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Morphoanatomy and ecophysiology of tree seedlings in semideciduous forest during high-light acclimation in nursery

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Abstract

The recomposition of deforested environments demands the acclimation of seedlings in nurseries. This process induces changes in physiological, anatomical, and morphological traits of plants, favouring their establishment after transplantation to the field. The present study aimed to verify the influence of full-sun acclimation on seedling hardiness. For the purpose, leaf gas-exchange, plant anatomical and morphological parameters of three tree species [*Ceiba speciosa* (A. St.-Hil.) Ravenna (Malvaceae), *Croton floribundus* Spreng. (Euphorbiaceae), and *Cecropia pachystachya* Trecul (Urticaceae)], which are used for reforestation in the Brazilian Atlantic biome, were evaluated. Seedlings were grown under 40% of total PPFD (shaded control) and under full sun (acclimated) for 168 days. The acclimation process induced a higher leaf production rate in *C. speciosa* and *C. floribundus*, whereas *C. pachystachya* seedlings replaced their leaves quickly, irrespective of the light conditions. The newly developed leaves of all three species presented a lower area and thicker palisade parenchyma, resulting in a reduced specific leaf area. The seedlings of *C. speciosa* and *C. pachystachya* showed increases in light-saturated net photosynthesis and transpiration rates, whereas water-use efficiency generally remained unchanged in all three species. The full-sun acclimated seedlings of *C. pachystachya* showed a reduced relative growth rate, lower height/stem diameter (H/D) and shoot to root dry mass ratios, characteristics that may result in greater physical resistance and ability for water and nutrient uptake to support the higher transpiratory demand under full sun. The reduction of the H/D ratio also occurred in the acclimated seedlings of *C. speciosa*. The seedlings of *C. floribundus* showed few changes during acclimation, but they did not seem to be affected by excessive light. In spite of the observed differences among the three species, all of them developed hardiness characteristics, mainly related to leaf anatomy, which should favour their establishment after transplantation to the field.

Additional key words: ecological anatomy; environmental restoration; mesophyll; plant ecophysiology.

Introduction

The microclimatic conditions of mature forests differ substantially from those of deforested areas (Craven *et al.* 2011), which are characterised by higher light intensities, air, soil temperatures, vapour pressure deficit (VPD), and large seasonal fluctuations in soil water potential (Guariguata and Ostertag 2001). As seedlings are vulnerable to environmental stresses (Walter *et al.* 2007), in order to use them for reforestation, it is necessary to produce them in controlled environments for proper acclimation, which favours their establishment, growth, and development after planting in the field (Davis and Jacobs 2005, Mendoza-Bautista *et al.* 2011). Seedlings submitted to acclimation under full sun at nurseries are subjected to long-term exposure to high light (Claussen 1996). If they survive any damage caused by excessive light, their morphology and physiology begin to resemble those of sun-grown plants (rustic seedlings), and such changes are referred as hardening (Claussen 1996).

Several plant species have exhibited high potential for acclimation (Rozendaal *et al.* 2006, Valladares and Niinemets 2008), through modifications in leaves, stems, and roots induced by environmental stimuli (Valladares *et al.* 2000). The most efficient way to acclimate to light environment possibly occurs by structural or physiological changes in leaves (Pandey and Kushwaha 2005, Oguchi *et al.* 2005, Rozendaal *et al.* 2006, Warren *et al.* 2007). Such alterations can occur both in fully expanded leaves (Yamashita *et al.* 2000, Oguchi *et al.* 2006) and in

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Abbreviations: *E* – transpiration rate; g_s – stomatal conductance; H/D – shoot height/stem diameter ratio; LA – leaf area; P_{Nmax} – light-saturated net photosynthetic rate; RGR – relative growth rate; SLA – specific leaf area; LDM – leaf dry mass; PDM – whole plant dry mass; RDM – root dry mass; STDM – stem dry mass; SDM/RDM – shoot-to-root dry mass ratio; WUE – water-use efficiency.

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new or immature ones (Strauss-Debenedetti and Bazzaz 1991), leading to the differentiation of sun leaves (Murchie and Horton 1997). These, when compared to shade leaves, are usually smaller, with thicker palisade parenchyma, lower specific leaf area, and higher stomatal density and carbon assimilation rate (Walters 2005, Niinemets 2007, Favaretto *et al.* 2011, Jiang *et al.* 2011, Murphy *et al.* 2012). Due to these traits, which provide a great ability to consume light energy, sun leaves are tolerant to high light, while shade leaves are more likely to suffer damage from excessive light (Demmig-Adams and Adams 1992, Lovelock *et al.* 1994).

Leaves are not the only structure influenced by increase in light intensity. The development of plants under high light may increase the storage capacity for carbohydrates and other nutrients in the stem (Johnson *et al.* 2002), as well as a decrease in stem elongation and increased thickness of this organ (Petritan *et al.* 2009), consequently favouring its resistance to physical damage (Cleary *et al.* 1978). High irradiance can also increase biomass distribution to root system, which contributes to water and nutrient uptake (Vilela *et al.* 2003).

When seedlings are grown under shade, they show characteristics that make them more susceptible to damage caused by high irradiance, drought, and high temperature (Lovelock *et al.* 1994). Consequently, the transfer of these seedlings to high-light environments can be disastrous and may even result in their death (Powles 1984, Demmig-Adams and Adams 1992). On the other hand, seedlings subjected to acclimation to full sun under the relatively controlled environment of nurseries develop greater tolerance to several types of stresses and show a better performance when transplanted to the field (Claussen 1996). The degree, to which exposure to different types and intensities of stress affects the development of seedlings, depends on the acclimation potential of each species (Griscom *et al.* 2005). Light-demanding species, such as *C. speciosa* (A. St. -Hil.) Ravenna (Malvaceae), *C. floribundus* Spreng. (Euphorbiaceae), and *C. pachystachya* Trecul (Urticaceae) are more likely to acclimate to high-light intensities than shade-tolerant species due to their lower susceptibility to photoinhibition (de Souza and Válio 2001, Favaretto *et al.* 2011). Since seedlings compete for space and limited resources in the field (Krause *et al.* 2001), the knowledge on the ability of a plant species to acclimate to high-light intensities becomes relevant. Thus, the study of seedling morphoanatomy and physiology in a variety of light environments should contribute to determining the best traits that would favour seedling establishment after planting in the field (Myers *et al.* 2000, Montgomery and Chazdon 2002), which is the most critical period for their survival (Ivancich *et al.* 2012).

