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Light acclimation in nursery: morphoanatomy and ecophysiology of seedlings of three light-demanding neotropical tree species

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Abstract Plants can acclimate to environmental changes by physiological and morphoanatomical responses. We aimed to evaluate the influence of high-light acclimation on the hardiness of neotropical tree species seedlings, through analysis of the gas exchange, morphology and anatomy of *Aegiphila integrifolia* (Jacq.) Moldenke, *Guazuma ulmifolia* Lam. and *Heliocarpus popayanensis* Kunth. Seedlings were grown in a shaded sector (40 % of photosynthetic photon flux density) of a nursery. After the growing period, part of the seedlings was kept in the shaded sector (shade) and another part was transferred to full sunlight (sun). The seedlings remained in the respective sectors for 168 days. *H. popayanensis* sun seedlings presented increases in net photosynthesis together with reduced stomatal conductance and transpiration, resulting in higher water-use efficiency. In contrast, the transfer to full sunlight led to a decrease in net photosynthesis of *A. integrifolia* and *G. ulmifolia* seedlings, but this parameter was recovered after development of new leaves in the sun. The hardening process under high irradiation levels induced morphoanatomical responses in all species, such as increased palisade parenchyma thickness and lower total leaf area (all species), higher stomatal density (*A. integrifolia* and *G. ulmifolia*), higher biomass allocation to roots (*H. popayanensis* and *G. ulmifolia*), and higher Dickson quality index (*H. popayanensis*). Thus, when submitted to a high radiation environment, the physiological and morphoanatomical acclimation increased seedlings hardiness and, in consequence, the probability of survival after planting in the field.

Keywords Dickson quality index · Dry mass · Gas exchange · Hardiness · Light · Mesophyll

Introduction

Although light is an indispensable resource (Poorter 2001), both low and high of light availability can limit plant performance (Valladares and Niinemets 2008). While low irradiance in leaves decreases carbon gain and reduces plant growth (Aleric and Kirkman 2005), its excess can cause deleterious effects by inducing photoinhibition (Jordan et al. 2005). However, plants often exhibit a remarkable ability to adjust their physiology, morphology, and anatomy in response to a particular light condition by acclimation, which allows plants to use efficiently the light energy, while avoiding photodamage to their leaves (Givnish 1988; Hanba et al. 2002; Walters 2005; Valladares and Niinemets 2008). The degree of response to increased light varies among species, and may result in altered patterns of growth and biomass allocation (Chazdon et al. 1996; Valladares and Niinemets 2008; Cano et al. 2011).

In response to different light intensities, many plant species are able to acclimate through the development of sun or shade leaves (Givnish 1988; Yano and Terashima 2004). Under low irradiance, plants tend to allocate more resources in leaves than in roots and develop larger and thinner leaves with lower stomatal density and

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photosynthetic rates (Poorter et al. 2012). In contrast, when exposed to high irradiance, plants have smaller and thicker leaves with higher stomatal density and carbon assimilation rates, investing more resources in root biomass (Givnish 1988; Poorter et al. 2012). Due to these characteristics, sun leaves have higher tolerance to high irradiance and enhanced capacity to dissipate the light excess, whereas shade leaves are prone to suffer the damage caused by excessive light (Demmig-Adams and Adams 1992; Gyimah and Nakao 2007).

The hardening process under high irradiance has been widely used in nurseries as a mechanism for increasing the robustness of seedlings of tropical tree species. Hardy plants are more likely to survive after planting than non-hardened ones and their development can be faster in the field (Jacobs et al. 2005). Studies on morphological, anatomical, and ecophysiological responses of different tropical tree species during the hardening process may contribute to the definition of a hardiness pattern for them. In nurseries that deal with homogenous populations of *Pinus* and *Eucalyptus* varieties, relationships between morphological parameters have been used to evaluate the hardiness of the seedling. The stem height by stem base diameter ratio (H/D), the shoot dry mass by root dry mass ratio (SDM/RDM), and the Dickson quality index (DQI), which considers the total dry mass production and its allocation in shoots and roots (SDM/RDM) as well as the H/D (Dickson et al.1960; Claussen 1996) are some examples. However, it is still a challenge to set a hardiness pattern for non-commercial tropical native seedlings based solely on quality indicators, due to the genetic variability of these species.

The knowledge of how plants respond morphologically and physiologically to contrasting light conditions can be useful to explain their occurrence and abundance patterns under specific environmental conditions (Yang et al. 2014), and it is also important for the production of tree seedlings for reforestation of degraded environments (Krause et al. 2001; Oguchi et al. 2006), especially in tropical areas. The understanding of morphological and photosynthetic traits of a desired species when planted in either shade or exposed sites is required for successful reforestation.

This study aimed to contribute to restoration programs by providing information about the high-light acclimation of three light-demanding tree species. For this, hardiness of the seedling was evaluated through the analysis of leaf gas exchange parameters and the morphology and anatomy of seedlings subjected to full sunlight in comparison with those maintained at shaded conditions. The following questions were raised: (1) which photosynthetic adjustments the three species develop in response to different light environments? (2) Which hardiness traits are developed in seedlings of light-demanding tree species during the high-light hardening process in nurseries?

Materials and methods

Study area, species, and experimental design

This research was carried out at the nursery of the "Laboratório de Biodiversidade e Restauração de Ecossistemas" (23°32′44″S, 51°19′83″W) of the Universidade Estadual de Londrina, Londrina, Paraná state, Southern Brazil. Three light-demanding tree species were selected due to their importance in reforestation programs in the region: *Heliocarpus popayanensis* Kunth (Malvaceae), *Guazuma ulmifolia* Lam. (Malvaceae), and *Aegiphila integrifolia* (Jacq.) Moldenke (Lamiaceae). Our purpose was to investigate the performance of seedlings under exactly the same conditions to which they are subjected in the nursery hardening routine. Thus, approximately 1000 seedlings of each species were grown in 50 cm^3 polyethylene tubes filled with a mixture of sieved organic compost (cattle manure and litter), carbonized rice husk, and washed sand, enriched with Osmocot® beads, a slow-release fertilizer (15 % N, 10 % P_2O_5, and 10 % K_2O). All plants remained in the growth sector of the nursery until they reached about 20 cm of height. This sector was covered with a polypropylene screen resulting in the incidence of only 40 % of total photosynthetic photon flux density (PPFD). After the growing period, about 40 % of seedlings were maintained in the growth sector (shade; control) and 60 % were transferred to full sunlight at the acclimation sector (sun). The seedlings were kept in these sectors for 168 days. In both sectors, they were watered for 30 min four times per day by an automatic irrigation system.

