

# Impact of phosphorus and luminosity in the propagation, photochemical reactions and quality of *Lippia alba* (Mill.) N.E.Br. seedlings

Impacto del fósforo y luminosidad en la propagación, reacciones fotoquímicas y calidad de plántulas de *Lippia alba* (Mill.) N.E.Br.



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**Propagation of *Lippia alba* by cutting.**

Photo: C.C. Santos

## ABSTRACT

*Lippia alba* (Mill.) N.E.Br. (lemongrass) is a spice and medicinal plant species, with fledgling studies related to mineral nutrition and luminosity on foliar and vegetative responses. Thus, the aim of this study was to assess the foliar aspects and production of *L. alba* under two light conditions (full sun and 50% shading) and four levels of phosphorus-P (0, 150, 300 and 450 mg kg<sup>-1</sup>). The *L. alba* seedlings presented changes in physiological indices and photochemical responses based on chlorophyll-*a* fluorescence. The phosphate fertilization helped mitigate the light stress for the synthesis of chlorophylls. The greatest leaf biomass production occurred with addition of P. The principal components analysis explained 74% of the variability, with leaf number, initial fluorescence and specific leaf area in principal component 1 (PC 1) and bud number, survival and leaf area in PC 2. Two groups formed in the cluster analysis, with lower distances between P300 full sun and P450 shading (2.31). The seedlings cultivated under full sun presented a higher survivability and seedling quality. The production of *L. alba* seedlings should be done under full sun with the addition of 450 mg kg<sup>-1</sup> of phosphorus.

**Additional key words:** acclimatization; chlorophyll-*a* fluorescence; medicinal plant; mineral nutrition.

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## RESUMEN

*Lippia alba* (Mill.) N.E.Br. (hierba de limón) es una especie de interés medicinal y aromática, con estudios incipientes en relación a la nutrición mineral y a la luminosidad en las respuestas foliares y vegetativas. El objetivo del trabajo fue evaluar los aspectos foliares y la producción de plántulas de *L. alba* bajo dos condiciones de luminosidad (pleno sol y 50% de sombreado) y cuatro niveles de fósforo (0, 150, 300 y 450 mg kg<sup>-1</sup>). Las plántulas presentaron alteraciones en los índices fisiológicos y respuestas fotoquímicas basadas en la fluorescencia de la clorofila-*a*. La fertilización fosfatada contribuyó en la mitigación del estrés luminoso en la síntesis de clorofilas. La mayor producción de biomasa de las hojas ocurrió con la adición de P. El análisis de componentes principales explicó el 74% de la variabilidad donde fueron características representativas el número de hojas, la fluorescencia inicial y el área foliar específica dentro del componente principal 1 (PC 1) y el número de brotes, supervivencia y el área foliar en el PC 2. En el análisis de conglomerados, se constató la formación de dos grupos, con distancias más bajas entre 300 pleno sol y 450 sombreado (2.31). Las plántulas cultivadas a pleno sol presentaron mayor capacidad de supervivencia y calidad. La producción de plántulas de *L. alba* debe ser realizada bajo pleno sol con adición de 450 mg kg<sup>-1</sup> del fósforo.

**Palabras clave adicionales:** aclimatización; fluorescencia de la clorofila-*a*; planta medicinal; nutrición mineral.

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## INTRODUCTION

*Lippia alba* (Mill.) N.E.Br. (lemongrass, Verbenaceae) is a plant of medicinal interest, and tea made from its leaves has a soothing effect (Hohlenwerger *et al.*, 2017). It can also be used as a natural preservative (Machado *et al.*, 2011). The essential oil from its leaves has shown an antifungal activity against *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma* (Glamočlija *et al.*, 2011). There are few studies on cultivation practices that must be established during the production of seedlings propagated with stem cuttings.

The abiotic factors that may influence the vegetative propagation of seedlings include light availability in the cultivation environment, which may affect the morphophysiological responses of plants since it acts directly in the process of photochemical and biochemical reactions of photosynthesis (Leal *et al.*, 2015).

However, light intensity may cause damage to the photosynthetic apparatus (Díez *et al.*, 2017; Taiz *et al.*, 2017) by changing the carboxylation speed of Ribulose 1.5 bisphosphate carboxylase/oxygenase, the assimilation of CO<sub>2</sub> and, consequently, the production of photoassimilates for seedling formation. The plants have foliar functional strategies based on chlorophyll-*a* fluorescence in photosystem II (PS II), enabling the efficient use of light (Jaimez *et al.*, 2018; Jiménez-Suanca *et al.*, 2015). Therefore, these

photochemical parameters may be used in assessing the integrity of photosynthetic machinery under eco-physiological disorders as a result of light stress.

In addition to light, another limiting factor for plants is soil in tropical regions, such as soil in Cerrado, which is highly weathered, has high levels of iron and aluminum oxides, and has high acidity (Souza *et al.*, 2013; Islas-Espinoza *et al.*, 2014), limiting the availability of some nutrients, particularly phosphorus (P). Greater attention is required for the low availability in soil in the tropics because of the greater fixation in soil (Quesada *et al.*, 2011; Gérard *et al.*, 2016).

Generally, P-deficient plants may suffer damage in diffusive and non-diffusive processes of photosynthetic metabolic pathways through a reduction in consumption and regeneration of Rubisco and production of ATP and NADPH (Andrade *et al.*, 2018), which may cause instability in the photochemical process in PS II. Thus, phosphate fertilization is an important agronomic practice since it may assist in photochemical stability (Carstensen *et al.*, 2018) and biomass increases in plants (Kuwahara *et al.*, 2016).

We tested the hypothesis that P may contribute to the production and quality of seedlings, mitigate damage and increase photochemical reactions in PS II under light stress. This study associated the mineral

nutrition and the ecophysiology of the plants in order to assess the photochemical and vegetative response and quality of *L. alba* seedlings produced with stem cuttings under different light availability and phosphate fertilization.

## MATERIAL AND METHODS

### Collection of plant material and preparation of cuttings

This species was identified and a voucher was deposited at the Herbarium DDMS of the Federal University of Grande Dourados (UFGD), under number 5226. The collection of plant material was performed from plant matrices in the Horto de Plantas Medicinais (22°11'43.7"S and 54°56'08.5"W, 452 m a.s.l.) of UFGD, in good phytosanitary conditions. The cuttings were removed from the median portion of the branches, standardized as 20 cm in length, mean diameter of 2.45 mm, and a pair of leaves at the apex; 1/3 of the cuttings was buried in the substrate.