This study aimed to verify the influence of the acclimation process under full sun on morphological, anatomical, and leaf gas exchange traits of seedlings of three tree species used for reforestation in the Brazilian Atlantic Forest biome. The following question was raised: does the acclimation method implemented in nurseries result in the hardening of seedlings of shade-intolerant species?

Materials and methods

Seedling production and treatments: Three light-demanding tree species native to Brazilian Atlantic biome were chosen, based on their importance in revegetation programs: *C. speciosa*, *C. floribundus*, and *C. pachystachya*.

The experiment was carried out in an outdoor nursery at Laboratório de Biodiversidade e Restauração de Ecossistemas, Universidade Estadual de Londrina, Londrina, PR, Brazil. Our focus was to verify the performance of seedlings under exactly the same conditions to which they are subjected in the nursery hardening routine. According to the Köppen system of classification, the climate of the region is type Cfa (humid subtropical), with an average annual rainfall of 1,200–1,600 mm, distributed unevenly throughout the year (IAPAR 2000). High-density polypropylene conical tubes with 2.65 cm internal diameter, 12.5 cm height, and 50 cm^3 volume were used for sowing, seedling growth, and during the entire experiment. A substrate composed of sieved cattle manure and vegetables, carbonised rice hull, sand, and a controlled release fertiliser (15% N, 10% P_2O_5, and 10% of K_2O) was used as a medium for plant growth. After germination and initial growth, an individual seedling of each species was maintained per tube. Prior to reaching a height of 15–20 cm (depending on the species), seedlings were kept in the growth sector, where they were exposed to 40% of PPFD. After the growing period (about 60 d for *C. speciosa*, 210 d for *C. floribundus*, and 150 d for *C. pachystachya*), a part of the tubes containing the seedlings was transferred to the acclimation sector, where the plants were exposed to full sun (acclimation treatment), whereas some of them remained in the growth sector (shaded control). The seedlings remained in the respective sectors for 168 d (DAT), where they were kept under natural conditions and temperature. The maximum irradiance incident on the seedlings on sunny days was about 1,800 and 710 µmol(photon) m^{-2} s^{-1}, respectively, for plants under full sunlight and shaded conditions. In both sectors, the seedlings were irrigated for 30-min periods, four times a day.

Gas-exchange measurements: Every 14 d, gas-exchange variables were assessed in one leaf of nine individuals of each species per treatment until 168 DAT. The penultimate, fully expanded, and nonsenescent leaf of randomly selected seedlings was used for the evaluation of gas-exchange parameters. The same criterion was always applied for the seedlings of the acclimation sector, regardless of whether the chosen leaf was partially or fully expanded under shade or full sun. The following parameters were measured: stomatal conductance (g_s), rate of light-saturated net photosynthesis (P_{Nmax}), transpiration (E), and instantaneous water-use efficiency (WUE), calculated as the P_N/E ratio. The measurements were carried out between 08:00 and 10:00 h, using the portable infrared gas-analyser *LI-COR 6400XT* (*Licor Inc*., Lincoln, NE, USA), with a 6 cm^2 chamber and a LED light source set to 1,900 $\mu mol\ m^{-2}\ s^{-1}$ PPFD, which saturated the photosynthesis of the three seedling species. This light intensity was determined from initial tests performed with each species to obtain the curve of net photosynthesis in response to PPFD (data not shown). The total net photosynthesis and transpiration of seedlings was estimated by multiplying the total leaf area to P_{Nmax} or E of the seedlings, respectively. Thereafter, we calculated the mean value for each treatment considering the data of all evaluation dates.

Anatomical and morphological measurements: The anatomical studies were conducted on the segments of the stem and on the third leaf from the apex at the end of the experiment (168 DAT). Segments of 0.5 cm^2 were placed in 50% formol:acetic acid:alcohol mixture and dehydrated in an alcohol serie. Transverse, 14 µm thick sections were obtained in a sliding microtome *SM2010R* (*Leica Microsystems*, Wetzler, Germany) and coloured by a combination of astra blue and basic fuchsine according to Johansen (1940) and Sass (1951) with minor modifications. Leaf segments were also submitted to the Jeffrey's maceration technique, to complete separation of adaxial and abaxial epidermis. The histological measurements were conducted using the *Image Pro-Plus* software, *v.2.0* (*Motic China Group Co. Ltd*., Xiamen, China) in an optical microscope (*Leica Microsystems*, Wetzler, Germany) coupled with a digital camera. For the anatomical studies, the variables analysed were medullary parenchyma, xylem, phloem, sclerenchyma, collenchyma, cortex, and periderm of stems, stomatal density, and thickness of abaxial and adaxial epidermis, palisade parenchyma, and spongy parenchyma of leaves.

Morphological variables were also evaluated in nine individuals of each species per treatment at the end of the experiment. The nondestructive morphological variables were the diameter of stem base and shoot height. The dry mass of leaves (LDM), stem (STDM), shoots (SDM), roots (RDM), and of the whole plant (PDM) were measured after keeping the tissues at 60ºC until constant mass was reached. The total leaf area (LA) of each individual was obtained by images in *ImageJ* software (National Institutes of Health, Bethesda, USA) and the specific leaf area (SLA) was calculated as the LA/LDM ratio. We also calculated the shoot height/stem diameter ratio (H/D) and shoot-to-root ratio (SDM/RDM). Additionally, the total leaf number was assessed every 14 d throughout the experimental period, along with the number of newly developed leaves up to 56 DAT. The relative growth rate (RGR) of seedlings was obtained according to Hunt (1982): $RGR = (\ln PDM_2 - \ln PDM_1)/(t_2 - t_1)$, where PDM_1 and PDM_2 are the whole plant dry mass of nine individuals harvested when part of the plants was transferred to the acclimation sector ($t_1 = 0$) and 168 DAT ($t_2 = 168$), respectively.

Statistical analysis: Gas-exchange and leaf number data were subjected to a two-way analysis of variance (*ANOVA*), and means were compared through a *Tukey*'s multiple comparison test, while anatomical and morphological data were subjected to a one-way *ANOVA*. Data expressed as percentage (stem tissues) were previously transformed to arcsine $\sqrt{\%/100}$. Statistical analyses were performed using *Statistica* software, *v. 10.0* (*Statsoft Inc*., Tulsa, USA), at the significance level of 5%.

Results

Sun-grown *Ceiba speciosa* seedlings showed increases in g_s (14, 112, 126, 140, and 154 DAT; Fig. 1*A*), P_{Nmax} (14, 126, 140, and 154 DAT; Fig. 1*B*), and E (112, 126, 140, and 154 DAT; Fig. 1*C*) compared to the shaded seedlings. The acclimated seedlings showed a lower WUE only during the last evaluation (168 DAT; Fig. 1*D*).