Leaf gas exchange measurements

Up to 168 days after beginning of the experiment, gas exchange parameters were determined every 14 days on the penultimate fully expanded leaves (without signs of senescence) from nine randomly selected seedlings of each treatment per species. The criterion for leaf choice was always applied even for seedlings of the acclimation sector, regardless the picked leaf has been initially developed under shade or full sun. Therefore, the first measurements were done in the standing leaves, which were developed under shaded conditions. Leaf gas exchange parameters were measured using a Portable Photosynthesis System LI-6400XT (LI-COR Biosciences Inc., Lincoln, NE, USA). A red-blue LED light source attached to the system was used to keep constant the PPFD at 1900 $\mu mol\ m^{-2}\ s^{-1}$, enough to saturate the CO_2 assimilation rate of the three species (as determined by light response curves). The light-saturated net photosynthetic rate (A_{max}, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), and transpiration rate (E, mmol m^{-2} s^{-1}) were measured between 8:00 a.m. to 10:00 a.m. The water-use efficiency (WUE, μmol $mmol^{-1}$) was calculated by the A_{max}/E ratio.

Anatomical analysis

Anatomical characteristics were evaluated at 168 days of the experiment in nine randomly selected seedlings of each treatment per species. We collected segments from the central area of the lamina of the penultimate fully expanded leaf, which were fixed in FAA 70 % (formaldehyde, glacial acetic acid, and ethanol 70 %, 5:5:90) for 72 h, then washed in distilled water, dehydrated in an ethanol-xylol series, and finally embedded in paraffin (Johansen 1940). Transverse 14-μm-thick sections were stained with astrablue—basic fuchsin and mounted in Canadian balsam (Macêdo 1997).

Additional leaf lamina segments (1 cm^2) were treated with Jeffrey's solution (1:1 chromic and nitric acids) for epidermis analyses (Macêdo 1997). Both epidermis faces were stained with astrablue—basic fuchsin and mounted in Canadian balsam (Macêdo 1997).

All slides were deposited at the Laboratório de Anatomia Vegetal, Universidade Estadual de Londrina. Observations and digital photomicrographs of the section were taken under a light microscope (Axioplan Zeiss, Germany). Digital photos were scaled and analyzed with Motic Images Plus 2.0 software (Motic China Group Co. Ltd., Xiamen, China). The thickness (μm) of the lamina, epidermis (adaxial and abaxial face), and mesophyll (palisade and spongy parenchyma) were measured, as well as the stomatal density (the number of stomata mm^{-2}) was calculated.

Morphological measurements

All morphological parameters were recorded in nine seedlings of each treatment per species. Plant height (H-from the base of the plant to the apex) and stem base diameter (D) were recorded at the end of the experiment. Stem base diameter was measured with digital calipers (±0.01 mm) at the soil surface, whereas the stem height was measured with millimeter ruler. We also counted the total number of leaves (TNL) and the number of newly developed leaves during the experiment (NDL).

At the end of the experiment, all leaves of each seedling were scanned in monochromatic format and the readings of the images were obtained by DDA 1.2.0.0 software (Ferreira et al. 2008) for the determination of the total leaf area (LA, cm^2) of each seedling. In order to measure the dry masses, the parts of the seedlings were separated (roots, stem, and leaves) and oven-dried at 70 °C to constant mass (approximately 72 h). Then, using a semi-analytical scales, we obtained the root (RDM), stem (StDM), leaves (LDM), shoot (SDM = StDM + LDM), and total (TDM = RDM + SDM) dry masses. Specific leaf area (SLA, cm^2 g^{-1}) of each seedling was calculated as the LA to LDM ratio. We also calculated H/D and SDM/RDM ratios and the Dickson quality index, given by DQI = TDM/(H/D + SDM/RDM) (Dickson et al. 1960).

Statistical analysis

Data were tested using one-way analysis of variance (ANOVA) with 5 % significance levels in Statistica 10.0 (Statsoft Inc., Tulsa, USA). The percentage data were transformed (arcsine of the square root of the ratio) to meet the assumption of ANOVA (normal distribution and equal variances).

Results

Irrespective of tree species, no seedling died during the experiment, but during the first weeks the seedlings transferred to the acclimation sector showed some signs of injury, such as yellowing and senescence of mature leaves (data not shown).

In the case of *H. popayanensis*, full sunlight acclimation induced increases in A_{max} at the beginning (14 and 42 days) and the end (126, 154, and 168 days) of the experiment (Fig. 1a). In comparison with shade seedlings, the sun plants showed lower g_s and E during a large extent of the experiment (28, 56 to 126 days, and 56 to 112 days, respectively) (Fig. 1b, c). As a result, higher WUE values were observed in sun seedlings from 56 to 126 days (Fig. 1d). In general, NDL and TNL did not differ between treatments, except at 14 days or at 28, 98, 112, and 126 days, when sun seedlings showed a decrease in NDL (Fig. 1e) or TNL (Fig. 1f), respectively.

Heliocarpus popayanensis has hypostomatic leaves. Although the stomatal density did not differ between treatments, the thickness of palisade and spongy parenchyma, adaxial epidermis, and leaf lamina was higher in sun plants than in shade ones (Table 1).

Sun seedlings of *H. popayanensis* showed lower H, but D did not differ in comparison with shade seedlings (Table 2). LA, SLA, and LDM were reduced during acclimation (Table 2). DQI was higher, while H/D and SDM/RDM were lower in sun seedlings (Table 2), indicating higher hardiness when compared to shade seedlings.

At initial phase of the experiment (up to 70 days), the hardening process reduced the A_{max} of *G. ulmifolia* seedlings (Fig. 2a). Additionally, lower g_s and E were detected