### Studied Factors and experiment design

This experiment was performed from October to December of 2017 in the Faculty Agrarian Science (22°11'43.7"S e 54°56'08.5"W, 452 m a.s.l.) of UFGD in Dourados, Mato Grosso do Sul, Brazil. The climate of the region is classified as Am (Alvares *et al.*, 2013) with an annual average rainfall over 1,500 mm. The factors consisted of two light conditions (full sun and 50% shading) and four levels of phosphorus in the form of simple superphosphate - 18% of P<sub>2</sub>O<sub>5</sub> (0, 150, 300 and 450 mg kg<sup>-1</sup>). The treatments were displayed in a 2×4 factorial scheme, in randomized blocks, with four replicates. The experiment unit consisted of five 500 mL plastic containers, with one cutting each.

Shading was simulated using polypropylene black screen with 50% retention of light. The soil was classified as Dystroferic Red Latosol (Santos *et al.*, 2018), clay texture, with the following chemical attributes: pH CaCl<sub>2</sub> = 4.76; P = 0.5 mg dm<sup>-3</sup>; Ca = 1.04 cmol<sub>c</sub> dm<sup>-3</sup>; K = 0.06 cmol<sub>c</sub> dm<sup>-3</sup>; Mg = 0.12 cmol<sub>c</sub> dm<sup>-3</sup>; Al = 1.2 cmol<sub>c</sub> dm<sup>-3</sup>; H+Al = 7.71 cmol<sub>c</sub> dm<sup>-3</sup>; sum of bases = 1.22 cmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity = 8.93 cmol<sub>c</sub> dm<sup>-3</sup> and base saturation = 13.68. No soil correction was performed. Base fertilization was performed in coverage with ammonium sulphate (20% N)

and potassium chloride (60% K<sub>2</sub>O). The cultivation practices consisted of daily irrigation at 70% of the substrate's field capacity.

### Chlorophyll-a fluorescence

At 30 and 60 days after burial (DAB) of cutting, the emission of chlorophyll-*a* fluorescence was assessed by subjecting the leaves to darkness for 30 min, using leaf clips, between 8:00 and 10:00 am. The initial ( $F_o$ ) and maximum ( $F_m$ ) chlorophyll-*a* fluorescence and photochemical efficiency of photosystem II ( $F_v/F_m$ ) were measured under flash of 1,500 μmol m<sup>-2</sup> s<sup>-1</sup> with a portable fluorometer (OS-30P; Opti-Sciences Chlorophyll Fluorometer, Hudson, NY). The variables fluorescence ( $F_v = F_m - F_o$ ), efficiency of absorbed energy conversion ( $F_v/F_o$ ), non-photochemical maximum yield ( $F_o/F_v$ ) and electron transport rate (ETR) were estimated (Baker, 2008).

### Quantification of photosynthetic pigments

At 60 DAB, fully expanded leaves were collected, with 1 g macerated in 8 mL of acetone (80%). Afterwards, the samples were centrifuged for 10 min at 3000 rpm, and the absorbance reading was taken at the wavelengths of 470, 645 and 663 nm using a spectrophotometer. The contents of chlorophylls *a*, *b*, total (*a* + *b*) and carotenoids were estimated (Arnon, 1949; Lichtenthaler and Buschmann, 2001).

### Growth indicators

After 65 DAB, the survival, length of the aerial part (distance from the collet to the inflection of the highest leaf), collar diameter - CD (± 1.0 cm above the substrate level), leaf thickness, and number of buds and leaves were recorded. Seedlings were removed from the containers, washed and assessed for length of the largest root and rooted cuttings (emission of adventitious root of at least 5.0 cm). The leaf area was also assessed (LA) using an area integrator (area meter LI-COR 3100 C; Licor, Lincoln, NE).

### Biomass, physiological and quality indices

Fresh material from the aerial and root parts were submitted to forced air circulation in an oven at 60±5°C to obtain the dry mass. Using the data for the LA and leaves and total dry biomass (LDM and

TDM, respectively), the physiological indices of leaf area ratio ( $LAR = LA/LDM$ ), specific leaf area and mass ( $SLA = LA/TDM$  and  $SFM = LDM/TDM$ , respectively) were calculated (Benincasa, 2003). From the data for total dry biomass, height/diameter ratio (RHD) and aerial part/root ratio (APRR), the Dickson quality index (DQI) was estimated (Dickson *et al.*, 1960).

### Statistical analysis

The rooting and survival data were transformed into arcsine of  $\sqrt{x + 0.5}$  and subjected to normal distribution with the Shapiro-Wilk test for normalization. The data for chlorophyll-*a* fluorescence were assessed in plots subdivided in time. All data were subjected to analysis of variance (ANOVA), and, when significant according to the F test, the averages were compared with Student's *t* test for the luminosity and evaluation periods, along with regression analysis for phosphorus ( $P \leq 0.05$ ), using SISVAR.