Both the number of newly developed leaves (Fig 1*E*) and the total leaf number (Fig. 1*F*) increased in the *C. speciosa* seedlings transferred to full sun, as verified at 28, 42, and 56 DAT. The LA and SLA decreased with acclimation in this species (Table 1). When LA was considered to estimate total net photosynthesis and the transpiration of seedlings, it was found that both parameters did not differ between the acclimated [23.3 ± 2.42 $nmol(CO_2)\ s^{-1}$; 7.98 ± 0.900 $\mu mol(H_2O)\ s^{-1}$, respectively] and shaded seedlings [29.2 ± 2.06 $nmol(CO_2)\ s^{-1}$; 7.75 ± 0.652 $\mu mol(H_2O)\ s^{-1}$, respectively], despite the increases in P_{Nmax} and E in the sun-grown plants.

Anatomically, the leaves of *C. speciosa* seedlings showed no differences in stomatal density or in the thickness of adaxial and abaxial epidermis between treatments, while the thickness of palisade parenchyma, spongy parenchyma, and leaf lamina increased in the acclimated seedlings (Table 1). In the stem of these seedlings, there was an increase in sclerenchyma percentage, compared with the shaded control (Table 2).

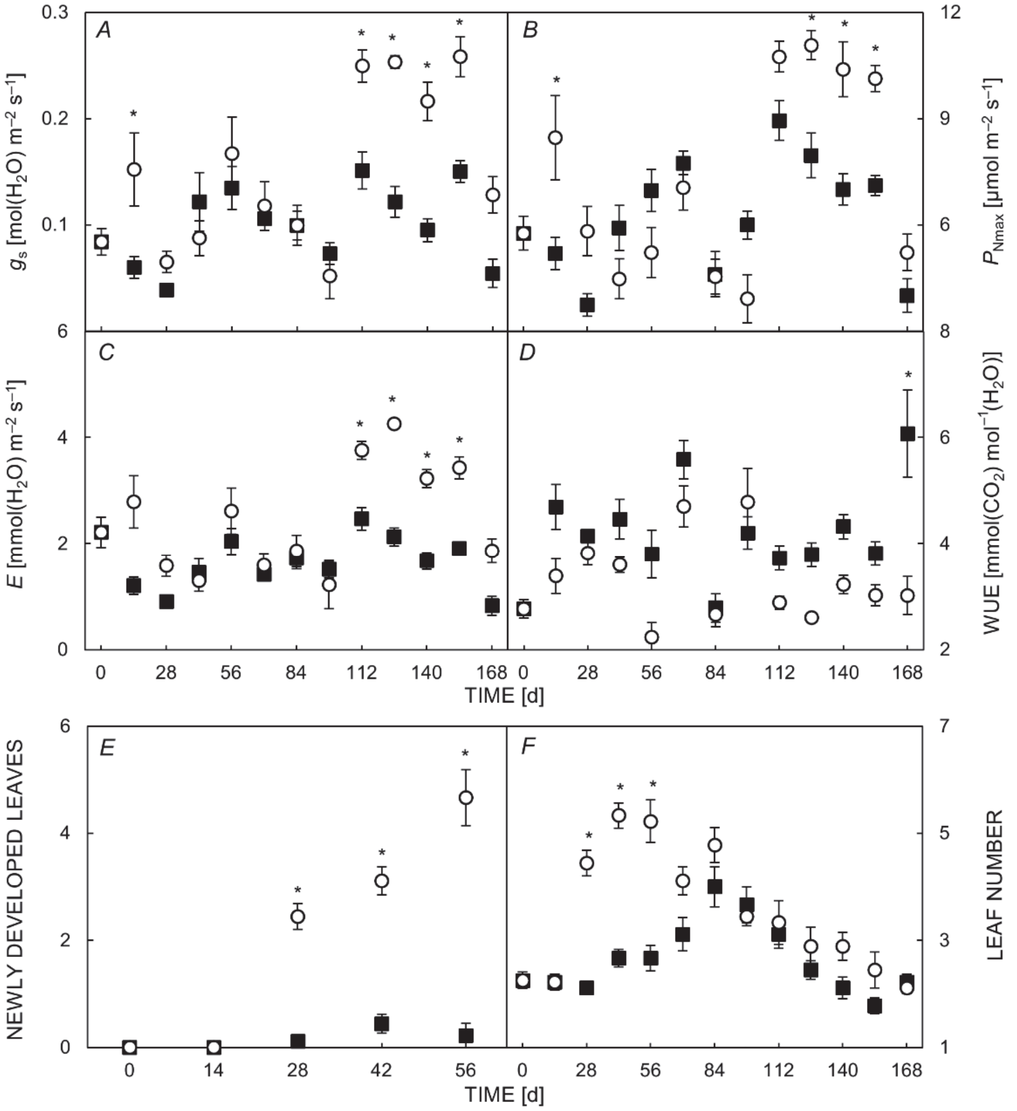

Fig. 1. Leaf number and gas-exchange parameters of *Ceiba speciosa* seedlings. *A*: stomatal conductance (g_s), *B*: light-saturated net photosynthetic rate (P_{Nmax}), *C*: transpiration rate (*E*), and *D*: water-use efficiency (WUE), over 168 days of experiment. *E*: newly developed leaves, and F: total leaf number, over 56 and 168 days of experiment, respectively. *Filled squares* are means of shaded control and *open circles* are means of acclimated treatment ($n = 9$). Bars are SE. Statistically significant differences ($p<0.05$) by *F*-test (*ANOVA*) are indicated by asterisks (*).

Table 1. Mean ± SE ($n = 9$) of morphoanatomical parameters of seedlings of *Ceiba speciosa*, *Croton floribundus*, and *Cecropia pachystachya* grown under shade (control) and under full sun (acclimated). LA – total leaf area; SLA – specific leaf area. Statistically significant differences ($p<0.05$) by *F*-test (*ANOVA*) are followed by asterisks (*).