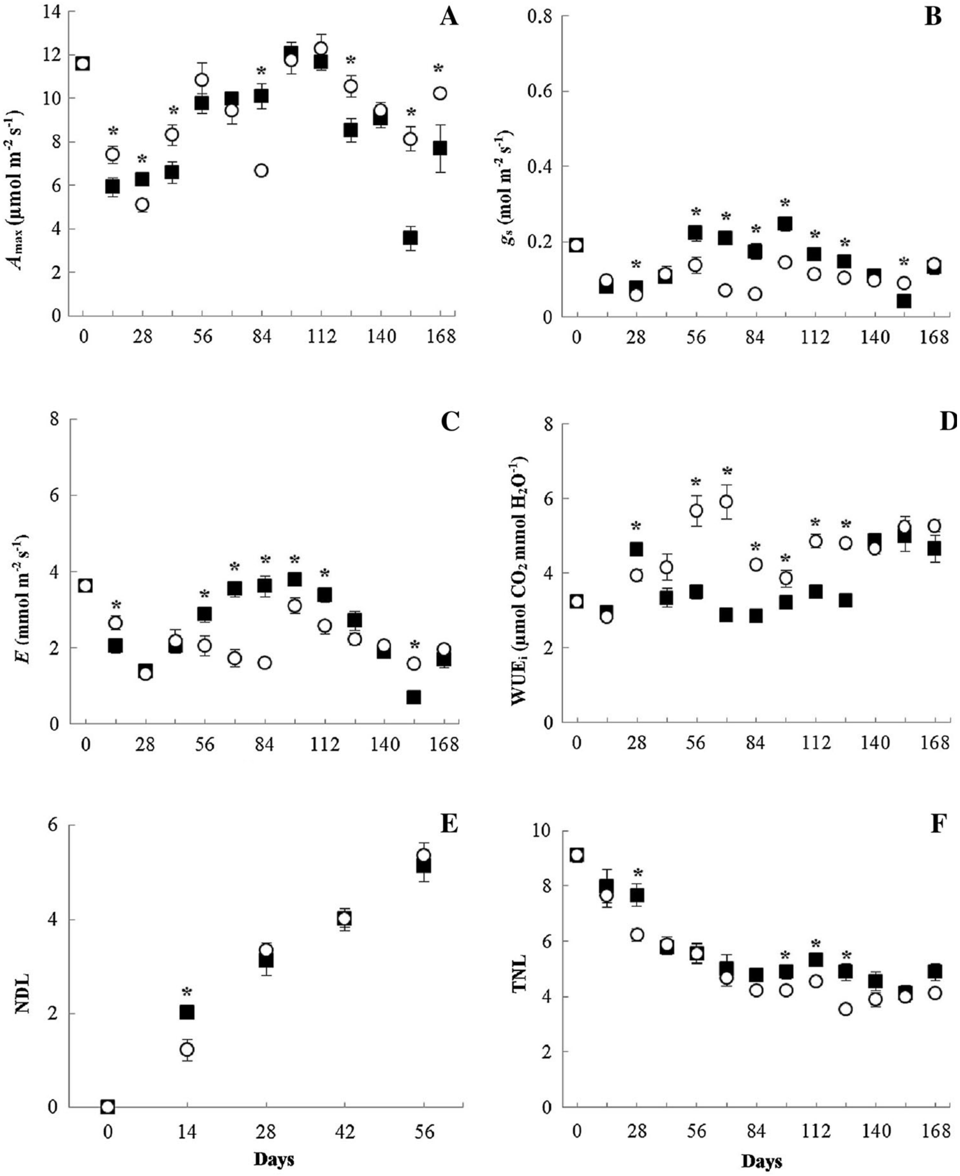

Fig. 1 Leaf gas exchange and leaf number of *Heliocarpus popayanensis* grown under 40 % of photosynthetic photon flux density (*filled square*—shade) or under full sun (*open circle*—sun) over 168 days of the experiment. **a** Maximum rate of net photosynthesis (A_{max}), **b** stomatal conductance (g_s), **c** transpiration rate (E), **d** water-use efficiency (WUE), **e** cumulative number of new developed leaves (NDL), and **f** total number of leaves (TNL), during 56 and 168 days of the experiment, respectively. *Asterisk* indicates significant differences ($P < 0.05$) by F test (ANOVA). *Verticals bars* indicate SE ($n = 9$)

Table 1 Leaf anatomic parameters (means ± standard errors, $n = 9$) of *Heliocarpus popayanensis* (Hp), *Guazuma ulmifolia* (Gu), and *Aegiphila integrifolia* (Ai) seedlings grown under 40 % of photosynthetic photon flux density (shade) or full sunlight (sun) after 168 days of experiment

Parameters	Species					
	Hp		Gu		Ai	
	Shade	Sun	Shade	Sun	Shade	Sun
Upper epidermis thickness (μm)	15.9 ± 0.73	18.7 ± 0.89**	37.2 ± 2.81	33.9 ± 2.20	17.0 ± 0.76	18.0 ± 0.87
Palisade parenchyma thickness (μm)	42.6 ± 2.09	66.3 ± 4.42*	22.8 ± 2.22	37.4 ± 4.56*	46.2 ± 5.11	63.7 ± 5.53*
Spongy parenchyma thickness (μm)	47.2 ± 1.86	60.5 ± 2.09*	32.1 ± 1.76	35.4 ± 2.50	79.6 ± 3.59	99.6 ± 6.00*
Lower epidermis thickness (μm)	13.3 ± 0.89	14.8 ± 0.82	15.4 ± 1.07	15.0 ± 0.90	10.9 ± 0.48	12.8 ± 0.49*
Leaf thickness (μm)	119.1 ± 3.45	160.4 ± 4.78*	107.5 ± 4.60	121.7 ± 5.22	153.8 ± 6.84	194.1 ± 9.87*
Stomatal density (n mm^{-2})	288.8 ± 23.0	272.2 ± 23.7	329.1 ± 21.2	494.8 ± 18.6*	251.3 ± 23.6	375.9 ± 15.1*

* Indicates significant differences ($P < 0.05$) by F test (ANOVA)

Table 2 Growth parameters (mean ± standard errors, $n = 9$) of *Heliocarpus popayanensis* (Hp), *Guazuma ulmifolia* (Gu), and *Aegiphila integrifolia* (Ai) seedlings, grown under 40 % of photosynthetic photon flux density (shade) or under full sun (sun) after 168 days of the experiment