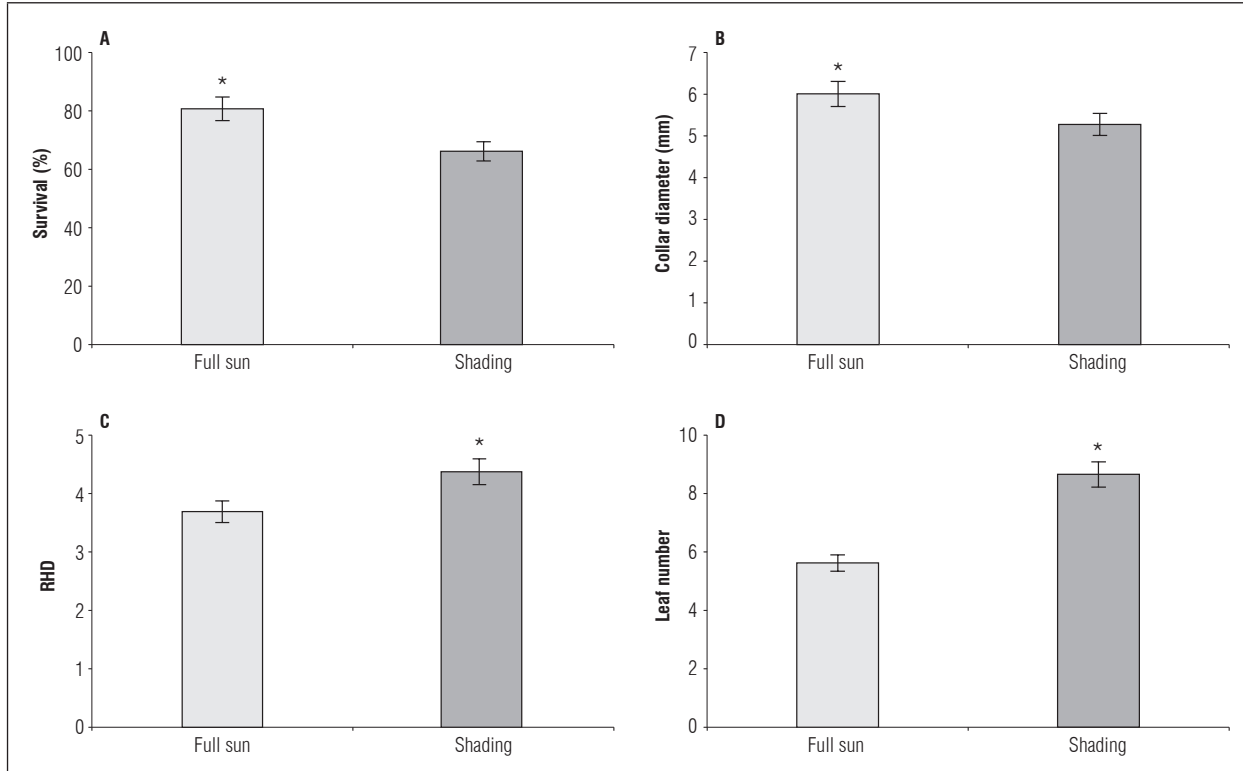
Additional multivariate analysis of principal components was carried out with variance and co-variance

arrays. A cluster analysis was also performed using the complete linkage method to describe the similarity between the factors, and the grouping was performed with the classical method using Euclidean distances.

### RESULTS

The greatest seedling survival and collet diameter were observed under full sun (Fig. 1A and B). The greatest height/diameter ratio (RHD) occurred in the shaded seedlings (Fig. 1C). The leaf number (LN) was influenced by the factors independently, with higher amounts under the shaded environment (Fig. 1D), without adjustment to phosphate fertilization (Tab. 1).

The characteristics height, rooting, length of the largest root, stem fresh and dry mass, root fresh mass and total mass were not influenced by the factors ( $P > 0.05$ ), with averages of 22.4 cm, 76%, 12.0 cm, 2.22, 1.04, 0.96 and 6.30 g/plant, respectively. The number of buds, chlorophyll *b* and fresh and dry mass of the leaves were influenced only by phosphorus, in



**Figure 1. Survival (A), collar diameter (B), height/diameter ratio – RHD (C) and leaf number – LN (D) in *L. alba* seedlings produced under light ambience. \* significant difference according to the Student's *t* test ( $P \leq 0.05$ ).**

**Table 1. Leaf and budding numbers, chlorophyll *b*, variable fluorescence, leaf fresh and dry mass in *L. alba* seedlings produced with different phosphorus doses.**

Characteristic	Equation	R <sup>2</sup>
Leaf number (LN)	$\hat{y}=\bar{y}= 7.18$ leaves/plant	W/adjust
Budding number (BN)	$\hat{y}=\bar{y}= 6.34$ budding/plant	W/adjust
Chlorophyll <i>b</i>	$\hat{y}=\bar{y}= 9.31$ $\mu\text{g cm}^2$	W/adjust
Variable fluorescence	$\hat{y} = 0.40832 + 0.00054*P - 0.00001*P^2$	0.98
Leaf fresh mass	$\hat{y} = 0.1564 + 0.0013*P - 0.00002*P^2$	0.98
Leaf dry mass	$\hat{y} = 0.14551 + 0.00079*P$	0.93

\* significant difference ( $P \leq 0.05$ ).

which the data for LN, NB and chlorophyll *b* had no adjustment to the mathematical models (Tab. 1) because of the low value of the determination coefficient ( $R^2$ ).

Chlorophyll *a* and total showed the same trend in the shaded environment, reducing the levels with increasing doses, with maximum levels (17.62 and 28.19  $\mu\text{g cm}^2$ ) and minimum levels (7.74 and 10.21  $\mu\text{g cm}^2$ ) without and with (450 mg kg<sup>-1</sup>) P, respectively (Tab. 2). On the other hand, under full sun, chlorophyll *a* and carotenoids had higher contents (9.96 and 1.84  $\mu\text{g cm}^2$ , respectively) with the addition of 450 mg kg<sup>-1</sup> of P.

Under the shaded environment, the data for  $F_v/F_m$  and  $F_o/F_v$  had no adjustment to the mathematical models (Tab. 2). Under full sun, the highest ratio was 0.750 and 0.338 electrons quantum<sup>-1</sup>, under 450 mg kg<sup>-1</sup> of P, respectively. The higher  $F_v/F_m$  values were observed at 60 DAB in full sun as a result of the lowest non-photochemical yield ( $F_o/F_v$ ) (Tab. 3). The greatest  $F_o$  occurred in the leaves of shaded seedlings (0.162 electron quantum<sup>-1</sup>) (Fig. 2A) and at 30 DAB (0.187 electrons quantum<sup>-1</sup>) (Fig. 2B).

The maximum  $F_v$ , 0.415 electron quantum<sup>-1</sup> was found with the addition of 27 mg kg<sup>-1</sup> of P (Tab. 1). The shaded seedlings had higher  $F_v$  and  $F_m$  values at 30 DAB, with a reduction of values at 60 DAB in both environments (Tab. 4). The ETR had lower values at 30 DAB (Tab. 4). The greatest ETR occurred in the leaves of seedlings cultivated under full sun at 60 DAB as a result of the higher incident light.