Parameter	Species *Ceiba speciosa* Shaded	 Acclimated	 *Croton floribundus* Shaded	 Acclimated	 *Cecropia pachystachya* Shaded	 Acclimated
LA [cm^2 plant^{-1}]	47.0 ± 3.82	32.7 ± 0.803*	43.5 ± 3.54	24.4 ± 1.05*	60.6 ± 5.11	17.5 ± 1.39*
SLA [cm^2 g^{-1}]	313 ± 11.9	279 ± 10.3*	198 ± 5.61	155 ± 4.18*	267 ± 4.36	191 ± 11.4*
Stomatal density [number mm^{-2}]	127 ± 8.78	133 ± 10.2	295 ± 17.7	426 ± 24.6*	268 ± 22.6	632 ± 23.8*
Adaxial epidermis thickness [μm]	35.0 ± 2.41	39.4 ± 1.69	21.5 ± 1.15	23.3 ± 1.03	19.8 ± 0.551	28.0 ± 1.65*
Abaxial epidermis thickness [μm]	15.8 ± 1.12	15.5 ± 1.04	12.4 ± 0.596	12.7 ± 0.472	7.10 ± 0.791	7.47 ± 0.456
Palisade parenchyma thickness [μm]	40.3 ± 3.03	51.0 ± 1.32*	58.4 ± 3.15	77.6 ± 2.39*	36.4 ± 1.41	50.7 ± 2.86*
Spongy parenchyma thickness [μm]	39.1 ± 2.29	51.7 ± 1.73*	50.8 ± 3.90	60.4 ± 4.24	26.1 ± 2.13	37.7 ± 2.58*
Leaf lamina thickness [μm]	130 ± 6.48	158 ± 3.24*	143 ± 5.19	174 ± 5.45*	89.4 ± 2.78	124 ± 3.58*

Table 2. Mean ± SE ($n = 9$) of the percentage of each tissue that composes the stem in seedlings of *Ceiba speciosa*, *Croton floribundus*, and *Cecropia pachystachya* grown under shade (control) and under full sun (acclimated). Statistically significant differences ($p<0.05$) by *F*-test (*ANOVA*) are followed by asterisks (*).

Parameter	Species *Ceiba speciosa* Shaded	 Acclimated	 *Croton floribundus* Shaded	 Acclimated	 *Cecropia pachystachya* Shaded	 Acclimated
Medullary parenchyma [%]	2.32 ± 0.373	2.71 ± 0.310	2.39 ± 0.218	2.32 ± 0.210	16.6 ± 1.09	16.7 ± 1.76
Xylem [%]	28.0 ± 1.53	28.5 ± 1.19	38.9 ± 1.37	37.4 ± 1.64	41.4 ± 1.22	35.5 ± 1.51*
Phloem [%]	9.98 ± 0.772	10.3 ± 0.719	13.5 ± 0.453	14.8 ± 0.855	9.28 ± 0.362	10.1 ± 0.462
Sclerenchyma [%]	3.69 ± 0.352	5.41 ± 0.428*	-	-	1.88 ± 0.141	2.24 ± 0.156
Collenchyma [%]	-	-	-	-	3.71 ± 0.188	4.32 ± 0.191*
Cortex [%]	56.0 ± 1.03	53.1 ± 1.00	38.5 ± 1.63	39.9 ± 1.71	20.7 ± 0.646	23.5 ± 0.728
Periderm [%]	-	-	6.72 ± 0.453	5.58 ± 0.686	6.37 ± 0.372	7.75 ± 0.570

With respect to morphological variables, it was found that acclimation stimulated the thickening of the stem, but did not influence the height of the *C. speciosa* plants (Table 3). The H/D ratio was lower in the acclimated seedlings, while the SDM/RDM ratio remained unchanged (Table 3). The STDM and RDM were not influenced by acclimation, but the LDM was found to be lower in the acclimated seedlings (Table 3). The PDM and RGR of seedlings of this species were similar in both treatments (Table 3).

Croton floribundus showed few leaf gas-exchange alterations during acclimation. The g_s (Fig. 2*A*) and P_{Nmax} (Fig. 2*B*) were similar in both treatments. In general, acclimation promoted only minor changes in gas exchange, such as the increase in *E* at 154 DAT (Fig. 2*C*) and the reduction of WUE at 168 DAT (Fig. 2*D*).

In *C. floribundus*, the number of newly developed leaves was higher at 56 DAT in the seedlings under acclimation (Fig. 2*E*), but the total leaf number did not differ between treatments (Fig. 2*F*). Under full sun, the LA, SLA (Table 1), and LDM (Table 3) were reduced. The estimation of total net photosynthesis and transpiration based on LA showed a reduction in both parameters in the acclimated seedlings [26.2 ± 2.09 nmol(CO$_2$) s^{-1}; 8.57 ± 0.776 µmol(H$_2$O) s^{-1}, respectively], compared to the shaded control [45.0 ± 3.07 nmol(CO$_2$) s^{-1}; 13.8 ± 1.13 µmol(H$_2$O) s^{-1}, respectively].

There were also increases in stomatal density and in the thickness of palisade parenchyma and leaf lamina with the proceeding acclimation (Table 1). However, adaxial and abaxial epidermis and spongy parenchyma thickness did not differ between treatments (Table 1). Stem tissue percentage in the *C. floribundus* seedlings remained unchanged between the acclimated and nonacclimated seedlings (Table 2). The seedlings of *C. floribundus* showed no differences in STDM, RDM, PDM, shoot height, stem diameter, and RGR between treatments (Table 3).

In *Cecropia pachystachya*, there were increases in g_s at 70 and 84 DAT (Fig. 3*A*), in P_{Nmax} at 42, 56, 70, 84, 126, and 140 DAT (Fig. 3*B*), and in *E* at 14, 28, 42, 56, 70, 84, and 112 DAT (Fig. 3*C*) in the seedlings under acclimation. In these individuals, there was also a reduction of WUE at 42 and 126 DAT (Fig. 3*D*).

Table 3. Mean ± SE ($n = 9$) of morphological parameters of seedlings of *Ceiba speciosa*, *Croton floribundus*, and *Cecropia pachystachya* grown under shade (control) and under full sun (acclimated). H/D – shoot height/stem diameter ratio; PDM – whole plant dry mass; RGR – relative growth rate; SDM/RDM – shoot-to-root dry mass ratio. Statistically significant differences ($p<0.05$) by *F*-test (*ANOVA*) are followed by asterisks (*).