Parameters	Species					
	HP		GU		AI	
	Shade	Sun	Shade	Sun	Shade	Sun
Stem height (cm)	48.1 ± 0.52	37.1 ± 0.17*	30.7 ± 0.77	29.0 ± 0.76	25.4 ± 0.58	25.6 ± 0.37
Stem base diameter (mm)	5.36 ± 0.15	5.34 ± 0.17	4.04 ± 0.08	3.36 ± 0.07*	3.99 ± 0.14	3.68 ± 0.19
H/D (cm mm^{-1})	9.04 ± 0.26	7.01 ± 0.21*	7.62 ± 0.28	8.65 ± 0.23*	6.44 ± 0.31	7.09 ± 0.35
Leaf dry mass (g)	0.204 ± 0.01	0.107 ± 0.01*	0.459 ± 0.03	0.126 ± 0.02*	0.168 ± 0.03	0.064 ± 0.01*
Stem dry mass (g)	0.861 ± 0.08	0.826 ± 0.05	0.533 ± 0.04	0.341 ± 0.01*	0.335 ± 0.03	0.236 ± 0.02*
Shoot dry mass (g)	1.070 ± 0.08	0.933 ± 0.05	0.992 ± 0.06	0.467 ± 0.19*	0.503 ± 0.05	0.301 ± 0.02*
Root dry mass (g)	0.589 ± 0.04	0.622 ± 0.03	0.764 ± 0.09	0.706 ± 0.06	1.060 ± 0.16	0.491 ± 0.07*
Total dry mass (g)	1.654 ± 0.11	1.555 ± 0.07	1.756 ± 0.11	1.173 ± 0.07*	1.563 ± 0.21	0.792 ± 0.08*
SDM/RDM (g g^{-1})	1.840 ± 0.12	1.510 ± 0.08*	1.470 ± 0.2	0.687 ± 0.04*	0.543 ± 0.81	0.688 ± 0.09
DQI	0.153 ± 0.01	0.183 ± 0.01*	0.196 ± 0.02	0.127 ± 0.01*	0.225 ± 0.03	0.105 ± 0.01*
Total leaf area (cm^2)	72.7 ± 4.82	28.9 ± 1.66*	119.8 ± 7.69	29.3 ± 2.83*	45.7 ± 6.83	14.7 ± 1.54*
Specific leaf area (cm^2 g^{-1})	355.7 ± 11.9	269.5 ± 4.3*	260.8 ± 7.2	242.1 ± 11.8	279.1 ± 7.8	238.9 ± 11.6*

H/D stem height by stem base diameter ratio; *SDM/RDM* shoot dry mass by root dry mass ratio; *DQI* Dickson quality index

* Indicates significant differences ($P < 0.05$) by F test (ANOVA)

in sun seedlings compared to shade ones until 42 days of the experiment (Figs. 2b, c). However, at the second part of the experiment, there was a recovery in the $A_{\max}$ of the sun seedlings (Fig. 2a), as well as they showed higher values of g_s and E in comparison with shade plants (Figs. 2b, c).

In general, WUE was lower in sun plants, especially at the end of the experiment (Fig. 2d). A TNL reduction was observed in sun seedlings from 28 days (Fig. 2f), while NDL reduction was observed only at 56 days in relation to shade seedlings (Fig. 2e).

Anatomical analysis showed that *G. ulmifolia* present hypostomatic leaves. The alterations induced by high-light acclimation were increases in stomatal density and palisade parenchyma thickness (Table 1).

The dry masses of most organs were lower in sun seedlings (only RDM remained unchanged) (Table 2). H and SLA did not differ between treatments, but D and LA were lower in sun seedlings (Table 2). In these plants, the *H/D* was higher, while SDM/RDM and DQI were lower when compared to shade seedlings (Table 2).

Sun seedlings of *A. integrifolia* showed $A_{\max}$ reduction at 14 and 28 days when compared to shade seedlings (Fig. 3a). However, after this initial phase, $A_{\max}$ of sun plants increased to values similar or even higher than the shaded control (Fig. 3a). High-light acclimation led to an increase in E (Fig. 3c), which was followed by higher g_s in some days (from 56 to 98 days, and 126 days) (Fig. 3b). In sun seedlings, WUE reduction was observed in comparison with shade seedlings, except from 140 and 168 days (Fig. 3d). In general, NDL and TNL did not differ between treatments (Fig. 3e, f). However, it should be noted that the species shows a deep decrease in the total leaf number during experiment in both treatments.

Aegiphila integrifolia also showed hypostomatic leaves. In sun seedlings, the stomatal density was higher and the abaxial epidermis, palisade and spongy parenchyma, and leaf lamina were thicker than shade seedlings (Table 1).

We did not observe differences in H and D between treatments, but the dry masses, LA, and SLA were lower in sun seedlings of *A. integrifolia* (Table 2). The *H/D* and SDM/RDM did not differ between treatments; however, the DQI was lower in sun plants (Table 2).

Discussion

Our study provided a characterization of the physiological, anatomical, and morphological changes induced by the full sunlight exposition of seedlings, in a nursery, of three light-demanding tree species used in reforestation programs in Brazil. The seedlings of *H. popayanensis*, *G. ulmifolia*, and *A. integrifolia* showed different responses to full sun exposition, showing species-specific acclimation in the evaluated parameters. However, all of them developed characteristics of hardiness, which may favor their initial establishment at the field.

The increase in light intensity after the transfer of seedlings to acclimation sector did not stimulate the