In the shaded environment, the largest LA was 16.43 cm<sup>2</sup> under 450 mg kg<sup>-1</sup> of P. Under full sun, the maximum leaf area (11.85 cm<sup>2</sup>) occurred in seedlings cultivated with 228.31 mg kg<sup>-1</sup> of P (Tab. 5). The leaf

**Table 2. Chlorophyll *a*, total chlorophyll, carotenoids, photochemical efficiency of the photosystem II ( $F_v/F_m$ ), maximum non-photochemical yield ( $F_o/F_v$ ) and efficiency of absorbed energy conversion ( $F_v/F_o$ ) in *L. alba* seedlings produced with phosphorus under light environment.**

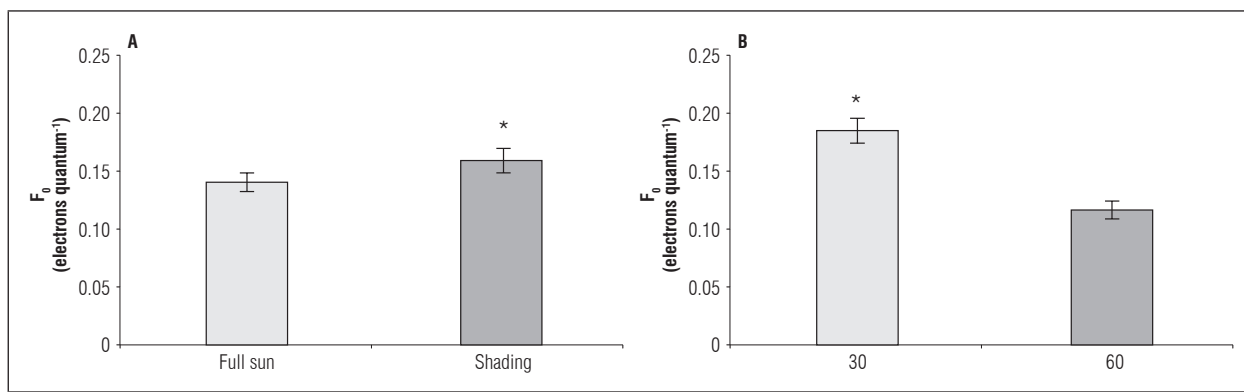
Chlorophyll <i>a</i> ( $\mu\text{g cm}^2$ )	
Full sun	Shading
$\hat{y} = 5.57350 + 0.00975*P$ $R^2 = 0.56$	$\hat{y} = 17.64521 - 0.02201*P$ $R^2 = 0.90$
Total chlorophyll ( <i>a</i> + <i>b</i> ) ( $\mu\text{g cm}^2$ )	
Full sun	Shading
$\hat{y} = \bar{y} = 16.73$ W/adjust	$\hat{y} = 33.33526 - 0.05138*P$ $R^2 = 0.89$
Carotenoids ( $\mu\text{g cm}^2$ )	
Full sun	Shading
$\hat{y} = 0.58725 + 0.00279*P$ $R^2 = 0.75$	$\hat{y} = \bar{y} = 1.51$ W/adjust
Photochemical efficiency of the photosystem II ( $F_v/F_m$ )	
Full sun	Shading
$\hat{y} = 0.71932 + 0.00007*P$ $R^2 = 0.57$	$\hat{y} = \bar{y} = 0.749$ W/adjust
$F_o/F_v$ (electrons quantum <sup>-1</sup> )	
Full sun	Shading
$\hat{y} = 0.39187 - 0.00013*P$ $R^2 = 0.50$	$\hat{y} = \bar{y} = 0.337$ W/adjust
$F_v/F_o$ (electrons quantum <sup>-1</sup> )	
Full sun	Shading
30 DAB	
$\hat{y} = 2.0480 + 0.00192*P$ $R^2 = 0.80$	$\hat{y} = \bar{y} = 2.958$ W/adjust
60 DAB	
$\hat{y} = \bar{y} = 3.642$ W/adjust	$\hat{y} = \bar{y} = 3.261$ W/adjust

\* significant difference ( $P \leq 0.05$ ).

**Table 3. Photochemical efficiency of the photosystem II ( $F_v/F_m$ ) and maximum non-photochemical yield ( $F_0/F_v$ ) in *L. alba* seedlings produced with phosphorus under light environment, at 30 and 60 days after burial (DAB).**

Environment	$F_v/F_m$		$F_0/F_v$	
	(electrons quantum <sup>-1</sup> )			
	30 DAB	60 DAB	30 DAB	60 DAB
Full sun	0.699 aB	0.774 aA	0.438 aA	0.284 bA
Shading	0.740 aA	0.759 aA	0.353 aB	0.312 aA
C.V. (%)	7.26		11.37	

Means followed by the same lower case letter in the rows, for day after burial, and upper case in the columns, for light environment, do not differ according to the Student's *t* test ( $P \leq 0.05$ ).

**Figure 2. Initial fluorescence –  $F_0$  in leaves of *L. alba* seedlings produced under phosphorus rates and light environment (a), at 30 and 60 days after burial (b). \*significant difference according to the Student's *t* test ( $P \leq 0.05$ ).****Table 4. Maximum ( $F_m$ ) and variable ( $F_v$ ) fluorescence and electron transport rate (ETR) in leaves of *L. alba* seedlings produced with phosphorus under light environment at 30 and 60 days after burial (DAB).**

Environment	$F_m$		$F_v$		ETR	
	(electrons quantum <sup>-1</sup> )					
	30 DAB	60 DAB	30 DAB	60 DAB	30 DAB	60 DAB
Full sun	0.611 aB	0.503 bA	0.433 aB	0.395 aA	248.38 bA	275.29 aA
Shading	0.768 aA	0.543 bA	0.572 aA	0.415 bA	107.94 aB	110.78 aB
C.V. (%)	8.33		10.22		6.12	

Means followed by different lower case letters in a row, for day after burial, and upper case letters in the columns, for light environment, indicate significant differences according to the Student's *t* test ( $P \leq 0.05$ ).

thickness data were not adjusted, and the sun leaves had higher values. The data for root dry mass and APRR of the seedlings under full sun were not adjusted to the mathematical models, but, under the shading, the higher mean values were 0.462 g/plant and

0.44 with 450 mg kg<sup>-1</sup> of P. The fresh and dry biomass of the leaves were influenced only by phosphorus, with higher values (0.177 and 0.0501 g/plant) with the addition of 32.5 and 450 mg kg<sup>-1</sup> of P, respectively (Tab. 1).