Parameter	Species *Ceiba speciosa* Shaded	 Acclimated	 *Croton floribundus* Shaded	 Acclimated	 *Cecropia pachystachya* Shaded	 Acclimated
Shoot height [cm]	22.7 ± 0.391	21.6 ± 0.733	19.0 ± 0.289	18.6 ± 0.869	41.2 ± 0.594	31.6 ± 0.608*
Stem diameter [mm]	7.40 ± 0.170	9.01 ± 0.183*	3.26 ± 0.170	3.47 ± 0.158	4.47 ± 0.049	4.13 ± 0.126*
H/D [cm mm^{-1}]	3.07 ± 0.085	2.41 ± 0.087*	5.95 ± 0.317	5.48 ± 0.414	9.22 ± 0.138	7.71 ± 0.308*
Leaf dry mass [g]	0.151 ± 0.012	0.118 ± 0.004*	0.222 ± 0.021	0.159 ± 0.009*	0.227 ± 0.019	0.094 ± 0.009*
Stem dry mass [g]	0.788 ± 0.077	0.792 ± 0.049	0.420 ± 0.039	0.417 ± 0.030	1.55 ± 0.068	0.735 ± 0.058*
Root dry mass [g]	1.31 ± 0.180	1.11 ± 0.054	0.681 ± 0.061	0.773 ± 0.059	0.422 ± 0.022	0.337 ± 0.031*
Shoot dry mass [g]	0.939 ± 0.081	0.910 ± 0.052	0.641 ± 0.050	0.575 ± 0.036	1.78 ± 0.066	0.829 ± 0.062*
PDM [g]	2.25 ± 0.249	2.02 ± 0.093	1.32 ± 0.086	1.35 ± 0.089	2.20 ± 0.072	1.17 ± 0.084*
SDM/RDM [g g^{-1}]	0.764 ± 0.060	0.823 ± 0.042	0.984 ± 0.099	0.760 ± 0.045	4.30 ± 0.242	2.54 ± 0.151*
RGR [mg g^{-1} d^{-1}]	10.2 ± 0.233	9.84 ± 0.502	1.60 ± 0.150	1.72 ± 0.159	6.49 ± 0.212	2.61 ± 0.164*

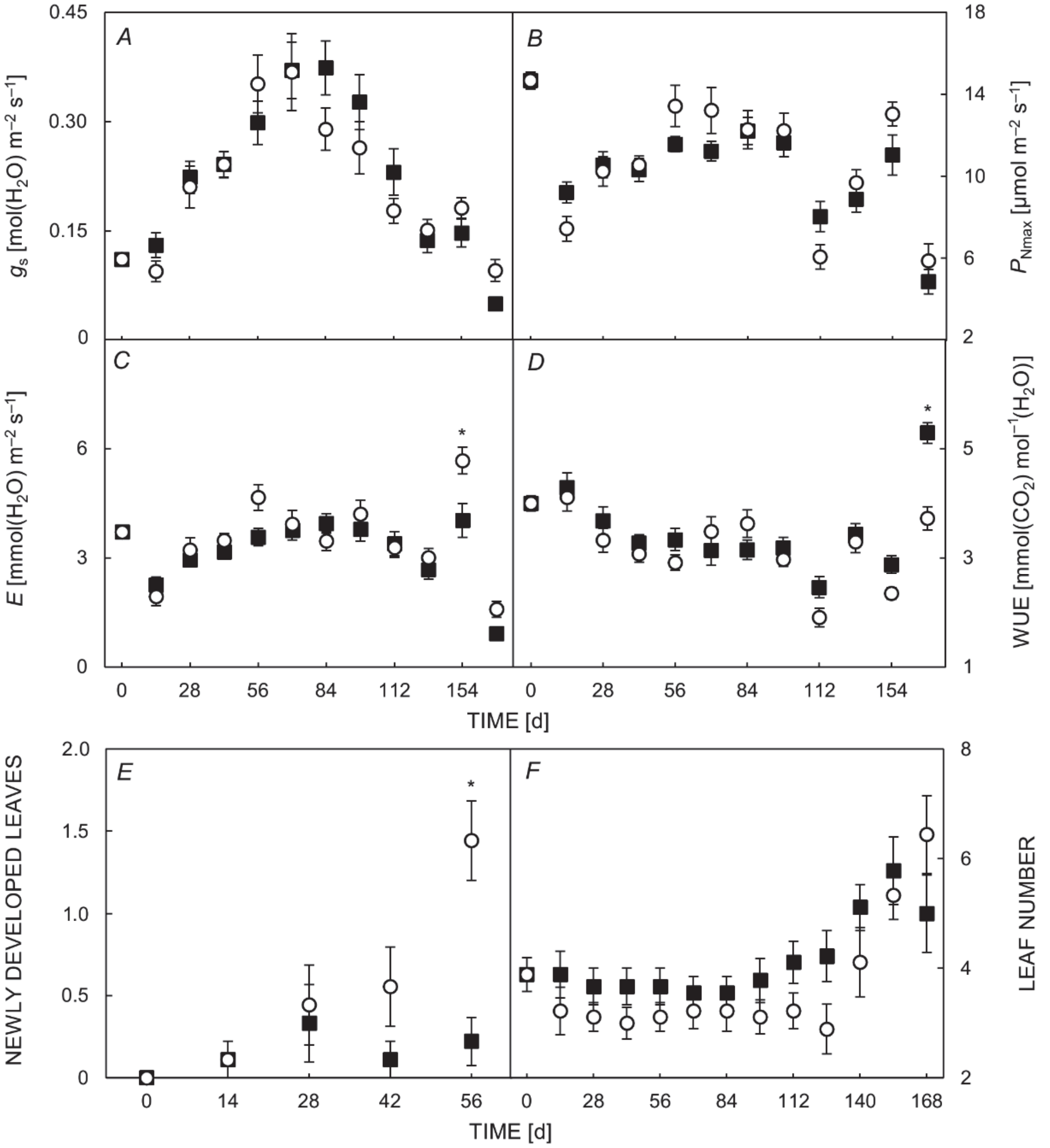

Fig. 2. Leaf number and gas-exchange parameters of *Croton floribundus* seedlings. *A*: stomatal conductance (g_s), *B*: light-saturated net photosynthetic rate (P_{Nmax}), *C*: transpiration rate (*E*), and *D*: water-use efficiency (WUE), over 168 days of experiment. *E*: newly developed leaves, and *F*: total leaf number, over 56 and 168 days of the experiment, respectively. *Filled squares* are means of shaded control and *open circles* are means of acclimated treatment ($n = 9$). Bars are SE. Statistically significant differences ($p<0.05$) by *F*-test (*ANOVA*) are indicated by asterisks.

In both treatments, the mature leaves of *C. pachystachya* seedlings were quickly replaced by new ones. Therefore, the number of newly developed leaves (Fig. 3*E*) and the total leaf number (Fig. 3*F*) did not differ between treatments. However, there was a reduction in LA, SLA (Table 1), and LDM (Table 3) during the acclimation. Considering the LA, it was found that the acclimated seedlings showed a reduction in total net photosynthesis and transpiration [17.9 ± 0.818 nmol(CO_2) s^{-1}; 9.60 ± 1.00 µmol(H_2O) s^{-1}, respectively], compared to the shaded control [49.3 ± 1.82 nmol(CO_2) s^{-1}; 21.2 ± 2.50 µmol(H_2O) s^{-1}, respectively].