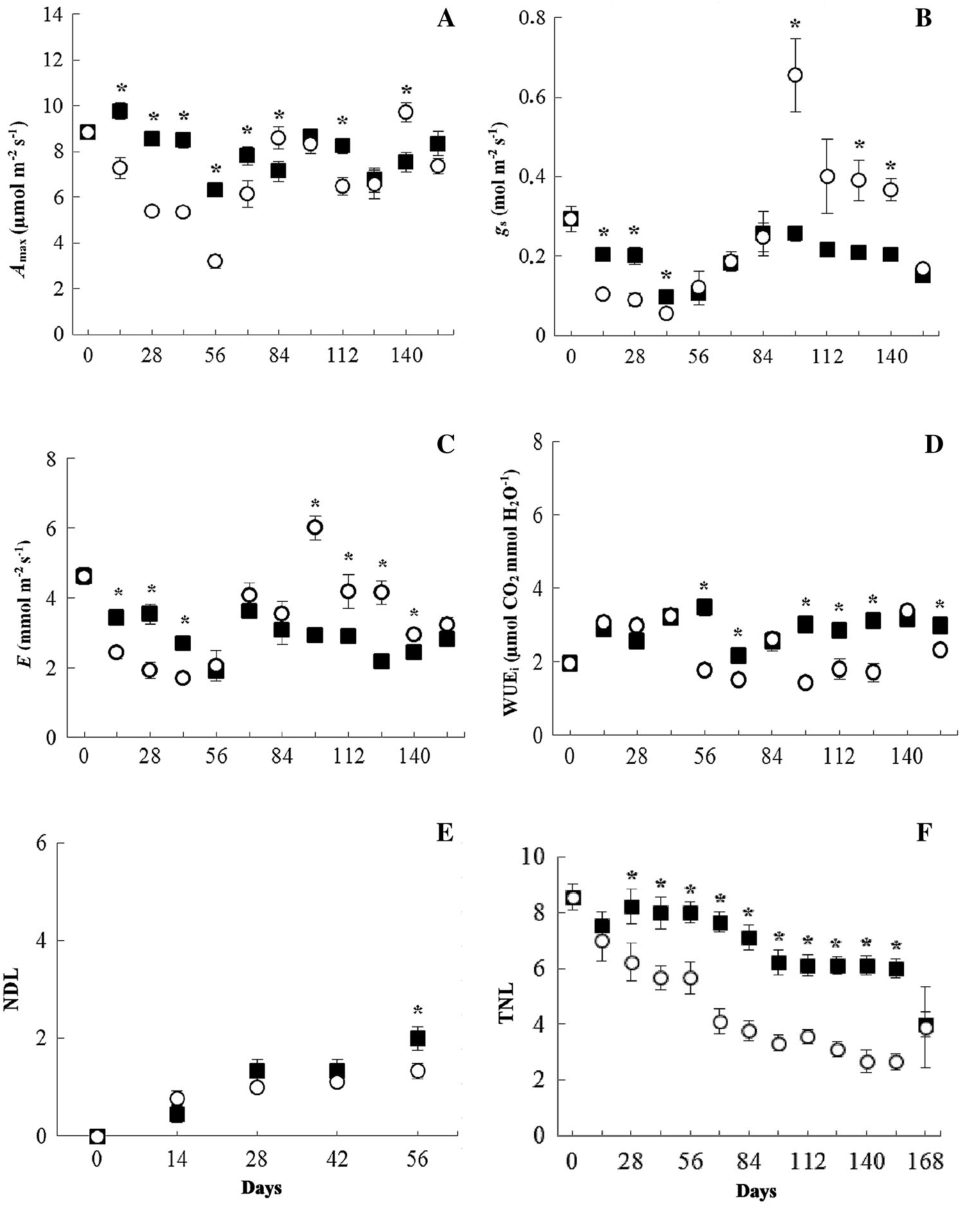

Fig. 2 Leaf gas exchange and leaf number of *Guazuma ulmifolia* grown under 40 % of photosynthetic photon flux density (*filled square*—shade) or under full sun (*open circle*—sun) over 168 days of the experiment. **a** Maximum rate of net photosynthesis (A_{max}), **b** stomatal conductance (g_s), **c** transpiration rate (E), **d** water-use efficiency (WUE), **e** cumulative number of new developed leaves (NDL) and **f** total number of leaves (TNL), during 56 and 168 days of the experiment, respectively. *Asterisk* indicates significant differences ($P < 0.05$) by F test (ANOVA). *Verticals bars* indicate SE ($n = 9$)

development of new leaves in any species. However, *G. ulmifolia* and *H. popayanensis* seedlings showed a reduction in TNL after the transfer, due to the shedding of older leaves, which indicated faster leaf senescence in sun plants. Indeed, sun-grown plants usually exhibit faster leaf senescence, but they also show higher leaf production than shade plants of the same species (Chabot and Hicks 1982; Sims and Pearcy 1992), a pattern that was not observed in this study. This may have occurred because in high-light environments, there is usually higher heat loads and vapor pressure deficits (Sessa and Givnish 2014), and seedlings possibly underwent drought stress in the acclimation sector, especially in warmer days, due to the low capacity of the tubes to retain water. Water shortage can induce leaf shedding, which should attenuate water stress and reduce the risk of hydraulic failure of plants (Cortina et al. 2013). At the end of the experiment, all the *A. integrifolia* seedlings were healthy. The decrease of leaf number in both treatments must have been caused by some factor inherent to the developmental process of the seedlings. Therefore, the progressive loss of leaves was not caused by contrasting abiotic conditions between treatments.

Leaf gas exchange analysis demonstrated an initial decrease in A_{max} of *G. ulmifolia* and *A. integrifolia* seedlings after the transfer to full sunlight. This depression in CO_2 assimilation rate was probably a consequence of the inability of mature leaves expanded under shade to dissipate excess excitation energy and develop effective

