensure better conditions for growth and raise the percentage of survival for recruitment of seedlings for reforestation using native tropical species. Analysis of soluble carbohydrates and amino acids associated with light stress may help in understanding the physiological mechanisms of brazilwood under the effect of high-radiation stress. The present data will enable to place this species in adequate position in ecological succession, which is very important for management and reforestation programs.

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