Stomatal density and the thickness of adaxial epidermis, palisade and spongy parenchyma, and leaf lamina were higher in the acclimated seedlings, but no difference in the abaxial epidermis thickness was found between treatments (Table 1). With the acclimation, there was a reduction in xylem and increase in collenchyma percentage in the stem, while other tissues maintained unchanged proportions (Table 2).

Under full sun, the shoot height and the stem diameter of the *C. pachystachya* seedlings were lower than those grown under shade (Table 3). The LDM, STDM, RDM, SDM, PDM, H/D ratio, SDM/RDM ratio, and RGR also decreased with the acclimation (Table 3).

Discussion

The responses of seedlings of *C. speciosa*, *C. floribundus*, and *C. pachystachya* to acclimation in the nursery induced plant hardiness, which is important to tolerate adverse conditions after planting in deforested areas.

The increase in light intensity through the transfer to the acclimation sector stimulated the development of new leaves in *C. speciosa* and *C. floribundus* seedlings, although this response was found later in *C. floribundus* (Figs. 1*E*, 2*E*). Despite the increase in leaf production in these species, the leaf number did not differ between treatments (in any period for *C. floribundus*, and after 56 DAT for *C. speciosa*), due to shedding of the older leaves, which indicated that leaf senescence was also faster in the acclimated seedlings. Generally, sun-grown plants exhibit higher leaf production, which would compensate the lower leaf longevity of these leaves compared to shade plants of the same species (Chabot and Hicks 1982, Sims and Pearcy 1992, Pearcy and Sims 1994).

In *C. pachystachya,* the number of the newly developed leaves and the total leaf number did not differ between treatments (Fig. 3*E,F*), since this species showed accelerated leaf production, even under shading. The shedding of basal leaves was also remarkable in both treatments. A rapid leaf turnover is positively related to a

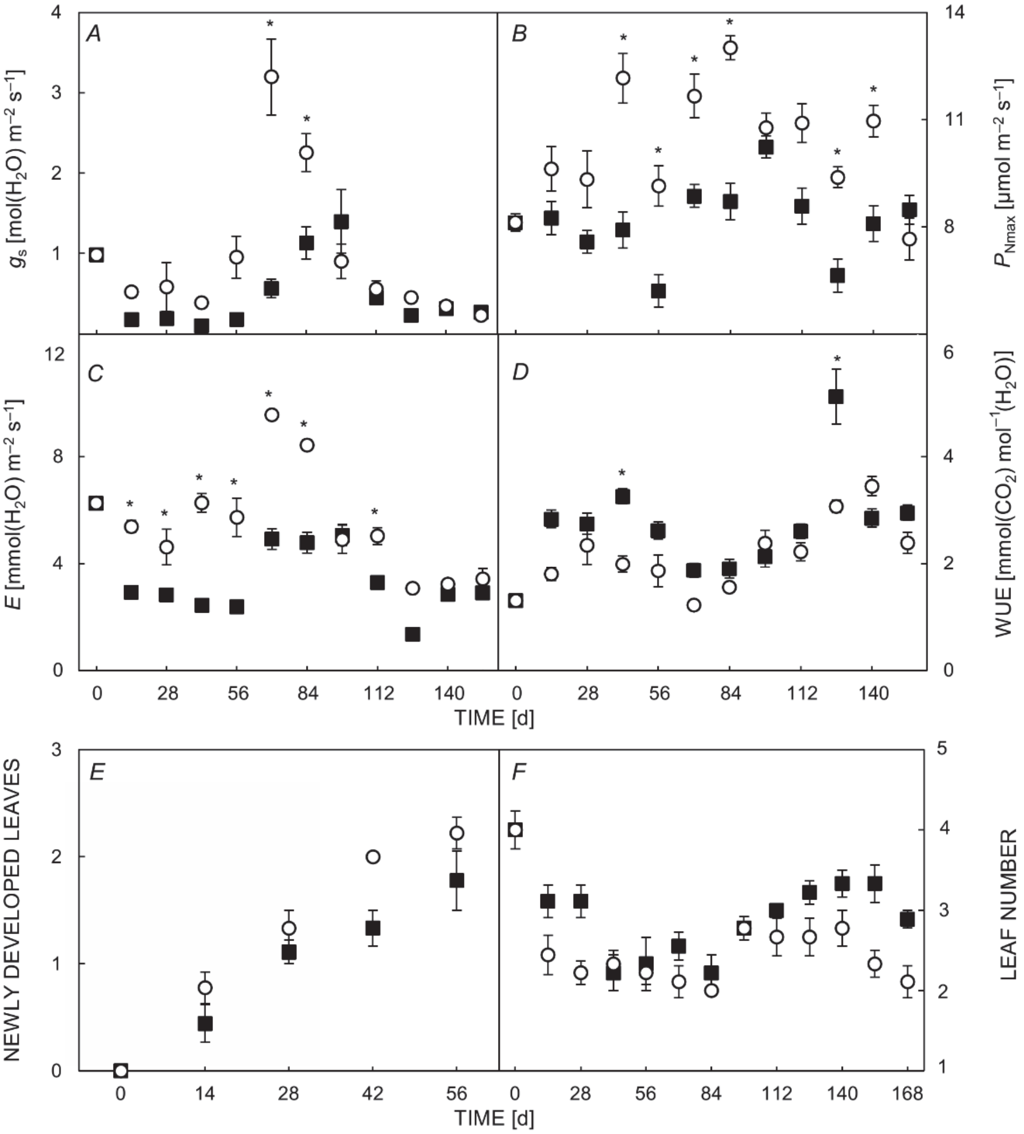

Fig. 3. Leaf number and gas-exchange parameters of *Cecropia pachystachya* seedlings. *A*: stomatal conductance (g_s), *B*: light-saturated net photosynthetic rate (P_{Nmax}), *C*: transpiration rate (*E*), and *D*: water-use efficiency (WUE), over 168 days of experiment. *E*: newly developed leaves, and *F*: total leaf number, over 56 and 168 days of experiment, respectively. *Filled squares* are means of shaded control and *open circles* are means of acclimated treatment (n = 9). Bars are SE. Statistically significant differences (p<0.05) by *F*-test (*ANOVA*) are indicated by asterisks (*).

high phenotypic plasticity, thus influencing plant acclimation. This feature is an important adaptive characteristic of typical gap species, due to its effect on the speed at which a given genotype can track environmental changes by replacing the existing leaves with phenotypically different ones (Valladares *et al.* 2000).