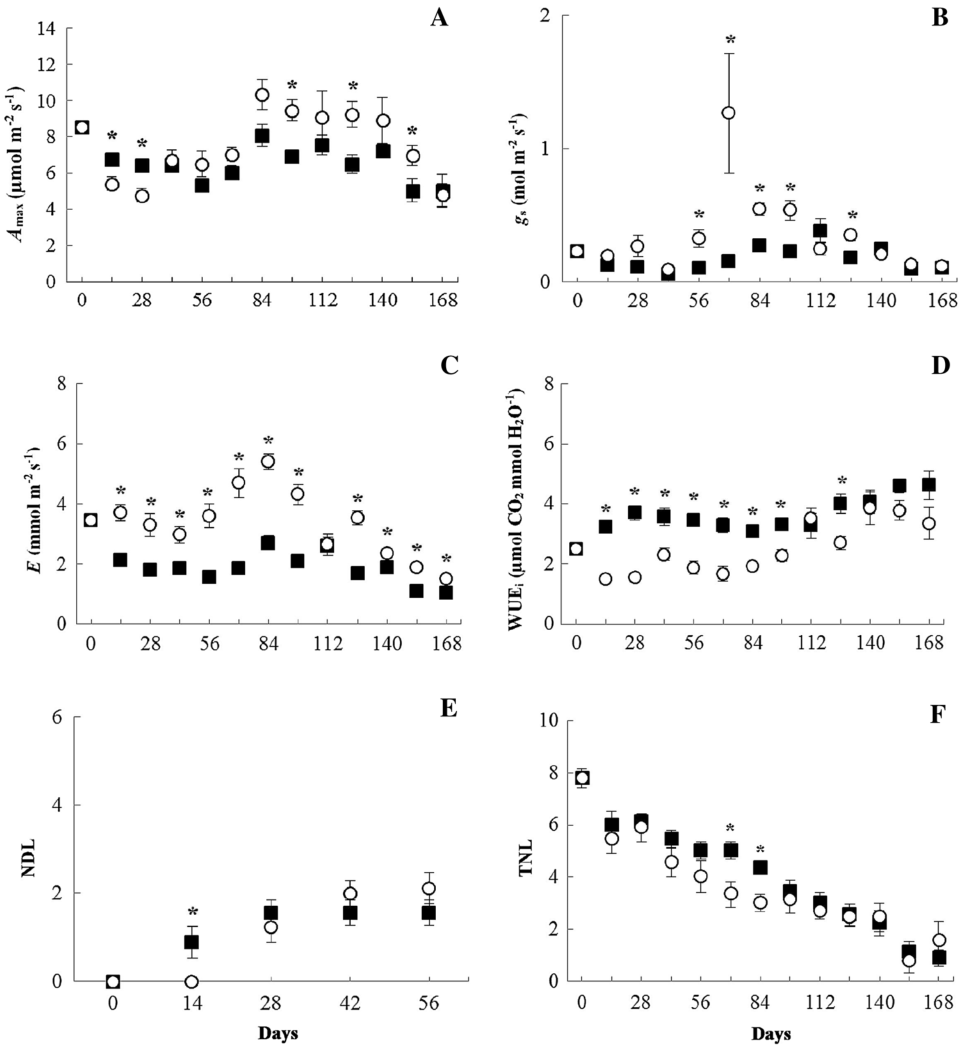

Fig. 3 Leaf gas exchange and leaf number of *Aegiphila integrifolia* grown under 40 % of photosynthetic photon flux density (*filled square*—shade) or under full sun (*open circle*—sun) over 168 days of the experiment. **a** Maximum rate of net photosynthesis (A_{max}), **b** stomatal conductance (g_s), **c** transpiration rate (E), **d** water-use efficiency (WUE), **e** cumulative number of new developed leaves (NDL), and **f** total number of leaves (TNL), during 56 and 168 days of the experiment, respectively. *Asterisk* indicates significant differences ($P < 0.05$) by F test (ANOVA). *Verticals bars* indicate standard errors ($n = 9$)

photoprotective mechanisms, thus resulting in photoinhibition, as previously proposed for other tree species (Claussen 1996; Kitao et al. 2000; Matsuki et al. 2003; Endres et al. 2010; Gonçalves et al. 2013). The effects of photoinhibition can be minimized by the production of new leaves with biochemical and structural traits that lead to acclimation to high irradiances (Mulkey and Pearcy 1992; Kitao et al. 2000; Yamashita et al. 2000; Cuzzol and Milanez 2012). Thus, the recovery of A_{max} of *G. ulmifolia* and *A. integrifolia* seedlings observed after the initial phase of the experiment might be a consequence of the production of sun-acclimated leaves. Similarly, the observation that, in general, *H. popayanensis* photosynthetic rates were not negatively affected by high irradiances may be related with faster rates of development of new leaves of this species.

Indeed, in the three studied species, the leaves expanded under full sunlight presented some of the morphoanatomical traits typical of sun leaves previously described in the literature (Boardman 1977; Givnish 1988; Ivancich et al. 2012), such as lower LA and SLA, thicker palisade and spongy parenchyma, and higher leaf thickness and stomatal density. In particular, the thickening of palisade parenchyma was observed in acclimated leaves of all species, which may represent a structural mechanism for increasing photosynthetic rates under high irradiances (Boardman 1977; Oguchi et al. 2005; Ivancich et al. 2012). The higher development of chlorenchyma is usually associated with a reduction of SLA (Poorter 1999; Hanba et al. 2002; Ivancich et al. 2012), a pattern that was observed in *H. popayanensis* and *A. integrifolia*. In these species, the higher SLA of shade seedlings may contribute to the increase of light absorption in environments of low irradiance (Poorter 1999). However, due to the greater surface area per leaf volume, this characteristic may decrease the control of water loss (Ivancich et al. 2012), thus augmenting the probability of dehydration after the transfer to the field.

In addition to the influence on SLA, the thickening of chlorenchyma induced by high irradiance stimulates the production of chloroplasts, what may result in an increase of CO_2 assimilation per leaf area (Evans and Poorter 2001; Terashima et al. 2001). This might explain the higher A_{max} values of *H. popayanensis* and *A. integrifolia* sun seedlings in comparison with shaded ones during the experiment, as suggested for other tree species (Martínez-Pastur et al. 2007; Ivancich et al. 2012), as well as the recovery of A_{max} values of *G. ulmifolia* and *A. integrifolia* sun seedlings. This ability of seedlings to develop physiological adjustments to high irradiances has been associated with higher probability of survival in the field (Gyimah and Nakao 2007).

The increase in photosynthetic rate at high light may occur at expenses of higher stomatal conductance, which causes greater water loss by transpiration (Lee et al. 2000; Hanba et al. 2002). In fact, the increase of E resulted in a WUE reduction in *G. ulmifolia* and *A. integrifolia* sun plants, as verified by Matsuki et al. (2003) and Endres et al. (2010) in the species studied by them. Nevertheless, the increased water loss by transpiration has been associated with a reduction of leaf temperature, therefore minimizing the risks of overheating and photoinhibition in plants under high light intensities (Krause et al. 2001).

Differently, together with higher A_{max} values, sun seedlings of *H. popayanensis* showed lower g_s in most days of analysis, which resulted in a reduction of E and an increase of WUE compared to shade seedlings. It suggests that this species was able to assimilate carbon with a lower water loss, as verified in *Acer rufinerve* Sieb. et Zucc. plants growing under high irradiance (Hanba et al. 2002). The reduction in g_s in *H. popayanensis* sun seedlings reinforces the possibility that plants have suffered water shortage in some periods in the acclimation sector. Stape et al. (2001) reported that in seedlings grown in the same tubes used here (50 cm^3), rapid root growth can cause restrictions in water drainage of tubes, and then decrease leaf water potential and increase stomatal resistance. Anyway, low leaf g_s in drier sites can protect the root-to-leaf hydraulic pathway from cavitation (Brodribb and Holbrook 2006), reduce plant transpiration rate, and favor seedling survival in water-stressed conditions after planting (Delpérée et al. 2003).

It is also noteworthy that high-light acclimation did not affect the total biomass of *H. popayanensis* seedlings, whereas the biomass of *G. ulmifolia* and *A. integrifolia* sun seedlings decreased when compared to control ones. The decrease in total dry mass in sun seedlings of these species may be a consequence of reduced CO_2 assimilation rates (per leaf area) observed at the first part of the experiment, together with the reduced leaf area at the end of the experiment (which decreases plant total photosynthesis). Similarly, the excess of light energy has been associated with photoinhibition and limited biomass production in different tree species (Long et al. 1994; Claussen 1996; Kitao et al. 2000; Gonçalves et al. 2005).

Despite the decrease in total dry mass, the hardening process did not affect root dry mass of *G. ulmifolia* seedlings, leading to decreased SDM/RDM ratio, a result that was also observed for *H. popayanensis* sun seedlings. In seedlings of *Eucalyptus* sp., the decrease in SDM/RDM ratio was attributed to the application of drought cycles in nursery, due to its negative effect on leaf expansion, but not on root development (Rhizopoulou and Davies 1993; Stape et al. 2001). A lower SDM/RDM ratio implies that biomass was preferentially allocated in roots in comparison with aerial organs. In plants growing under high irradiances, this response favors water and nutrient absorption in detriment to water loss by transpiration (Poorter 2001; Mielke and Schaffer 2010), which would contribute to initial growth of the plants after their transfer to the field (Claussen 1996; Moraes et al. 2010; Freitas et al. 2012).