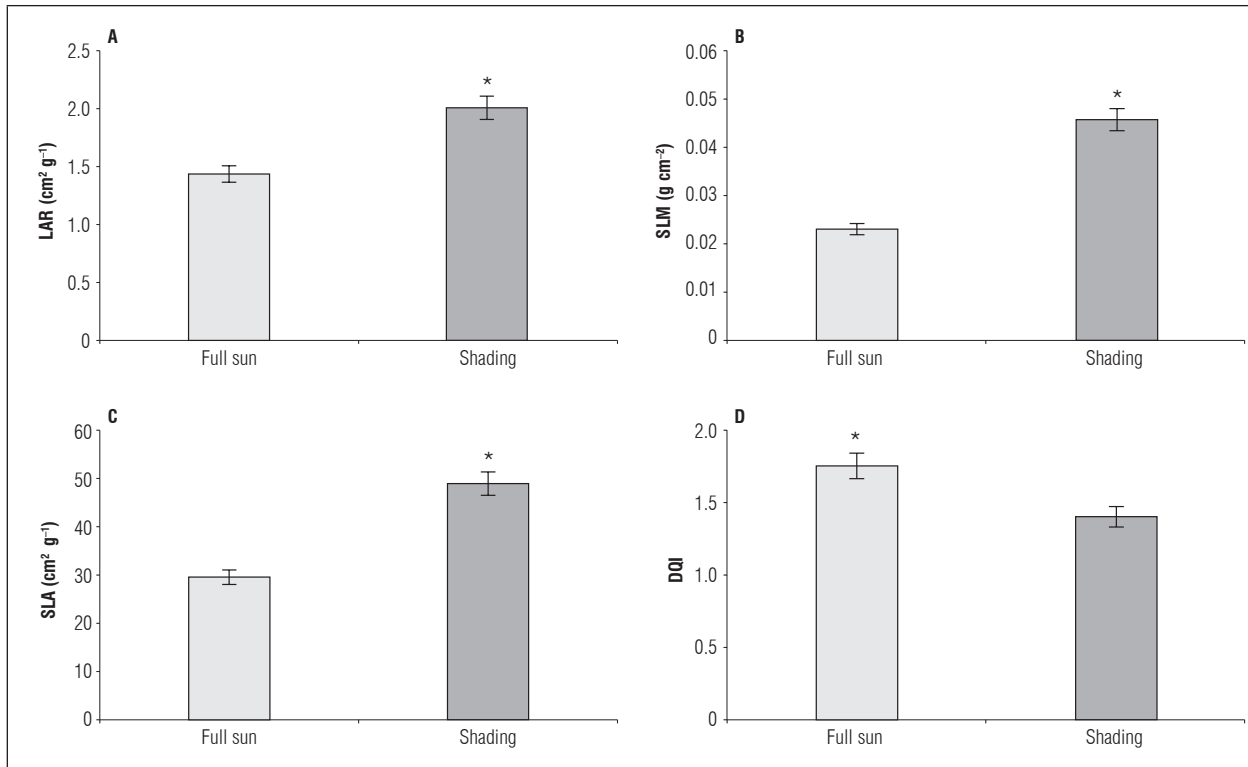
**Table 5. Leaf area, thickness, root dry mass and aerial part/root ratio in *L. alba* seedlings produced with phosphorus under light environment.**

Leaf area – LA (cm <sup>2</sup> per plant)	
Full sun	Shading
$\hat{y} = 7.68317 + 0.03653 * P - 0.00008 * P^2$ $R^2 = 0.86$	$\hat{y} = 8.50657 + 0.01762 * P$ $R^2 = 0.72$
Leaf thickness – LT (mm)	
Full sun	Shading
$\hat{y} = \bar{y} = 0.33$ W/adjust	$\hat{y} = \bar{y} = 0.26$ W/adjust
Root dry mass – RDM (g per plant)	
Full sun	Shading
$\hat{y} = \bar{y} = 0.537$ W/adjust	$\hat{y} = 0.21012 + 0.00056 * P$ $R^2 = 0.60$
Aerial part/root ratio – APRR	
Full sun	Shading
$\hat{y} = \bar{y} = 0.093$ W/adjust	$\hat{y} = 0.03605 + 0.0009 * P$ $R^2 = 0.63$

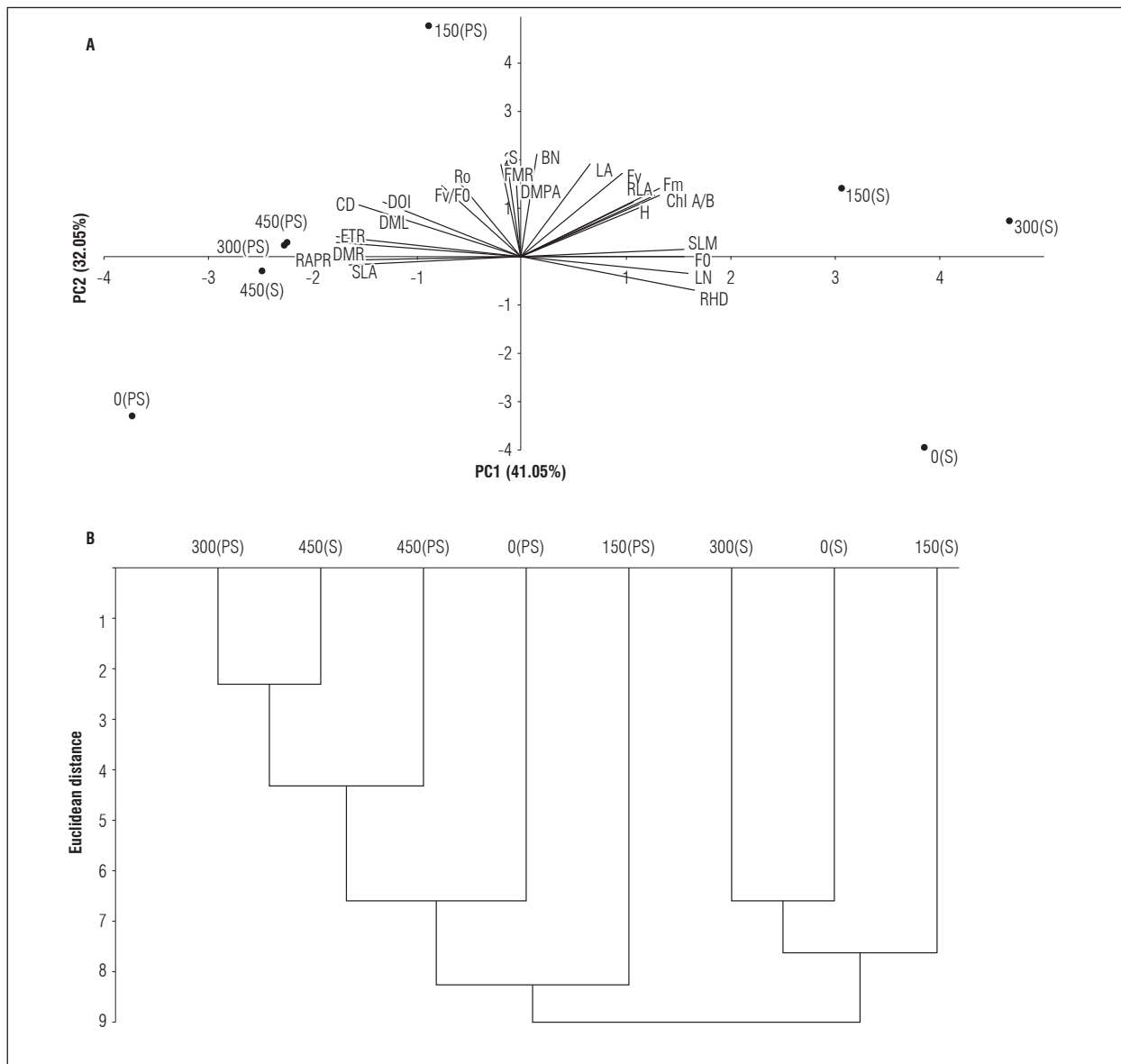
\* significant regression ( $P \leq 0.05$ )