In addition to the importance of developing newly acclimated leaves, we found that the previously expanded leaves (at the time of transfer) of the *C. speciosa* seedlings lasted for a long time and had a significant role in the initial acclimation phase in this species. These leaves showed the increase in P_{Nmax} 14 days after seedling transfer, and showed no reduction in this variable compared to shaded control in the subsequent gas-exchange evaluations (Fig. 1*B*). Yamashita *et al.* (2000) and Oguchi *et al.* (2006) also reported increases in the photosynthetic rate in mature leaves exposed to increased light intensity. This characteristic provides rapid growth to plants when subjected to high light (Kursar and Coley 1999), and is likely to have allowed *C. speciosa* seedlings to maintain RGR after transferring to acclimation conditions.

However, other species do not exhibit the ability to increase the photosynthetic rate in mature leaves when exposed to enhanced light intensity (Sims and Pearcy 1992, Yamashita *et al.* 2000), as observed in the case of *C. floribundus* (Fig. 2*B*). The expanded leaves of *C. floribundus* seedlings transferred to the acclimation sector maintained P_{Nmax} similar to the shaded control, *i.e.*, showed no evidence of chronic photoinhibition. This type of photoinhibition negatively affects carbon assimilation in the morning as well as in the afternoon (Pearcy *et al.* 2005), and is common in tropical tree species exposed to high light (Houter and Pons 2005).

The new leaves produced by the seedlings of the three species after the increase in irradiance were thicker and showed the reduction in SLA (Table 1). Only *C. speciosa* did not show the increase in stomatal density in the leaves developed under full sun, which often occurs in response to light increase (Boardman 1977, Sun *et al.* 2003, Sarijeva *et al.* 2007). In *C. floribundus*, the palisade parenchyma was the only leaf tissue that became thicker during the acclimation. In *C. speciosa* and *C. pachystachya*, in addition to palisade parenchyma, the higher leaf thickness also resulted from the thickening of spongy parenchyma in the acclimated seedlings. Moreover, in *C. pachystachya*, the larger epidermal cells of adaxial face also contributed, to a lesser extent, to the increase in leaf thickness (Table 1). As also verified in other species by Matos *et al.* (2009) and Ivancich *et al.* (2012), in the present study, the thickening of palisade parenchyma in response to increased light intensity resulted from cell elongation and was not due to the differentiation of an additional cell layer. Le Roux *et al.* (2001), Robakowski *et al.* (2003), and Aranda *et al.* (2005) suggested that the thickening of this parenchyma facilitates light acclimation. Generally, a larger development of this tissue is associated with SLA reduction (Marques *et al.* 2000, Hanba *et al.* 2002, Rodríguez-Calcerrada *et al.* 2008, Ivancich *et al.* 2012), which was similar to the pattern identified in the species in the present study (Table 1). The increased SLA observed in shade-grown seedlings resulting from the lower thickness of their leaves is considered important for light capture under low light conditions (Aranda *et al.* 2007). However, it makes leaves more susceptible to water loss under dry conditions due to higher exposure of area per leaf volume (Corcuera *et al.* 2002, Aranda *et al.* 2005, Ivancich *et al.* 2012), which increases the susceptibility of seedlings to fluctuations in the soil water potential at the planting site.

In addition to the influence on SLA, the thickening of spongy and palisade parenchyma can provide an increased potential for light absorption, since there is stimulation of chloroplast production under high irradiance (Terashima *et al.* 2001). This may provide higher mesophyll CO_2 conductance, since it would allow a higher chloroplast surface area set next to intercellular spaces, reducing the resistance to gas diffusion to carboxylation sites (Hanba *et al.* 2002, Warren *et al.* 2007). Therefore, an increased potential for light absorption, a higher internal CO_2 conductance, and a greater accumulation of photosynthetic components in the thicker mesophyll (Martínez-Pastur *et al.* 2007, Ivancich *et al.* 2012) should have favoured the increase in P_{Nmax} of the *C. speciosa* and *C. pachystachya* seedlings under acclimation (Figs. 1*B*, 3*B*).

The increased P_{Nmax} in *C. speciosa* under high irradiance was dependent on g_s ($r^2 = 0.79$, $p<0.01$), as it was also verified in other species (Aranda *et al.* 2007, Sarijeva *et al.* 2007, Sessa and Givnish 2014). Hanba *et al.* (2002) reported that higher g_s inevitably causes increases in water loss through transpiration, as verified in *C. speciosa*. This occurs mainly in high-light environments, where there is usually a higher water vapour pressure deficit and leaf temperature increases (Sessa and Givnish 2014). In spite of the higher g_s, being a typical characteristic of leaves from high-light environments and a pre-requisite for the increase of photosynthetic rate (Sun *et al.* 2003, Sarijeva *et al.* 2007), there was the elevation of P_{Nmax} and E regardless of the increase in g_s in the *C. pachystachya* seedlings under full sun (Fig. 3*A,B*).

In *C. floribundus,* no increases in g_s or P_{Nmax} were found in the acclimated seedlings (Fig. 2*A,B*), although they developed the new leaves with lower SLA, thicker palisade parenchyma, and higher stomatal density compared to the shaded control (Table 1). This might occurr because in brightly lit sites with high heat loads and vapour pressure deficits, plants have to balance the increased photosynthetic carbon gain due to increased light and temperature with greater water loss, which can lead to desiccation (Sessa and Givnish 2014). For this reason, g_s can be negatively or positively influenced by PPFD (Favaretto *et al.* 2011, Sessa and Givnish 2014). Therefore, as previously suggested by Hanba *et al.* (2002), it is possible that CO_2 diffusion from the atmosphere to Rubisco, which comprises processes such as g_s, limited the P_{Nmax} increase of the *C. floribundus* seedlings under acclimation (Fig. 2*B*). As g_s also limits *E* (Sessa and Givnish 2014), there was no increase in this variable or variations in WUE in this species (Fig. 2*C,D*).

WUE was slightly altered only in *C. speciosa* and *C. pachystachya* with the acclimation (Figs. 1*D*, 3*D*). Hanba *et al.* (2002) reported that the acclimation to high-light intensity in some species is characterised by the maximisation of WUE, instead of the increase in leaf net carbon gain, and this must be related to the water deficit faced by leaves under high irradiance. However, in light-demanding species, such as those of this study, WUE may not increase or can even become lower when subjected to high-light intensity. Possibly, this occurs because the concomitant increases in photosynthetic and transpiration rates in *C. speciosa* and *C. pachystachya* contribute to reduction of the negative effects caused by the exposure to excessive irradiance. High CO_2 assimilation and transpiration rates would allow the maintenance of a high photochemical efficiency of PSII and a decrease of leaf temperature, which reduces leaf overheating and photoinhibition risk (Kitajima 1994, Poorter 1999, Krause *et al.* 2001, Cano *et al.* 2011).

Despite the increase in *E* during the acclimation of the *C. speciosa* and *C. pachystachya* seedlings, when the reduction in total leaf area of each seedling was considered, we found that the water loss by transpiration of the acclimated seedlings decreased in *C. floribundus* and *C. pachystachya* in absolute terms (taking into account the total transpiration of each individual). Moraes *et al.* (2010) reported that the reduction in total leaf area contributes significantly to the decrease in water loss through transpiration. According to Murphy *et al.* (2012), shade leaves, due to their large area and their inherent limitation in photosynthetic capacity, reach high temperatures when transferred to high irradiance, which results in disproportionate increases in transpiration relative to photosynthesis. In comparison, sun leaves have higher hydraulic capacity influenced by their higher g_s and stomatal density, and by their reduced leaf area (Murphy *et al.* 2012). These characteristics help to explain the prevalence of these leaves under conditions of high photosynthetic and evaporative demand (Murphy *et al.* 2012). On the other hand, with the reduction of total leaf area, the net photosynthesis of seedlings under acclimation (considering the total leaf area) also became lower, which may have contributed to the decrease in RGR of the *C. pachystachya* acclimated seedlings (Table 3), a species, where leaf area reduction was expressive.

The higher RGR of the *C. pachystachya* seedlings under shade compared to the acclimated seedlings resulted from the maximisation of vertical growth, a behaviour exhibited by several light-demanding species in the understory (Henry and Aarssen 2001, Taugourdeau *et al.* 2011, Valladares *et al.* 2012). However, the increases in height and stem diameters were not proportional (Table 3), thus the acclimated seedlings of *C. pachystachya* had a lower H/D ratio, as also verified in *C. speciosa* in this study, and in other species by Gratzer *et al.* (2004). A high H/D ratio resulting from the development of plants in shady spots can result in etiolated seedlings that are likely to have problems in sustaining their aerial part after planting at field (Pacheco *et al.* 2013). Furthermore, the proportion of collenchyma in the stem decreased in the shaded *C. pachystachya* seedlings (Table 2), which could reduce its resistance to factors that may cause deformation in this organ, such as the disturbance by winds (Niklas 1999). On the other hand, the increased xylem proportion in the stem of the *C. pachystachya* nonacclimated seedlings (Table 2) could be important both for the sustenance of their bigger shoots and for the water supply to their broader leaves, since the xylem is the main tissue responsible for water transport and structural support in secondary growth stems (Evert 2006).

For reforestation purposes, the lower SDM/RDM ratio of the *C. pachystachya* acclimated seedlings (Table 3) may also indicate that they are more favourable for planting than the shade-grown ones. Plants with a lower SDM/RDM ratio should proportionally have greater access to water and nutrients, which, coupled with the seedling total transpiration reduction (considering the lower total leaf area), would allow for an effective acclimation after transplantation to the field (Moraes *et al.* 2010). Poorter and Nagel (2000) reported that the elevated SDM/RDM ratio may indicate spatial restriction of root growth and the loss of balance between root and shoot development. In this case, the roots could not sufficiently supply the shoots with water (Poorter and Nagel 2000), because the transpiration rate is high in plants with great aerial biomass (Harvey and van den Driessche 1997). Therefore, the *C. pachystachya* shade-grown seedlings (with higher SDM/RDM ratio), despite the higher total dry mass and shoot height (Table 3), would possibly have reduced water supply for aerial parts, and for this reason their development was negatively affected after planting (Moraes *et al.* 2010).

Ceiba speciosa seedlings did not have their height influenced by light increase, although there was stem thickening in the acclimated seedlings, which resulted in the H/D ratio reduction (Table 3), corroborating the results of Pacheco *et al.* (2013) for the same species. A large stem diameter has been considered an indicator of greater resistance to physical damage (Cleary *et al.* 1978). Furthermore, large-diameter seedlings may store more carbohydrates and nutrients (Johnson *et al.* 2002), which are immediately available following planting to meet sink demands (Salifu and Timmer 2001) until plants re-establish root-soil contact to exploit resources from the site (Jacobs *et al.* 2005). Anatomical analysis of the stem revealed that, in addition to thickening, there was the increase in stem sclerenchyma percentage in the acclimated seedlings of *C. speciosa* (Table 2), which should result in higher stiffness of this organ and provide greater mechanical resistance to the seedlings (Niklas 1999).

The changes that occurred during the acclimation in the *C. speciosa* and *C. pachystachya* seedlings should favour their success in the field compared to the nonacclimated ones. This inference can be made considering the higher P_{Nmax}, which is probably related to a high photoprotection capacity of the new leaves developed under full sun (Moraes *et al.* 2010), the lower leaf transpiration surface, which should reduce water loss by seedlings after planting (Claussen 1996, Moraes *et al.* 2010), and the lower H/D ratio, which can favour their support and physical damage resistance (Cleary *et al.* 1978, Pacheco *et al.* 2013). For *C. speciosa*, the higher sclerenchyma proportion in the stem should also be considered, as this is a trait that is also related to the higher mechanical resistance of this organ (Niklas 1999). Moreover, the increased stem diameter may indicate greater nutrient and carbohydrate storage in acclimated seedlings (Johnson *et al.* 2002). For *C. pachystachya*, the lower SDM/RDM ratio, a trait that can be related to a greater ability for water and nutrients uptake (Poorter and Nagel 2000), should be also taken into account.

In *C. floribundus*, the acclimation did not promote responses in any of the analysed stem or root parameters (Tables 2, 3). However, the new leaves were developed with typical characteristics of sun leaves (reduced LA, SLA, LDM, and increased palisade parenchyma, leaf lamina thickness, and stomatal density), such as those reported by Oguchi *et al.* (2006), Matos *et al.* (2009), Ivancich *et al.* (2012) and Murphy *et al.* (2012), *i.e.*, the seedlings had invested in the development of foliar traits that may be favourable under the adversities of the planting site. Moreover, the acclimated seedlings showed no signs that they were affected by excessive light, such as the reduction of RGR or leaf necrosis (Powles 1984). Therefore, it can be proposed that hardiness traits cited above the developed *C. floribundus* seedlings during the acclimation process would probably favour their establishment in the field, compared to the nonacclimated seedlings.

Overall, the acclimation process, by which seedlings were subjected to stresses (particularly high irradiance) stimulated different responses among the studied species, even though the seedlings of the three light-demanding species were able to acclimate to the new light environment. In fact, when subjected to acclimation process, the plants acquired some hardiness characteristics, mainly related to the leaf structure, which would likely favour their establishment after planting in the field.

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