Despite not presenting the thickening of stem base diameter, *H. popayanensis* sun plants presented reduced stem height, resulting in lower *H/D* ratio in comparison with shade seedlings. It suggests that these seedlings showed a greater equilibrium in the development of the different parts and that the stem base diameter was compatible with the height, what might imply in seedlings that would be more tolerant to the conditions found at the field (Campos and Uchida 2002). Sun seedlings of *H. popayanensis* also presented a higher DQI value when compared to shade seedlings. Taken together with the lower *H/D* and SDM/RDM ratios, it indicates that analysis of these morphological parameters was adequate for expressing the quality of *H. popayanensis* sun seedlings, suggesting higher hardiness at the end of the experiment. However, it was not valid for *G. ulmifolia* and *A. integrifolia* seedlings.

In *G. ulmifolia,* sun seedlings presented higher H/D, whereas the hardening process did not affect this ratio in *A. integrifolia* (as well as the SDM/RDM). Moreover, the DQI was lower in sun seedlings of *G. ulmifolia* and *A. integrifolia* in comparison with shaded ones. These results do not necessarily indicate low quality of the seedlings, given that there are not in the literature reference values for the seedlings of different tropical tree species (Leles et al. 2006; Ferraz and Engel 2011). However, Fonseca et al. (2002) recorded higher DQI in seedlings of *Trema micrantha* (L.) Blume grown under full sunlight. Thus, the quality indexes are not necessarily adequate for expressing the hardiness of all tropical tree seedlings, especially if we consider that they constitute more genetically heterogeneous populations. More studies are necessary for the proposal of quality indexes more adequate for nurseries that deal with different tropical tree species.

More importantly, irrespective of the quality indexes, the seedlings of the three species analyzed in the present study developed traits of hardiness after acclimation under full sunlight. Sun plants of *H. popayanensis* presented physiological (increases in $A_{\max}$ and WUE) and morphoanatomical responses (thicker mesophyll and palisade parenchyma), in addition to the reduction of H/D and SDM/RDM, and higher DQI. In *A. integrifolia* seedlings subjected to high irradiances, leaves also developed morphoanatomical traits of hardiness (thicker mesophyll and palisade parenchyma and higher stomatal density) and presented higher $A_{\max}$ values at the end of the experiment (in spite of the initial drop in this parameter). In *G. ulmifolia*, at the end of the hardening process, although $A_{\max}$ did not increase compared to shade seedlings, the leaves developed under full sunlight did not present signs of photoinhibition and exhibited structural characteristics of hardiness, such as thickening of palisade parenchyma, higher stomatal density, and lower LA and SDM/RDM. Overall, these characteristics induced by the hardening process in each species might allow better conditions of growth and survival for these seedlings in the field.

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References

Aleric KM, Kirkman K (2005) Growth and photosynthetic responses of the federally endangered shrub, *Lindera melissifolia* (Lauraceae), to varied light environments. Am J Bot 92:682–689

Boardman NK (1977) Comparative photosynthesis of sun and shade plants. Annu Rev Plant Physiol 28:355–377

Brodribb TJ, Holbrook NM (2006) Declining hydraulic efficiency as transpiring leaves desiccate: two types of response. Plant Cell Environ 29:2205–2215

Campos MAA, Uchida T (2002) Influência do sombreamento no crescimento de mudas de três espécies amazônicas. Pesqui Agropecu Bras 37:281–288

Cano FJ, Sánchez-Gómez D, Gascó A, Rodríguez-Calcerrada J, Gil L, Warren CR, Aranda I (2011) Light acclimation at the end of the growing season in two broadleaved oak species. Photosynthetica 49:581–592

Chabot BF, Hicks DJ (1982) The ecology of leaf life spans. Annu Rev Ecol Syst 13:229–259

Chazdon RL, Pearcy RW, Lee DW, Fetcher N (1996) Photosynthetic responses of tropical forest plants to contrasting light environments. In: Mulkey SS, Chazdon RL, Smith AP (eds) Tropical forest plant ecophysiology. Chapman and Hall, New York, pp 5–55

Claussen JW (1996) Acclimation abilities of three tropical rainforest seedlings to an increase in light intensity. For Ecol Manag 80:245–255

Cortina J, Vilagrosa A, Trubat R (2013) The role of nutrients for improving seedling quality in drylands. New For 44:719–732

Cuzzol GRF, Milanez CRD (2012) Morphological and physiological adjustments in juvenile tropical trees under contrasting sunlight irradiance. In: Najafpour MM (ed) Advances in photosynthesis—fundamental aspects. InTech, Rijeka, pp 501–519

Delpérée C, Kinet JM, Lutts S (2003) Low irradiance modifies the effect of water stress on survival and growth-related parameters during the early developmental stages of buckwheat (*Fagopyrum esculentum*). Physiol Plant 119:211–220

Demmig-Adams B, Adams WW (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol 43:599–626

Dickson A, Leaf AL, Hosner JF (1960) Quality appraisal of white spruce and white pine seedling stock in nurseries. For Chron 36:10–13

Endres L, Câmara CA, Ferreira VM, Silva JV (2010) Morphological and photosynthetic alterations in the yellow-ipe, *Tabebuia chrysotricha* (Mart. ex DC.) Standl., under nursery shading and gas exchange after being transferred to full sunlight. Agrofor Syst 78:287–298. doi:10.1007/s10457-009-9235-9

Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ 24:755–767

Ferraz AV, Engel VL (2011) Efeito do tamanho de tubetes na qualidade de mudas de jatobá (*Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Lee et Lang.), ipê-amarelo (*Tabebuia chrysotricha* (Mart. ex Dc.) Sandl.) e guarucaia (*Parapiptadenia rigida* (Benth.) Brenan). Rev Árvore 35:413–423

Ferreira OGL, Rossi FD, Andrighetto C (2008) DDA: software para determinação de área foliar, índice de área foliar e área de olho de lombo versão 1.2. Santo Augusto

Fonseca ÉP, Valéri SV, Miglioranza É, Fonseca NAN, Couto L (2002) Padrão de qualidade de mudas de *Trema micrantha* (L.) Blume, produzidas sob diferentes períodos de sombreamento. Rev Árvore 26:515–523

Freitas GA, Vaz-de-Melo A, Pereira MAB, Andrade CAO, Lucena GN, Silva RR (2012) Influência do sombreamento na qualidade de mudas de *Sclerolobium paniculatum* Vogel para recuperação de área degradada. J Biotechnol Biodivers 3:5–12