The greatest LAR (2.027 cm<sup>2</sup> g<sup>-1</sup>) and SLM (0.0458 g cm<sup>-2</sup>) occurred in the shaded seedlings (Fig. 3A and B) as a result of the greatest leaf thickness (Tab. 5). The greatest SLA was 48.54 cm<sup>2</sup> g<sup>-1</sup> in the seedlings produced in the shaded environment (Fig. 3C). For the DQI, the greatest value (1.70) was observed in the seedlings cultivated under full sun (Fig. 3D), regardless of phosphorus.

The principal components analysis explained 74% of the variability, in which PC1 and PC2 contributed 41.05% and 32.05%, respectively, of the remaining variance of the characteristics in the *L. alba* seedlings (Fig. 4A). In the cluster analysis, two groups were formed for the P dose (G1 and G2), with six subgroups (Fig. 4B). The subgroups with lower distances between P dose and light environment (S - shading and PS - full sun) were 300 PS and 450 S (2.31), followed by 450 S and 450 PS (4.61) and 300 S and 0 S (6.65).



**Figure 3. Leaf area ratio – LAR (A), specific leaf mass – SLM (B), specific leaf area – SLA (C) and Dickson quality index – DQI (D) in *L. alba* seedlings under light environment at 65 days after burial. \*significant difference according to the Student's *t* test ( $P \leq 0.05$ ).**



**Figure 4. Principal components analysis (A) and dendrogram of similarity, based on Euclidean distances (B) of characteristics evaluated in the *L. alba* seedlings produced with phosphorus doses (0, 150, 300 and 450 mg kg<sup>-1</sup>) and light environment (S - shading and PS - full sun).**

## DISCUSSION

The greatest survival was associated with the amount of photoassimilates as a function of greater incident light. Seedlings under excessive light lose more water through transpiration because of an increased temperature, with the greater diameter allowing a greater ability to transport water and photoassimilates to other organs in order to maintain turgidity (Taiz *et al.*, 2017). The greatest RHD occurred in the shaded seedlings, but the values did not indicate that

there was etiolation in the seedlings under limited light.

Generally, plants under shading tend to expand the number of leaf limbs, enabling greater light interception (Oliveira *et al.*, 2017) and maximizing photosynthesis. These characteristics are important in the vegetative propagation of the species since the greater the amount of buds, the greater the formation of leaves, contributing to photoassimilate distribution to developing organs.



The reduction of pigments, except carotenoids, in leaves under full sun can be explained by photoinhibition of chlorophylls as a result of excessive light (Fu *et al.*, 2012). However, shaded leaves tend to increase the content of chlorophyll and, thereby, maximize the photosynthetic capacity (Díez *et al.*, 2017). Similar results were observed by Soares *et al.* (2017) when assessing young *Tamarindus indica* L. plants under shading, which presented higher concentrations of chlorophylls.

An increase in chlorophyll content is desirable because it is responsible for capturing light photons and transmitting energy to reaction centers (Monteiro *et al.*, 2018), and carotenoids are responsible for chlorophyll photoprotection and reduction of membrane harm (Taiz *et al.*, 2017), maximizing the photosynthetic capacity. Under full sun cultivation, phosphate fertilization can be a potential mitigation strategy for ecophysiological disorders from light stress in the synthesis of chlorophylls.

P, in the form of Pi, is responsible for the control of enzymatic reactions and metabolic regulation in the cytosol and chloroplast (Hawkesford *et al.*, 2012). Higher photochemical indicators, especially the  $F_v/F_m$  ratio, with phosphorus result because P favors the speed of ATP synthesis, contributing to the export of protons to the chloroplast stroma and thylakoid lumen (Carstensen *et al.*, 2018), favoring the maintenance of electron mobility in PS II. Increasing  $F_v/F_v$  can be a photoprotective mechanism of the photosynthetic apparatus for excessive incident radiation (Blind *et al.*, 2018), i.e. a greater use of electrons produced as a result of higher efficiency in absorbed energy conversion ( $F_v/F_0$ ), regardless of the shading level during this period (60 DAB) (Tab. 2).

The higher  $F_0$  at 30 DAB is desirable in order to mitigate damage in the photosynthetic apparatus (Fig. 2B). Fu *et al.* (2012) described a higher  $F_0$  in *Lactuca sativa* L. plants cultivated under  $100 \mu\text{mol m}^{-2}\text{s}^{-1}$  (low irradiance) at the initial stage of the experiment, stating that this mechanism can mitigate an increase in reactive oxygen and D1 protein degradation. As for phosphorus in  $F_v$ , P is involved in phosphorylation reactions and pyrophosphate release, acting in the activation of catalytic enzymes, forming ATP (Hawkesford *et al.*, 2012, Xing and Wu 2014; Andrade *et al.*, 2018) and favoring photochemical stability because  $F_v$  is related to the ability to transfer electrons.

The results for  $F_m$  and ETR is important in the photochemical process since larger values favor the flow of chlorophyll molecules between acceptors in photosystems (Farias *et al.*, 2016). The higher photochemical efficiency in PS II effectively contributes to maintaining the integrity of the photosynthetic apparatus and increasing vegetative characteristics.

Plants exposed to low luminosity enhance the expansion of leaves (Gondim *et al.*, 2018) as a strategy for use of light. Increasing APRR and RDM may be associated with the fact that under high irradiance, substrate and leaves tend to lose more water through evapotranspiration and leaf transpiration. Thus, an increase in these characteristics under full sun promotes water absorption for the maintenance of metabolic processes and nutrient transport (Sarto *et al.*, 2018).

The responses of plants to abiotic variants can change according to the species. Oliveira *et al.* (2017), when assessing the physiological and productive aspects of *Origanum vulgare* L. plants, found greater root biomass under shaded cultivation. On the other hand, there was greater biomass allocation in *Physalis minima* L. seedlings under full sun (Silva *et al.* 2016), similar to *L. alba* seedlings (Fig. 3).

P plays an important role in plant biomass allocation and is a structural component of nucleic acids, phospholipids and ATP formation, favoring primary metabolism reactions and constituting  $\sim 0.2\%$  of plant mass (Kuwahara *et al.*, 2016). For seedling production in *Dalbergia nigra* Benth., it was found that the addition of  $500 \text{ mg kg}^{-1}$  of P favored an increase in biomass (Carlos *et al.*, 2018), similar to the *L. alba* seedlings in this study (Tab. 1).

LAR and SLM show greater biomass allocation through leaf area with greater thickness (Oliveira *et al.*, 2016). These authors found that *Melissa officinalis* L. plants, at 120 d after transplanting, showed a greater SLM when cultivated under full sun. For LAR, Ribeiro *et al.* (2018) found higher values in *Pogostemon cablin* cultivated under shaded conditions. The increase in SLA under this condition indicated the adaptive ability of leaf tissues in optimizing light capture (Guzmán *et al.*, 2016; Liu *et al.*, 2016) because it promotes  $\text{CO}_2$  assimilation and stomatal control (Gommers *et al.*, 2013). However, leaves with a smaller thickness are less heavy. Similar results were observed in *Enterolobium contortisiliquum* (Vell.) Morong

(Souza *et al.*, 2017) and *Colocasia esculenta* L. Schott (Gondim *et al.*, 2018). Both authors described higher values in plants under artificial shading.

The DQI demonstrated that this species has survival and stability capacity when exposed to high irradiance, an important fact since the initial cultivation under this condition reduced the acclimatization period (rustification) of the seedlings, i.e. these will be less susceptible to weather under field conditions, such as excessive sunlight. The use of DQI has been constant when assessing seedling quality since it is an easy implementation analysis, performed by calculating the morphometric stability level, distribution and biomass allocation.

In PC 1, the characteristics that showed positive scores in descending order were RHD (0.271), LN (0.263),  $F_o$  and SFM (0.255), and the characteristics with negative scores were APRR (-0.292), ETR and RDM (-0.288) and SLA (-0.270), which were the most similar. In PC 2, the components that most contributed with positive factorial scores were the number of buds (0.352), survival (0.330), leaf area (0.319) and  $F_v$  (0.289), with no negative absolute scores > 0.30. The cluster analysis consisted of sample classification in order to verify the similarity within the groups and between-group heterogeneity, considering all evaluated characteristics (Araújo *et al.*, 2013). Thus, there was greater heterogeneity between the luminous environments, with G1 comprising the association of P with shading, except for 450 S and G2 under full sun.

## CONCLUSION

The *Lippia alba* seedlings responded positively to the phosphate fertilization when vegetative propagation was used with stem cuttings. The association of 450 mg of phosphorus with the cultivation under full sun contributed substantially to mitigating ecophysiological disorders in the photosynthetic apparatus as the result of light stress, providing greater photochemical stability, survival and quality in the *Lippia alba* seedlings. No acclimatization process was required.

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## BIBLIOGRAPHIC REFERENCES

- Alvares, C.A., J.L. Stape, P.C. Sentelhas, J.L. de Moraes Gonçalves, and J.L. Sparovek. 2013. Köppen's climate classification map for Brazil. *Meteorol. Z.* 22(6), 711-728. Doi: 10.1127/0941-2948/2013/0507
- Andrade, F.H.A., W.E. Pereira, R.R. Morais, A.F. Silva, and M.A. Barbosa Neto. 2018. Effect of phosphorus application on substrate and use of saline water in sugar-apple seedlings. *Pesqui. Agropecu. Trop.* 48(2), 190-199. Doi: 10.1590/1983-40632018v48s2035
- Araújo, E.C., M.A. Uribe-Opazo, and J.A. Johann. 2013. Análise de agrupamento da variabilidade espacial da produtividade da soja e variáveis agrometeorológicas na região oeste do Paraná. *Eng. Agríc.* 33(4), 782-795. Doi: 10.1590/S0100-69162013000400018
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24(1), 1-15. Doi: 10.1104/pp.24.1.1
- Baker, B. 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Ann. Rev. Plant Biol.* 59, 8-113. Doi: 10.1146/annurev.arplant.59.032607.092759
- Benincasa, M.M.P. 2003. Análise do crescimento de plantas (noções básicas). FUNEP, Jaboticabal, Brazil.
- Blind, M.R., K.C.P. Costa, C.E.M. Silva, P.T.B. Sampaio, and J.F.C. Gonçalves. 2018. Fotossíntese de espécies Anibae em resposta à exposição a ambientes contrastantes de luz. *Rodriguésia* 69(2), 397-407. Doi: 10.1590/2175-7860201869211
- Carlos, L., N. Venturin, R.P. Venturin, J.M. Alves, and P.R. Silva. 2018. Liming and phosphating in *Dalbergia nigra* (Vell.) Allemão ex Benth. seedlings. *Floresta Ambient.* 25(4), e20170239. Doi: 10.1590/2179-8087.023917
- Carstensen, A., A. Herdean, S.B. Schmidt, A. Sharma, C. Spetea, M. Pribil, and S. Husted. 2018. The impacts of phosphorus deficiency on the photosynthetic electron transport chain. *Plant Physiol.* 3, 1-38. Doi: 10.1104/pp.17.01624
- Dickson, A., A.L. Leaf, and J.F. Hosner. 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. *Forest. Chron.* 36(1), 10-13. Doi: 10.5558/tfc36010-1

- Díez, M.C., F. Moreno, and E. Gantiva. 2017. Effects of light intensity on the morphology and CAM photosynthesis of *Vanilla planifolia* Andrews. *Rev. Fac. Nac. Agron. Medellín* 70(1), 8023-8033. Doi: 10.15446/rfna.v70n1.61736
- Farias, M.E., E.G. Martinazzo, and M.A. Bacarin. 2016. Chlorophyll fluorescence in the evaluation of photosynthetic electron transport chain inhibitors in the pea. *Rev. Ciênc. Agron.* 47(1), 178-186. Doi: 10.5935/1806-6690.20160021
- Fu, W., P. Li, and Y. Wu. 2012. Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. *Sci. Hortic.* 135, 45-51. Doi: 10.1016/j.scienta.2011.12.004
- Gérard, F. 2016. Clay minerals, iron/aluminum oxides, and their contribution to phosphate sorption in soils - a myth revisited. *Geoderma* 262, 213-226. Doi: 10.1016/j.geoderma.2015.08.036
- Glamočlija, J., M. Soković, V. Tešević, G.A. Linde, and N.B. Colauto. 2011. Chemical characterization of *Lippia alba* essential oil: an alternative to control green molds. *Braz. J. Microbiol.* 42(4), 1537-1546. Doi: 10.1590/S1517-83822011000400041
- Gommers, C.M.M., E.J.W. Visser, K.R.S. Onge, L.A.C.J. Voesenek, and R. Pierik. 2013. Shade tolerance: when growing tall is not an option. *Trends Plant Sci.* 18(2), 65-71. Doi: 10.1016/j.tplants.2012.09.008
- Gondim, A.R.O., M. Puiatti, F.L. Finger, and P.R. Cecon. 2018. Artificial shading promotes growth of taro plants. *Pesqui. Agropecu. Trop.* 48(2), 83-89. Doi: 10.1590/1983-40632018v48i2.1355
- Guzmán, J.A., R.A. Cordeiro, and E. Corea. 2016. Biomass allocation and gas exchange are affected by light conditions in endangered *Cedrela salvadorensis* (Meliaceae) seedlings. *Rev. Biol. Trop.* 64(3), 1143-1154. Doi: 10.15517/rbt.v64i3.19606
- Hawkesford, M., W. Horst, T. Kichey, H. Lambers, J. Schjoerring, I.S. Møller, and P. White. 2012. Functions of macronutrients. pp. 135-189. In: Marschner, P. (ed.). *Marschner's mineral nutrition of higher plants*. 3<sup>rd</sup> ed. Elsevier; Academic Press, Amsterdam. Doi: 10.1016/B978-0-12-384905-2.00006-6
- Hohlenwerger, J.C., B. Baldissotto, R.D. Couto, B.M. Heinzmann, D.T. Silva, B.O. Caron, D. Schmidt, and C.E. Copatti. 2017. Essential oil of *Lippia alba* in the transport of Nile tilapia. *Cienc. Rural.* 47(3), e20160040. Doi: 10.1590/0103-8478cr20160040
- Islas-Espinoza, M., L. Solís-Mejía, and M.V. Esteller. 2014. Phosphorus release kinetics in a soil amended with biosolids and vermicomposto. *Environ. Earth Sci.* 71(3), 1441-1451. Doi: 10.1007/s12665-013-2549-y
- Jaimez, R.E., F.P. Amores, A. Vasco, R.G. Loor, O. Tarqui, G. Quijano, J.C. Jimenez, and W. Teraza. 2018. Photosynthetic response to low and high light of cacao growing without shade in an area of low evaporative demand. *Acta Biol. Colomb.* 23(1), 95-103. Doi: 10.15446/abc.v23n1.64962
- Jiménez-Suanca, S.C., O.H. Alvarado, and H.E. Balaguera-López. 2015. Fluorescencia como indicador de estrés en *Helianthus annuus* L. Una revisión. *Rev. Colomb. Cienc. Hortíc.* 9(1), 149-160. Doi: 10.17584/rcch.2015v9i1.3753
- Kuwahara, F.A., G.M. Souza, K.A. Guidorizi, C. Costa, and P.R.L. Meirelles. 2016. Phosphorus as a mitigador o the effects of water stress on the growth and photosynthetic capacity of tropical C4 grasses. *Acta Sci. Agro.* 38(3), 363-370. Doi: 10.4025/actasciagron.v38i3.28454
- Leal, C.C.P., S.B. Torres, R.M.O. Freitas, N.W. Nogueira, and R.M. Farias. 2015. Light intensity and type of container on producing *Cassia grandis* L. f. seedlings. *Rev. Bras. Eng. Agríc. Ambient.* 19(10), 939-945. Doi: 10.1590/1807-1929/agriambi.v19n10p939-945
- Lichtenthaler, H.K. and C. Buschmann. 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Curr. Protoc. Food Anal. Chem.* 1(Supl. 1), F4.3.1-F4.3.8. Doi: 10.1002/0471142913.faf0403s01
- Liu, Y., W. Dawson, D. Prati, E. Haeuser, Y. Feng, and M. Kleunen. 2016. Does greater specific leaf area plasticity help plants to maintain a high performance when shaded? *Ann. Bot.* 118(7), 1329-1336. Doi: 10.1093/aob/mcw180
- Machado, T.F., M.F. Borges, and L.M. Bruno. 2011. Aplicação de antimicrobianos naturais na conservação de alimentos. *Empresa Agroindústria Tropical*, Fortaleza, Brazil.
- Monteiro, D.R., H.F. Melo, C.M.T. Lins, P.R.M. Dourado, H.R.B. Santos, and E.R. Souza. 2018. Chlorophyll a fluorescence in saccharine Sorghum irrigated with saline water. *Rev. Bras. Eng. Agríc. Ambient.* 22(10), 673-678. Doi: 10.1590/1807-1929/agriambi.v22n10p673-678
- Oliveira, V.C., A.R. Santos, G.S. Souza, and R.M. Santos. 2017. Respostas fisiológicas de plantas de orégano (*Origanum vulgare* L.) cultivadas sob malhas coloridas e fertilizantes orgânicos. *Rev. Colomb. Cienc. Hortíc.* 11(2), 400-407. Doi: 10.17584/rcch.2017v11i2.7591
- Oliveira, G.C., W.L. