

## Biomass allocation and gas exchange are affected by light conditions in endangered *Cedrela salvadorensis* (Meliaceae) seedlings

J. Antonio Guzmán Q.<sup>1,3\*</sup>, Roberto A. Cordero S.<sup>1</sup> & Eugenio Corea A.<sup>2</sup>

1. Laboratorio de Ecología Funcional y Ecosistemas Tropicales, Escuela de Biología, Universidad Nacional de Costa Rica. PO Box 86-3000, Heredia, Costa Rica; antguz06@gmail.com\*, ticolamb@gmail.com
2. Instituto de Investigación y Servicios Forestales, Universidad Nacional de Costa Rica. PO Box 86-3000, Heredia, Costa Rica; eugeniocorea@hotmail.com
3. Programa Regional de Posgrado en Biología, Sistema de Estudios de Posgrado, Universidad de Costa Rica. PO Box 2060, San José, Costa Rica.

\* Correspondence

Received 16-VI-2015. Corrected 03-III-2016. Accepted 04-IV-2016.

**Abstract:** The determination of favorable light habitat conditions per species and life stage is transcendental, for both *ex situ* and *in situ* conservation strategies of endangered forest tree species, and for their utilization as plantation trees. This becomes especially important when planting material is scarce. We studied the multivariate responses in biomass allocation and in gas exchange to light and to CO<sub>2</sub> in *Cedrela salvadorensis* seedlings, grown under similar light conditions as those this species faces in nature. During a period of 135 days, groups of ten seedlings were put under 75, 45, 15 and 3.5 % of full sun exposure obtained with neutral shade cloth, under nursery conditions. A series of biomass allocation variables and detailed gas exchange parameters (photosynthesis response curves to light and to internal carbon concentration) were measured at the end of the growth period in plants of the four treatments. According to the principal component analyses, highest values of gas exchange response were associated with the lower values of biomass allocation traits. These changes can be associated with resource-conservative and resource-acquisitive strategies, where the *C. salvadorensis* seedlings acclimatize their traits for the exploration and exploitation of light, to high or to dim light environment, respectively. The multivariate analyses also showed that the plants had a high performance at 45 % of light environments. These results suggest that 45 % of light environment was the optimal light habitat of this species at the tested developing stage. Our results have important implications to choose the best natural habitat for a successful establishment of *C. salvadorensis*. We propose practical considerations for programs of reforestation or reintroduction where this species be involved. Rev. Biol. Trop. 64 (3): 1143-1154. Epub 2016 September 01.

**Key words:** CO<sub>2</sub> response curve, endangered tree species, light preferences, light responses curve; tropical trees.

Applied tree ecophysiology is focused on the study of optimal environmental or habitat requirements to improve the success of tropical tree plantations and forest restoration programs. In past decades, strategies development for endangered plant species management using ecophysiological techniques under nursery conditions, has become a center of interest for many conservationists, because of its low costs, great amount of replicates that can be held in a little space, and precise results that describe response of tree species to different

factors (Cooke et al., 2013; Corea, Arnaez, Moreira, Cordero, & Castillo, 2014; Guzmán & Cordero, 2013).

When looking to the habitat requirements of tree species, there are many resources involved to determine optimal conditions for each species, such as the water availability (Comita & Engelbrecht, 2009), nutrient supply (Walters & Reich, 2000), and soil conditions (Passioura, 2002). However in tropical forests, light requirements have been considered as the major environmental factor influencing

the establishment, growth, development and reproduction of many tree species (Lambers, Chapin, & Pons, 2008). Presently, response of growth and photosynthetic traits of different endangered species when planted in either shade or exposed sites must be known in order to improve successful reforestation practices and conservation strategies (Aleric & Kirkman, 2005; Smith, Wu, & Green, 1993). To accomplish this, the use of artificial light treatments under nursery conditions can be useful to evaluate optimal habitat conditions for endangered species. In addition, population conditions of these species are typically in such a reduced condition, that field and community surveys are not enough to precisely extrapolate their habitat requirements.

Some photosynthetic and morphological traits of endangered species stand out as key features to classify their light preferences, mostly because they are plastic traits that respond multidimensionally to several light regimes, and whose variation trends under natural light environments can be inferred (Valdadares & Niinemets, 2008). For example, it is a general trend that rates of carbon assimilation and specific leaf area under nursery conditions of shade-intolerant and tolerant species vary accordingly with the behavior found under natural conditions (Aleric & Kirkman, 2005). However, those trends in growth and survival rates of species under natural conditions are not necessarily similar as under controlled conditions, because these depend on whole habitat features. These kind of responses have led to consider that physiological and morphological traits are entities with a multidimensional response to light preferences.

The present study was conducted to analyze the effects of light regimes on *Cedrela salvadorensis* Standl. seedlings under nursery conditions by considering a set of morphological, biomass relations and detailed gas exchange variables. In Costa Rica, *C. salvadorensis* is critically endangered (MINAE Decree N° 25700, 1997) and their distribution is very limited, because it has a high habitat reduction (74 %) with a high rate of exploitation

(Estrada-Chavarria, Rodriguez-Gonzales, & Sanchez-Gonzales, 2005). Because of this, this study focused on the ecophysiological response of this species to different light environments, and how this response could have implications in programs of reforestation or reintroduction where this species is involved. Two main central questions were addressed: (1) How does *C. salvadorensis* adjust photosynthetic performance, growth, and biomass to different light environments? and (2) Is there a multidimensional response of biomass allocation and foliar physiology to different light regimes?. These two questions were responded through a biomass allocation analysis along with leaf photosynthesis changes due to light response and internal CO<sub>2</sub> response curves. More than an ecophysiological perspective, we wanted to enhance and interpret the results to exalt and recommend procedures for optimizing seedling establishment and growth of this species in forest restoration programs.

## MATERIALS AND METHODS

**Study species:** Work was done with seedlings of the neotropical endangered tree *Cedrela salvadorensis* Standl (Meliaceae), a tree species distributed from Mexico to Panama between 0 to 1000 masl. In Costa Rica, the species is found in wet and dry deciduous and semi-deciduous forests, where a large wood extraction and limited natural regeneration have occurred (Jiménez, 1999); for this reason, the Costa Rican government proclaimed an executive decree declaring this species as forbidden for extraction.