Givnish TJ (1988) Adaptation to sun and shade: a whole plant perspective. Aust J Plant Physiol 15:63–92

Gonçalves JFC, Barreto DCS, Santos-Junior UM, Fernandes AV, Sampaio PTB, Buckeridge MS (2005) Growth, photosynthesis and stress indicators in young rosewood plants (*Aniba rosaeodora* Ducke) under different light intensities. Braz J Plant Physiol 17:325–334

Gonçalves ER, Souza FC, Santos LN, Silva JV, Ferreira VM, Endres L (2013) Morphological and photosynthetic adaptations of *Tabebuia aurea* seedlings in the nursery. Rev Bras Eng Agríc Ambient 17:1201–1209

Gyimah R, Nakao T (2007) Early growth and photosynthetic responses to light in seedlings of three tropical species differing in successional strategies. New Forest 33:217–236. doi:10.1007/s11056-006-9028-1

Hanba YT, Kogami H, Terashima I (2002) The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. Plant Cell Environ 25:1021–1030

Ivancich HS, Lencinas MV, Pastur GJM, Esteban RMS, Hernández L, Lidstrom I (2012) Foliar anatomical and morphological variation in *Nothofagus pumilio* seedlings under controlled irradiance and soil moisture levels. Tree Physiol 32:554–564. doi:10.1093/treephys/tps024

Jacobs DF, Salifu KF, Seifert JR (2005) Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. New For 30:235–251. doi:10.1007/s11056-005-5419-y

Johansen DA (1940) Plant microtechnique. McGraw- Hill, New York

Jordan GJ, Dillon RA, Weston PH (2005) Solar radiation as a factor in the evolution of scleromorphic leaf anatomy in Proteaceae. Am J Bot 92:789–796

Kitao M, Lei TT, Koike T, Tobita H, Maruyama Y (2000) Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. Plant Cell Environ 23:81–89

Krause GH, Koroleva OY, Dalling JW, Winter K (2001) Acclimation of tropical tree seedlings to excessive light in simulated tree-fall gaps. Plant Cell Environ 24:1345–1352

Lee DW, Oberbauer SF, Johnson P, Krishnapilay B, Mansor M, Mohamed H, Yap SK (2000) Effects of irradiance and spectral quality on leaf structure and function in seedlings of two southeast Asian *Hopea* (Dipterocarpaceae) species. Am J Bot 87:447–455

Leles PSS, Lisboa AC, Oliveira Neto SN, Grugiki MA, Ferreira MA (2006) Qualidade de mudas de quatro espécies florestais produzidas em diferentes tubetes. Floresta Ambient 13:69–78

Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Annu Rev Plant Physiol Plant Mol Biol 45:633–662

Macêdo NA (1997) Manual de Técnicas em Histologia Vegetal. Editora UEFS, Feira de Santana

Martínez-Pastur G, Lencinas MV, Peri PL, Arena M (2007) Photosynthetic plasticity of *Nothofagus pumilio* seedlings to light intensity and soil moisture. For Ecol Manag 243:274–282

Matsuki S, Ogawa K, Tanaka A, Hara T (2003) Morphological and photosynthetic responses of *Quercus crispula* seedlings to high-light conditions. Tree Physiol 23:769–775

Mielke MS, Schaffer B (2010) Photosynthetic and growth responses of *Eugenia uniflora* L. seedlings to soil flooding and light intensity. Environ Exp Bot 68:113–121. doi:10.1016/j.envexpbot.2009.11.007

Moraes GABK, Chaves ARM, Martins SCV, Barros RS, DaMatta FM (2010) Why is it better to produce coffee seedlings in full sunlight than in the shade? A morphophysiological approach. Photosynthetica 48:199–207

Mulkey SS, Pearcy RW (1992) Interactions between acclimation and photoinhibition of photosynthesis of a tropical forest understory herb, *Alocasia macrorrhiza*, during simulated canopy gap formation. Funct Ecol 6:719–729

Oguchi R, Hikosaka K, Hirose T (2005) Leaf anatomy as a constraint for photosynthetic acclimation: differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. Plant Cell Environ 28:916–927

Oguchi R, Hikosaka K, Hirura T (2006) Leaf anatomy and light acclimation in woody seedlings after gap formation in a cool-temperate deciduous forest. Oecologia 149:571–582. doi:10.1007/s00442-006-0485-1

Poorter H (1999) Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. Funct Ecol 13:396–410

Poorter L (2001) Light-dependent changes in biomass allocation and their importance for growth of rain forest tree species. Funct Ecol 15:113–123

Poorter H, Niklas KJ, Reich PB, Olesksyn PP, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol 193:30–50. doi:10.1111/j.1469-8137.2011.03952.x

Rhizopoulou S, Davies WJ (1993) Leaf and root growth dynamics in *Eucalyptus globulus* seedlings grown in drying soil. Trees Struct Funct 8:1–8

Sessa EB, Givnish TJ (2014) Leaf form and photosynthetic physiology of *Dryopteris* species distributed along light gradients in eastern North America. Funct Ecol 28:108–123

Sims DA, Pearcy RW (1992) Response of leaf anatomy and photosynthetic capacity in *Alocasia macrorrhiza* (Araceae) to a transfer from low to high light. Am J Bot 79:449–455

Stape JL, Gonçalves JLM, Gonçalves AN (2001) Relationships between nursery practices and field performance for *Eucalyptus* plantations in Brazil. New For 22:19–21

Terashima I, Miyazawa SI, Hanba Y (2001) Why are sun leaves thicker than shade leaves? Consideration based on analyses of CO_2 diffusion in the leaf. J Plant Res 114:93–105

Valladares F, Niinemets Ü (2008) Shade tolerance, a key plant feature of complex nature and consequences. Annu Rev Ecol Evol Syst 39:237–257. doi:10.1146/annurev.ecolsys.39.110707.173506

Walters RG (2005) Towards an understanding of photosynthetic acclimation. J Exp Bot 56:435–447. doi:10.1093/jxb/eri060

Yamashita N, Ishida A, Kushima H, Tanaka N (2000) Acclimation to sudden increase in light favoring an invasive over native trees in subtropical islands, Japan. Oecologia 125:412–419. doi:10.1007/s004420000475

Yang SJ, Sun M, Zhang YJ, Cochard H, Cao KF (2014) Strong leaf morphological, anatomical, and physiological responses of a subtropical woody bamboo (*Sinarundinaria nitida*) to contrasting light environments. Plant Ecol 215:97–109. doi:10.1007/s11258-013-0281-z

Yano S, Terashima I (2004) Developmental process of sun and shade leaves in *Chenopodium album* L. Plant Cell Environ 27:781–793