**Study site and experimental design:** We conducted this study at the Instituto de Investigación y Servicios Forestales of the Universidad Nacional de Costa Rica (10° 01' 20"N - 84° 06' 36", 1270 masl), from June 21 to November 4, 2011. We exposed forty seedlings of *C. salvadorensis* to four light treatments (10 for each treatment) created with superimposed neutral shade cloth: 75, 45, 15 y 3.5 % of light, respect to full sun exposure. We determined

the light conditions by measuring photosynthetic photon flux density (PPDF,  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ) with a quantum sensor during short morning periods, which presented the maximum PPDF values of the studied season. Seedlings were obtained from seeds collected from five mother trees dispersed in a seasonal deciduous forests located at Valle del Sol, Southwest area of Costa Rica Central Valley. Seeds were germinated using fine river sand on the top of long concrete tables (10 m long x 1 m side x 10 cm depth) inside a greenhouse. Sand was carefully washed and sieved in a 2.0 mm mesh size sieve. Seedlings were transferred to plastic bags of 1 647  $\text{cm}^3$  filled with a Typic distrandept soil (US Department of Agriculture, 1982) taken from the A horizon of nearby coffee plantations and mixed with sand (3:1 soil to sand). We maintained the seedlings under nursery conditions at 78 % shade for a period of six months, until they were used for this experiment.

Each treatment consisted of five pairs of seedlings from five different mother trees. The placement of individuals within each treatment followed a Latin Square Design with occasional rotation. After treatment set up, we monitored the acclimation progress following plant growth by measuring plant height and recording the presence of new leaves on June 21, August 18, October 06 and November 4 2011. To avoid water and nutritional deficiencies, the plants were kept moist every day with an automatic watering system, and plants were fertilized with four grams of complete commercial low-release (5-6 months) fertilizer (Osmocote Plus, 15-09-12 with minor elements). After the 135-day acclimation period, leaf physiological traits were measured by gas exchange analysis from light-response and  $\text{CO}_2$  response curves, using a Photosynthesis

Systems Li6400XT (LiCor Inc, Nebraska, U.S.A.). Final biomass and allocation variables were measured after harvesting.

**Biomass allocations:** We harvested each plant to measure the total fresh leaf area ( $LA$ ,  $\text{cm}^2$ ) with a leaf area meter (Li-3100, LICOR Inc., USA), and then we dried the harvested plants at 58 °C for more than 72 hours to measure the root ( $R$ , g), shoot ( $S$ , g), total dry biomass ( $TB$ , g), and total leaf dry biomass ( $LB$ , g). From this, we calculated the following ratios: root to shoot ratio ( $R/S$ ), specific leaf area ( $SLA$ , as  $LA/LB$ ,  $\text{cm}^2/\text{g}$ ), leaf area ratio ( $LAR$ , as  $LA/TB$ ,  $\text{cm}^2/\text{g}$ ), leaf mass ratio (LMR, as  $LB/TB$ ), shoot mass ratio ( $SMR$ , as  $S/TB$ ) and root mass ratio ( $RMR$ , as  $R/TB$ ) (Lambers et al., 2008).

**Gas exchange:** We selected four plants per treatment to measure physiological characteristics through leaf gas exchange analysis. Measurements were made using a portable photosynthesis system LI-6400 (LI-COR, USA), open configured, equipped with an artificial light source and  $\text{CO}_2$  assembly, on mature and fully expanded leaves. We performed light and  $\text{CO}_2$  response curve following Tambussi and Graciano (2010). Leaf temperature was maintained between 25-27 °C and relative humidity between 65-80 % for both kinds of curves. Light response curves were made at 385-395 ppm  $\text{CO}_2$  concentration. We measured the carbon assimilation uptake at several irradiances ranging from 1500 to 0  $\mu\text{mol}/\text{m}^2\cdot\text{s}$  by pre-illuminating leaves at 1250  $\mu\text{mol}/\text{m}^2\cdot\text{s}^1$  for 2-3 min. Carbon assimilation ( $A$ ,  $\mu\text{molCO}_2/\text{m}^2\cdot\text{s}$ ) versus irradiance ( $I$ ,  $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ) data was fitted by a non-rectangular hyperbola model (Marshall & Biscoe, 1980; Thornley & Johnson, 1990):

$$A = R_{\text{dark}} + \frac{Q_{\text{app}} I + (A_{\text{max}} + R_{\text{dark}}) - \sqrt{(Q_{\text{app}} I + (A_{\text{max}} + R_{\text{dark}}))^2 - 4\theta Q_{\text{app}} I (A_{\text{max}} + R_{\text{dark}})}}{2\theta}$$

where  $A$  is rate of carbon assimilation ( $\mu\text{molCO}_2/\text{m}^2\cdot\text{s}$ ),  $R_{\text{dark}}$  is the dark respiration rate ( $\mu\text{molCO}_2/\text{m}^2\cdot\text{s}$ ),  $Q_{\text{app}}$  is apparent quantum yield (mol/mol),  $I$  is the applied photosynthetic photon flux density ( $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ),  $A_{\text{max}}$  is the

maximum assimilation rate ( $\mu\text{molCO}_2/\text{m}^2\cdot\text{s}$ ) and  $\theta$  describes the curvature. This model was fit by *nls2* package (Grothendieck, 2014) of R software (R Development Core Team, 2014). All fitted curves showed  $r^2$  values greater

than 0.95. Likewise, we calculated independently the light compensation point ( $I_c$ ,  $\mu\text{mol}/\text{m}^2\text{s}$ ) by interpolation at “x” line interception of linear regression analysis of data points between 0 and 60  $\mu\text{mol}/\text{m}^2\text{s}$ . Because the values of  $A_{\text{max}}$  were presented close to values of light saturation of 1000  $\mu\text{mol}/\text{m}^2\text{s}$ , we extracted from irradiances of 1000  $\mu\text{mol}/\text{m}^2\text{s}$  the stomatal conductance ( $g$ ,  $\text{mmol H}_2\text{O}/\text{m}^2\text{s}$ ), transpiration ( $E$ ,  $\text{mmol H}_2\text{O}/\text{m}^2\text{s}$ ), and  $\text{CO}_2$  assimilation ( $A_{1000}$ ), and then we estimated the water use efficiency (WUE,  $\mu\text{mol CO}_2/\text{m}^2\text{s}/\text{mmol H}_2\text{O}/\text{m}^2\text{s}$ ) by the ratio of  $A_{1000}/E$ .

We carried out a  $\text{CO}_2$  response curve after the light response curve in the same leaf, varying  $\text{CO}_2$  concentration between 50-900 ppm at a light irradiance of 1000  $\mu\text{mol}/\text{m}^2\text{s}$  and atmospheric pressure of 86-87 Pa. We fitted the  $A$  versus internal carbon concentration ( $C_i$ ) data following Ethier and Livingston (2004) version of Farquhar’s model (Farquhar, von Caemmerer, & Berry, 1980) to extract different leaf biochemical parameters: maximum rate of carboxylation ( $V_{\text{cmax}}$ ,  $\mu\text{mol CO}_2/\text{m}^2\text{s}$ ), maximum photosynthetic electron flow rate ( $J_{\text{max}}$ ,  $\mu\text{mole}/\text{m}^2\text{s}$ ), day respiration rate ( $R_{\text{day}}$ ,  $\mu\text{mol CO}_2/\text{m}^2\text{s}$ ), chloroplast  $\text{CO}_2$  compensation point ( $\Gamma$ ,  $\mu\text{mol}/\text{mol}$ ) and mesophyll conductance ( $g_m$ ,  $\text{mol CO}_2/\text{m}^2\text{s}$ ).

We applied three mixed design of analysis of variance to determine the effects of time, treatments, and their interaction on the height, amount of leaves and amount of new leaves. Also we compared the treatments effects on the biomass allocation, light response and  $A-C_i$  response curves parameters by analysis of variance (ANOVA). We performed simple linear regressions to show the biochemical effects of  $V_{\text{cmax}}$ ,  $J_{\text{max}}$  and  $\Gamma$  on  $A_{\text{max}}$ . We also performed two principal component analyses (PCA) from a correlation matrix of biomass allocation and leaf gas exchange parameters to evidence differential weight on the total value variation. From the first principal component of each PCA, an analysis of covariance (ANCOVA) was performed of the first components of biomass allocations and leaf gas exchange factor scores as continuous variables using the light

treatments as categorical factor, used as an indirect test of a multivariate response due to the light treatments. We conducted the previous analyses using R software version 3.1.1 (R Development Core Team, 2014).

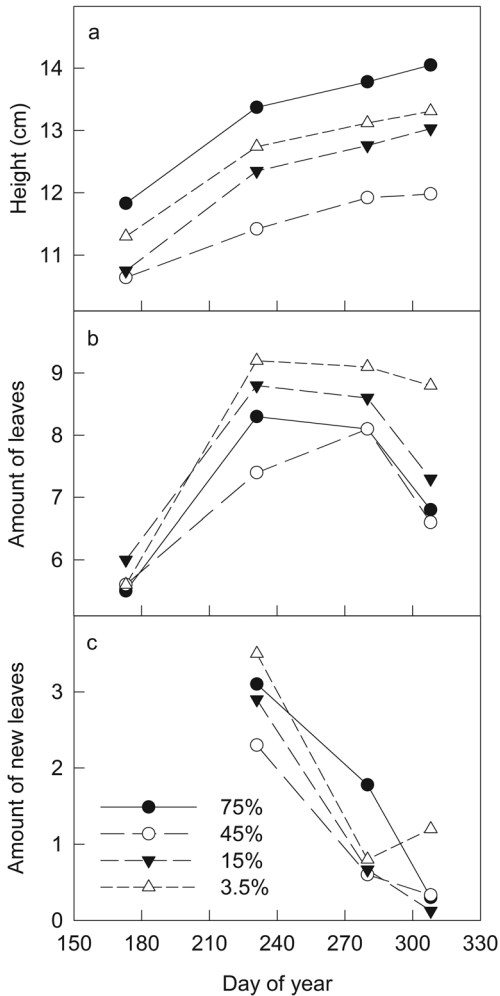
## RESULTS

### Growth and biomass allocations:

Throughout the 135 days of study, all plants showed increases in height (Fig. 1a), where plants at 75 % light treatment showed more increases than other treatments. Independently of treatment, plants presented reductions in the stem height increase by the end of the period, along with a final reduction of the amount of leaves and new leaves were produced (Fig. 1b and Fig. 1c). At day 58, plants increased the amount of leaves by  $2.5 \pm 1.69$  leaves, but plants decreased this mean to  $1.58 \pm 1.92$  leaves compared to baseline. However, lower light treatment presented plants with more leaves than other treatments. In the same way, the number of new leaves produced after 58 days was higher ( $2.95 \pm 1.47$  leaves) than at the end of the study ( $0.51 \pm 1.71$  leaves). Mixed design ANOVA showed a significant temporal effects on plant height and amount of leaves and new leaves, with no significant treatments\*time interaction effect (Table 1). Only in the amount of leaves, the mixed design ANOVA showed a significant treatment effect.

Comparisons of four light treatments evidenced that there is a strong light influence on some biomass allocations parameters (Table 2), where  $TB$ ,  $SLA$ ,  $LAR$  and  $LMR$  showed significant differences between light treatments; meanwhile, the lighter treatments of 75 and 45 % presented greater  $TB$  and lower  $SLA$ ,  $LAR$ ,  $LMR$  values.  $SLA$ ,  $LAR$  and  $LMR$  increased and  $TB$  decreased with decreasing irradiance, respectively.  $SMR$ ,  $RMR$ , and  $R/S$  showed no significant light effect between treatments.

**Gas exchange:** Only  $A_{\text{max}}$  presented significant differences between light treatments in the  $A-I$  response curve (Table 3). Highest and lowest values of  $A_{\text{max}}$  were measured in 45 %



**Fig. 1.** Mean values of height (a), amount of leaves (b) and amount of new leaves produced (c) of seedlings of *Cedrela salvadorensis* exposed to four light treatments through during a 135 days of growth period.

and 3.5 % light treatments, respectively. All other parameters extracted from light response curves presented slightly higher values in lighter treatments, with no significant effects between them.  $Q_{app}$  showed a non-significant inverse trend. The  $A-C_i$  response curve parameters differ significantly between treatments (Table 3), except  $R_{day}$ . Highest values of  $V_{cmax}$ ,  $J_{max}$  and  $g_m$ , and lowers values of  $\Gamma$  were found in 45 % light treatments. Linear relationships (Fig. 2) evidenced significant responses of  $A_{max}$

to biochemical leaf properties, showing high values of  $V_{cmax}$  and  $J_{max}$  and lower values of  $\Gamma$  associated with high rates of assimilation.

**Whole-plant response:** PCA statistics for growth and biomass allocation (Fig. 3a) indicated that more than 66 % of variation was explained by the first two components. The first component is mostly influenced by  $LAR$ ,  $LMR$  and  $SLA$  (eigenvector= 0.50, 0.42 and 0.41, respectively), and the second component by  $SMR$ ,  $RMR$ , and  $SLA$  (eigenvector= -0.69, 0.63 and 0.26, respectively). For the foliar physiology variables, PCA explained 67 percent of the variation (Fig. 3b), where the first component is mostly influenced by  $V_{cmax}$ ,  $J_{max}$  and  $A_{max}$  (eigenvector= 0.42, 0.41 and 0.38, respectively) and the second component by  $\Gamma$ ,  $R_{day}$  and  $WUE$  (eigenvector=-0.46, 0.35 and 0.33, respectively). Linear relationship between the first principal components from the two PCA showed that high leaf physiological scores were related with low scores of biomass allocations (Fig. 3c). ANCOVA comparisons showed that the biomass allocation traits were affected by light treatments ( $F_{3,16} = 7.96$ ;  $P < 0.001$ ) and foliar physiology ( $F_{1,16} = 20.10$ ,  $P < 0.001$ ), without interaction between these ( $F_{3,16} = 0.75$ ;  $P > 0.05$ ), suggesting the existence of a multivariate response.

## DISCUSSION

Results of monitoring leaf production showed a strong light-dependent acclimation of *C. salvadorensis* to light treatments. Although this study was conducted during a shorter period than a year, the responses of biomass allocation and gas exchange suggested a quick acclimation of this species to light regimes. During the monitoring, no differences were found in height, number and production of leaves within each treatment, but a reduction in the production and the number of leaves. Surprisingly during the early stage of development of these seedlings, the reduction in the production and number of leaves presented, could be associated to the inherent phenological

TABLE 1  
Mixed design ANOVA to compare the effect of time, treatments, and their interaction on the height, amount of leaves and new leaves produced by seedlings of *Cedrela salvadorensis* exposed to four light treatments during a 135 days growth period

Parameters	Factors <sub>(df)</sub>	F	P
Height	Time <sub>(3, 160)</sub>	2.72	0.04
	Treatment <sub>(1, 160)</sub>	0.71	0.40
	Time*treatment <sub>(3, 160)</sub>	0.01	0.99
Amount of leaves	Time <sub>(3, 160)</sub>	5.25	<0.01
	Treatment <sub>(1, 160)</sub>	6.53	<0.01
	Time*treatment <sub>(3, 160)</sub>	0.69	0.56
Amount of new leaves	Time <sub>(3, 160)</sub>	14.54	<0.001
	Treatment <sub>(1, 160)</sub>	0.23	0.63
	Time*treatment <sub>(3, 160)</sub>	1.85	0.14

TABLE 2  
Means ( $\pm$ SD) and probability values of ANOVA to compare the effects of four light treatments on biomass allocation of seedlings *Cedrela salvadorensis* after 135 days growth period

Parameter	Light treatment				ANOVA	
	75 %	45 %	15 %	3.5 %	F-ratio df=3.36	P-value
TB (g)	7.49 $\pm$ 3.36 <sup>a</sup>	6.23 $\pm$ 2.50 <sup>ab</sup>	4.35 $\pm$ 1.96 <sup>b</sup>	4.49 $\pm$ 1.79 <sup>ab</sup>	3.43	0.03
R/S	0.72 $\pm$ 0.22 <sup>c</sup>	0.91 $\pm$ 0.30	0.82 $\pm$ 0.16	0.73 $\pm$ 0.38	1.35	0.27
SLA (cm <sup>2</sup> /g)	322.68 $\pm$ 45.61 <sup>c</sup>	340.79 $\pm$ 95.99 <sup>c</sup>	414.51 $\pm$ 62.80 <sup>b</sup>	513.71 $\pm$ 64.18 <sup>a</sup>	16.17	<0.001
LAR (cm <sup>2</sup> /g)	39.03 $\pm$ 11.52 <sup>c</sup>	53.29 $\pm$ 21.47 <sup>bc</sup>	62.36 $\pm$ 20.93 <sup>b</sup>	104.53 $\pm$ 32.27 <sup>a</sup>	12.79	<0.001
LMR	0.12 $\pm$ 0.03 <sup>b</sup>	0.15 $\pm$ 0.09 <sup>ab</sup>	0.15 $\pm$ 0.05 <sup>ab</sup>	0.20 $\pm$ 0.06 <sup>a</sup>	3.80	0.02
SMR	0.47 $\pm$ 0.07	0.39 $\pm$ 0.07	0.40 $\pm$ 0.08	0.39 $\pm$ 0.07	2.79	0.05
RMR	0.41 $\pm$ 0.08	0.46 $\pm$ 0.09	0.45 $\pm$ 0.05	0.40 $\pm$ 0.10	1.40	0.25

Abbreviation: Total biomass (TB), root/shoot ratio (R/S), specific leaf area (SLA), leaf area ratio (LAR), leaf mass ratio (LRM), stem mass ratio (SMR) and root mass ratio (RMR). Significant differences of Tukey test are indicated by the letter code.

patterns of the species, which is described as a deciduous species from November to March (Corea et al., 2014; Rojas-Rodríguez & Torres-Cordoba, 2013). This latter could have major implications in the reforestation programs, so according to the plant phenology, we recommend three practices for the transplantation and reforestation: i) keep the areas of reforestation within the premontane deciduous and semi-deciduous forests with long dry season (4-6 months), ii) avoid dry season during their early leaf loss, and iii) avoid dimer habitats. These types of practices are highly recommended for deciduous and other tropical tree species in some studies (Elliott et al., 2003; Wishnie et al.,

2007), because dry sites can reduce significantly the growth rates, survival and acclimation of the species, especially in seedlings stage.

The continuous monitoring of seedlings of *C. salvadorensis* to different light conditions in terms of plant height, leaf number and production, might not be effective indicators of the acclimation of the species to different light environments, at least during the short period investigated, contrary of what happened with variables from foliar physiology and biomass allocation. In general, changes in the size and the amount of leaf in trees have been associated in long-term studies where the environmental conditions like drought or light regimen can

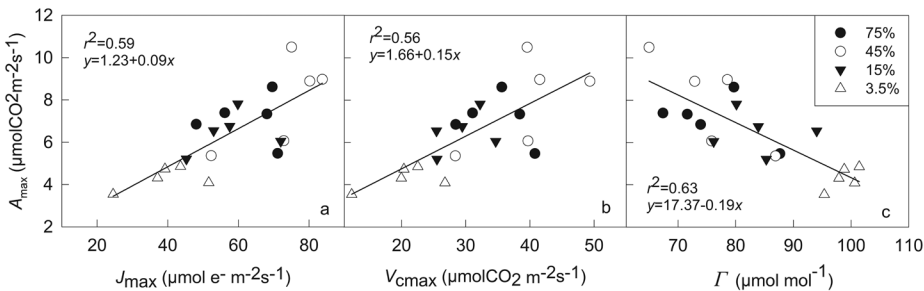
TABLE 3

Means ( $\pm$ SD) and probability values of ANOVA to compare the effects of four light treatments on the light and CO<sub>2</sub> curve response parameters of seedlings of *Cedrela salvadorensis* exposed to 135 days growth period

Parameter	75 %	45 %	15 %	3.5 %	ANOVA	
					F-ratio df=3.16	P-value
<b>Light response curve</b>						
$A_{max}$ ( $\mu\text{mol CO}_2/\text{m}^2\text{s}$ )	7.13 $\pm$ 1.14 <sup>a</sup>	7.96 $\pm$ 2.16 <sup>a</sup>	6.48 $\pm$ 0.96 <sup>ab</sup>	4.30 $\pm$ 0.53 <sup>b</sup>	6.85	0.003
$R_d$ ( $\mu\text{mol CO}_2/\text{m}^2\text{s}$ )	-0.64 $\pm$ 0.53	-0.63 $\pm$ 0.46	-0.64 $\pm$ 0.44	-0.36 $\pm$ 0.31	0.50	0.69
$I_c$ ( $\mu\text{mol}/\text{m}^2\text{s}$ )	11.94 $\pm$ 8.94	11.52 $\pm$ 8.17	11.50 $\pm$ 7.82	6.54 $\pm$ 6.04	0.53	0.66
$Q_{app}$ ( $\mu\text{mol CO}_2/\text{m}^2\text{s} / \mu\text{mol}/\text{m}^2\text{s}, 10^{-2}$ )	5.5 $\pm$ 1.6	5.2 $\pm$ 1.0	5.8 $\pm$ 0.4	6.1 $\pm$ 0.7	0.61	0.62
$\theta$ ( $10^{-2}$ )	41.6 $\pm$ 38.9	76.5 $\pm$ 15.4	71.2 $\pm$ 9.0	52.5 $\pm$ 30.2	2.07	0.14
$g$ (mmol H <sub>2</sub> O/m <sup>2</sup> s)	0.09 $\pm$ 0.05	0.09 $\pm$ 0.03	0.09 $\pm$ 0.02	0.07 $\pm$ 0.02	0.28	0.83
$E$ (mmol H <sub>2</sub> O/m <sup>2</sup> s)	1.24 $\pm$ 0.39	1.11 $\pm$ 0.21	1.24 $\pm$ 0.38	0.97 $\pm$ 0.34	0.78	0.51
WUE ( $\mu\text{mol CO}_2/\text{m}^2\text{s} / \text{mmol H}_2\text{O}/\text{m}^2\text{s}$ )	5.15 $\pm$ 1.37	6.12 $\pm$ 0.55	4.69 $\pm$ 1.08	4.25 $\pm$ 1.44	2.37	0.10
<b>CO<sub>2</sub> response curve</b>						
$V_{cmax}$ (mmol CO <sub>2</sub> /m <sup>2</sup> s)	34.87 $\pm$ 5.09 <sup>ab</sup>	39.71 $\pm$ 7.49 <sup>a</sup>	29.47 $\pm$ 4.07 <sup>b</sup>	20.37 $\pm$ 5.28 <sup>c</sup>	10.85	<0.001
$J_{max}$ ( $\mu\text{mol}/\text{e}^-\text{m}^2\text{s}$ )	62.59 $\pm$ 10.05 <sup>a</sup>	72.83 $\pm$ 12.25 <sup>a</sup>	57.52 $\pm$ 9.81 <sup>ab</sup>	39.19 $\pm$ 9.90 <sup>b</sup>	8.89	0.001
$R_{day}$ (mmol CO <sub>2</sub> /m <sup>2</sup> s)	2.35 $\pm$ 0.79	2.49 $\pm$ 0.46	2.27 $\pm$ 0.10	2.14 $\pm$ 0.66	0.32	0.81
$\Gamma$ ( $\mu\text{mol}/\text{mol}$ )	76.05 $\pm$ 7.86 <sup>b</sup>	75.83 $\pm$ 8.01 <sup>b</sup>	83.91 $\pm$ 6.68 <sup>b</sup>	98.83 $\pm$ 2.39 <sup>a</sup>	13.29	<0.001
$g_m$ (mol CO <sub>2</sub> /m <sup>2</sup> s, 10 <sup>-2</sup> )	6.78 $\pm$ 1.4 <sup>ab</sup>	7.5 $\pm$ 2.9 <sup>a</sup>	4.3 $\pm$ 0.9 <sup>bc</sup>	2.9 $\pm$ 0.6 <sup>c</sup>	8.04	0.002

Maximum assimilation rate ( $A_{max}$ ), dark respiration rate ( $R_d$ ), light compensation point ( $I_c$ ), apparent quantum yield ( $Q_{app}$ ), the curvature ( $\theta$ ), stomatal conductance ( $g$ ), transpiration rate ( $E$ ), water-use efficiency (WUE), maximum rate of carboxylation ( $V_{cmax}$ ), rate of photosynthetic electron flow ( $J$ ), day respiration rate ( $R_{day}$ ), CO<sub>2</sub> compensation point ( $\Gamma$ ), mesophyll conductance ( $g_m$ ).

Significant differences of Tukey test are indicated by the letter code.

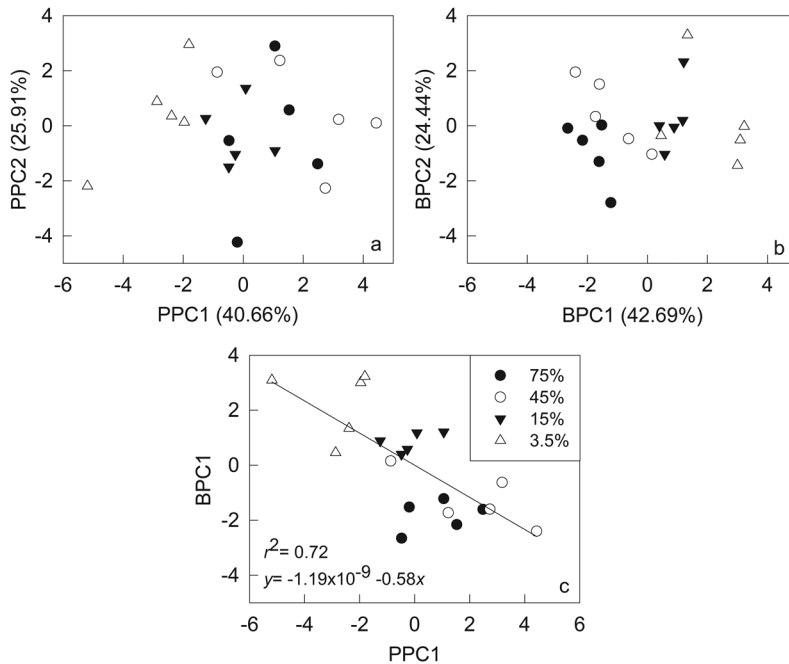


**Fig. 2.** Linear relationship between maximum photosynthetic rates ( $A_{max}$ ) and maximum rate of photosynthetic electron flow ( $J_{max}$ ) (A), maximum rate of carboxylation ( $V_{cmax}$ ) (B) and chloroplast CO<sub>2</sub> compensation point ( $\Gamma$ ) (C) of endangered seedlings of *Cedrela salvadorensis* exposed to four light treatment for 135 days. Linear significance relationship has describe by a:  $F_{2,18} = 26.16$ ,  $P < 0.001$ , b:  $F_{2,18} = 23.17$ ,  $P < 0.001$ , c:  $F_{2,18} = 31.69$ ,  $P < 0.001$ .

have strong repercussions on the increases or decreases of these traits during the growth (Wishnie et al., 2007). However, in short-term studies like this, changes in the biomass allocation and gas exchange due to light availability in traits like *SLA* and  $A_{max}$  are related to the direct advantage for light interception in higher

or low-light environment (Poorter, 1999, 2001), where this interception have strong implications on the plant performance and the assignment of structures to capture resources.

General observations from the results indicated that the biomass allocation had a great weight in the acclimation of this species to



**Fig. 3.** Eigenvalues of the principal component analysis for foliar physiology (a) and biomass allocations parameters (b) and linear relationship between two first principal components (c) of seedlings of *Cedrela salvadorensis* exposed to four light treatments for 135 days. Linear significance relationship of C is described by:  $F_{2,18}=20.10$ ,  $P < 0.001$ .

contrasting light regimes, especially the biomass allocation in leaf traits. Some studies showed that leaf characteristics such as higher *SLA* may allow seedlings to harvest light more effectively at low irradiance (Loach, 1970) which can maximize the carbon gain per unit leaf mass (Evans & Poorter, 2001). However, the higher *SLA* and *LMR* in *C. salvadorensis* in the treatment with low light could be associated with a small growth or lower biomass accumulation in a dim light environment. Although variables such as *R/S*, *SMR* and *RMR* apparently did not respond to light treatments, it is clear that these variables are important in the balance between investment in light interception and water and nutrient uptake by organs (Poorter, 2001). Therefore, we do not discard that these variables in long-term studies and with other environmental factors also play an important role in the acclimation or survival of the species to different light regimes, or they will become important when planted under field conditions.

From a general perspective, we considered that the changes in the biomass allocation to high or dim light environments in *C. salvadorensis*, can be related to an economic advantage to exploit and efficient use of the light resource according to its availability. In this sense it is likely that the observed changes in the biomass allocation are related to resource-conservative and resource-acquisitive strategies (Adler, Fajardo, Kleinhesselink, & Kraft, 2013). For example, in this study, increases in the *LMR* in the low light treatments could be associated with resource-conservative strategy for the exploration of light in a low light environment (Givnish, 1988; Loach, 1970). Likewise, increases in *SLA* in the high light treatments could be associated with resource-acquisitive strategy to harvest more light per area in high light environments (Adler et al., 2013). We suggest that if the adaptation in the biomass allocation respond to conservative-acquisitive strategies in face to different light environments, it could prevent the environmental



filtering of high or dim light environment and it could promote their success in the forest.

When considering the gas exchange performance, the differences between the biochemical characteristics of leaf physiology to light treatments, evidenced a joint response of leaf biochemical capacity and  $A_{\max}$ . In general, higher  $A_{\max}$  values found in treatments with higher irradiance, showed typical responses of light-demanding species (Schaedle, 1975); however, the responses to the light treatment observed in this species can have different explanations. For example, studies suggest that the resistance in the molecules ( $\text{CO}_2$  and  $\text{H}_2\text{O}$ ) transport inside and outside leaf mesophyll, can limit different physiological processes (Medrano, Escalona, Bota, Gulias, & Flexas, 2002), for this, it is possible that conductance effects like  $g$  and in particular the lower values of  $g_i$  in the 3.5 % light treatment could be the responsible for the differences in  $A_{\max}$ ,  $V_{\text{cmax}}$ ,  $J_{\max}$ , and  $\Gamma$ . Likewise, the differences in these parameters could suggest that the assimilation capacity, and the biochemical ability of carboxylation and electron flow exhibit symptoms of photosynthetic stress like photoinhibition. In many cases, differences in the assimilation, carboxylation and electron flow in leaves are the result of environmental stress like a high radiation or high temperature, and they will have strong implications in other processes like respiration or the light compensation point (Long, Humphries, & Falkowski, 1994; Niinemets, Kull, & Tenhunen, 1998). Either by changes in the leaf conductance or environmental triggers which induce a physiological stress, here the changes in  $A_{\max}$ ,  $V_{\text{cmax}}$  and  $J_{\max}$  suggest that *C. salvadorensis* presents a greater capacity of gas exchange in 45 % light treatments. From an ecological perspective, it is possible that these results obtained at the 45 % shade treatment seems to coincide with light regime perceived inside natural large gaps (>400 m<sup>2</sup>) in tropical forests (Chazdon & Fetcher, 1984).

The multidimensional response found evidences that light acclimation of the species is directly related to concomitant changes in leaf gas exchange physiology and biomass

allocation patterns (Bazzaz & Pickett, 1980; Pearcy, 2000). In this sense, it is likely that precise monitoring of single physiological or biomass allocation traits may help to follow a species light acclimation trend. For example, changes in plant *LAR*, *LMR* or *SLA* in response to different ranges of natural conditions could prove to be indicative of the gas exchange performance of the plant, which can provide a simple protocol for monitoring of the species under different conditions. Likewise, although the degree of phenotypic plasticity of this species to light treatments was not measured, it is possible that the great variations of their trait in terms of biomass allocation and gas exchange could be indicators of the great phenotypic plasticity induced by changes in the light regimens as shade-intolerant species. This suggestion has been clearly demonstrated in this kind of species and associated with changes in *SLA*,  $A_{\max}$ , and *LAR* (Rozenaal, Hurtado, & Poorter, 2006; Valladares, Martinez-Ferri, Balaguer, Perez-Corona, & Manrique, 2000).

There is a strong light-dependent acclimation of *C. salvadorensis* to light regimes, where the overall performance of this species under nursery conditions seems to be optimized at light levels corresponding to 45 % shade environment. This light condition is easy to obtain with low cost and low time-demanding practices in a simple and rustic nursery or in natural conditions like forest edge, agroforestry systems with low density of trees, or secondary forests. Caution must be taken to extrapolate these results to open field conditions in a greater scale initiative, where water availability and fertilization activities may be reduced. With this in mind, the use of this species in any forest restoration programs has to be more careful in the selection of sites with suitable light conditions for the growth and establishment of species, such as avoiding dry season during their early leaf loss and avoiding lighter and dimer habitats. Any effort in this sense will help reduce the speed of biodiversity genetic loss of this neotropical endangered tree species. Future work must be directed to demonstrate to what extent these acclimation responses

may change during field establishment under natural or plantations systems, and how these responses can allow the survival of this species in the highly diverse tropical forests.

#### ACKNOWLEDGMENTS

We thank Maricruz Torres, Leonel Orozco and Jairo Hidalgo for their assistance with the plants and data collection. Thanks to Elmer García who provided valuable comments and recommendations on the manuscript. This study was supported by two grants from the Fondo Especial para la Educación Superior through the (Consejo Nacional de Rectores de Costa Rica), one to Roberto Cordero and other equipment grant (Licor-6400XT system) to Escuela de Ciencias Agrarias, both to Universidad Nacional of Costa Rica.

#### RESUMEN

**Asignación de biomasa e intercambio de gases son afectados por las condiciones de luz en plántulas de *Cedrela salvadorensis* (Meliaceae) en peligro de extinción.** La determinación de las condiciones lumínicas favorables por especie y estado de vida es trascendental para las estrategias de conservación *ex situ* y *in situ* de especies de árboles en peligro de extinción, y su utilización como plantaciones forestales. Esto se vuelve especialmente importante cuando el material de siembra es escaso. Aquí, nosotros estudiamos las respuestas en asignación de biomasa y en intercambio de gases a luz y CO<sub>2</sub> en plántulas de *Cedrela salvadorensis* crecidas bajo condiciones lumínicas similares a las que esta especie enfrenta en la naturaleza. Durante 135 días, grupos de diez plántulas fueron colocadas bajo condiciones de 75, 45, 15 y 3.5 % de exposición total al sol obtenidas por medio de serán bajo condiciones de vivero. Una serie de variables de asignación de biomasa y parámetros de intercambio de gases (fotosíntesis según curvas de respuesta a la luz y a la concentración de carbono interno) fueron medidas al final del periodo de crecimiento en las plantas de los cuatro tratamientos. De acuerdo con los análisis de componentes principales, valores altos de respuesta de intercambio de gases están asociados con valores bajos de rasgos de asignación de biomasa. Los cambios observados se encuentran asociados con las estrategias recurso-conservativas y recurso-adquisitivas donde *C. salvadorensis* aclimata sus rasgos para la exploración y explotación de luz en ambientes con escasa o excesiva radiación lumínica, respectivamente. Los análisis multivariados muestran también que las plantas tienen un alto rendimiento a 45 % de luz ambiental. Estos resultados

sugieren que el 45 % de ambiente lumínico es el hábitat lumínico óptimo de esta especie en el estado de desarrollo estudiado. Nuestros resultados tienen importantes implicaciones para escoger el mejor hábitat natural para un exitoso establecimiento de *C. salvadorensis*. Por esto, proponemos consideraciones prácticas para programas de reforestación y reintroducción donde esta especie estaría involucrada.

**Palabras clave:** curvas de respuesta al CO<sub>2</sub>, árboles en peligro de extinción, preferencias lumínicas, curvas de respuesta a la luz, árboles tropicales.

#### REFERENCES

- Adler, P. B., Fajardo, A., Kleinhesselink, A. R., & Kraft, N. J. (2013). Trait-based tests of coexistence mechanisms. *Ecology Letters*, 16(10), 1294-1306.
- Aleric, K., & Kirkman, L. (2005). Growth and photosynthetic responses of the federally endangered shrub, *Lindera melissifolia* (Lauraceae), to varied light environments. *American Journal of Botany*, 92, 682-689.
- Bazzaz, F., & Pickett, S. (1980). Physiological ecology of tropical succession: a comparative review. *Annual Review of Ecology and Systematic*, 11, 287-310.
- Chazdon, R., & Fetcher, N. (1984). Photosynthetic light environments in a lowland tropical rain forest in Costa Rica. *Journal of Ecology*, 72(2), 553-564.
- Comita, L., & Engelbrecht, B. (2009). Seasonal and spatial variation in water availability drive habitat associations in a tropical forest. *Ecology*, 90(10), 2755-2765.
- Cooke, S., Sack, L., Franklin, C., Farrell, A., Beardall, J., Wikelski, M., & Chown, S. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conservation Physiology*, 1, 1-23.
- Corea, E., Arnaez, E., Moreira, I., Cordero, R., & Castillo, M. (2014). *Recurso forestal amenazado: seis especies en peligro crítico de extinción en Costa Rica*. Cartago, Costa Rica: Editorial Tecnológica de Costa Rica.
- Elliott, S., Navakitbumrung, P., Kuarak, C., Zanglum, S., Anusarnsunthorn, V., & Blakesley, D. (2003). Selecting framework tree species for restoring seasonally dry tropical forests in northern Thailand based on field performance. *Forest Ecology and Management*, 184, 177-191.
- Estrada-Chavarria, A., Rodriguez-Gonzales, A., & Sanchez-Gonzales, J. (2005). *Evaluación y categorización del estado de conservación de plantas en Costa Rica*. San José, Costa Rica: Museo Nacional de Costa Rica, Instituto Nacional de Biodiversidad, Sistema Nacional de Áreas de Conservación.

- Ethier, G. J., & Livingston, N. J. (2004). On the need to incorporate sensitivity to CO<sub>2</sub> transfer conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model carbon gain. *Plant, Cell & Environment*, 27(2), 137-153.
- Evans, J., & 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 & Environment*, 24(8), 755-767.
- Farquhar, G. D., von Caemmerer, S. V., & Berry, J. A. (1980). A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C3 species. *Planta*, 149(1), 78-90.
- Givnish, T. (1988). Adaptation to sun and shade: a whole-plant perspective. *Functional Plant Biology*, 15(2), 63-92.
- Grothendieck, G. (2014). nls2: Non-linear regression with brute force. Retrieved from <http://cran.r-project.org/web/packages/nls2/>
- Guzmán, J., & Cordero, R. (2013). Growth and photosynthetic performance of five tree seedlings species in response to natural light regimes from the Central Pacific of Costa Rica. *Revista de Biología Tropical*, 61(3), 1433-1444.
- Jiménez, Q. (1999). *Árboles maderables en peligro de extinción en Costa Rica* (2nd ed.). Santo Domingo de Heredia, Costa Rica: Instituto Nacional de Biodiversidad.
- Lambers, H., Chapin, F. S., & Pons, T. L. (2008). *Plant physiological ecology* (2nd ed.). New York, USA: Springer.
- Loach, K. (1970). Shade tolerance in tree seedlings. II. Growth analysis of plants raised under artificial shade. *New Phytologist*, 69, 273-286.
- Long, S. P., Humphries, S., & Falkowski, P. G. (1994). Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology*, 45(1), 633-662.
- Marshall, B., & Biscoe, P. V. (1980). A model for C3 leaves describing the dependence of net photosynthesis on irradiance. *Journal of Experimental Botany*, 31(1), 29-39.
- Medrano, H., Escalona, J. M., Bota, J., Gulias, J., & Flexas, J. (2002). Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of Botany*, 89(7), 895-905.
- Ministerio de Ambiente y Energía. (1997). Decreto Ejecutivo N° 25700-MINAE. La Gaceta. Diario Oficial (CR) Vol. 119 (11). Enero 16, 9-10.
- Niinemets, Ü., Kull, O., & Tenhunen, J. D. (1998). An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. *Tree Physiology*, 18(10), 681-696.
- Passioura, J. (2002). "Soil conditions and plant growth". *Plant, Cell and Environment*, 25(2), 311-318.
- Pearcy, R. (2000). Acclimation to sun and shade. In A. Raghavendra (Ed.), *Photosynthesis: a Comprehensive Treatise* (pp. 250-263). England: Cambridge University Press.
- Poorter, L. (1999). Growth responses of 15 rain-forest tree species to a light gradient: The relative importance of morphological and physiological traits. *Functional Ecology*, 13(3), 396-410.
- Poorter, L. (2001). Light-dependent changes in biomass allocation and their importance for growth of rain forest tree species. *Functional Ecology*, 15(1), 113-123.
- R Development Core Team. (2014). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.r-project.org>
- Rojas-Rodríguez, F. & Torres-Cordoba, G. (2013). Árboles del Valle Central de Costa Rica: reproducción Cedro (*Cedrela salvadorensis* Stadl.). *Revista Forestal Mesoamericana Kurú*, 10, 34-35.
- Rozendaal, D., Hurtado, V., & Poorter, L. (2006). Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Functional Ecology*, 20, 207-216.
- Schaedle, M. (1975). Tree photosynthesis. *Annual Review of Plant Physiology*, 26, 101-115.
- Smith, M., Wu, Y., & Green, O. (1993). Effect of light and water-stress on photosynthesis and biomass production in *Boltonia decurrens* (Asteraceae), a threatened species. *American Journal of Botany*, 80, 859-864.
- Tambussi, E. A., & Graciano, C. (2010). Técnicas de medición de intercambio de gases en plantas: curvas de respuesta a la luz y al CO<sub>2</sub>. In M. E. Fernández & J. E. Gyenge (Eds.), *Técnicas de medición en ecofisiología vegetal: conceptos y procedimientos* (pp. 109-115). Buenos Aires, Argentina: Ediciones INTA.
- Thornley, J. H., & Johnson, I. R. (1990). *Plant and crop modelling*. Oxford University, USA: Blackbur Prees.

- US. Department of Agriculture. Soil Conservation Service 1982. Soil Taxonomy. SMSS Technical Monograph No. 5. p. 139.
- Valladares, F., Martinez-Ferri, E., Balaguer, L., Perez-Corona, E., & Manrique, E. (2000). Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytologist*, 148(1), 79-91.
- Valladares, F., & Niinemets, Ü. (2008). Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 237-257.
- Walters, M., & Reich, P. (2000). Seed size, nitrogen supply, and growth rate affect tree seedling survival in deep shade. *Ecology*, 81(7), 1887-1901.
- Wishnie, M., Dent, D., Mariscal, E., Deago, J., Cedeno, N., Ibarra, D., ..., & Ashton, P. (2007). Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama. *Forest Ecology and Management*, 243, 39-49.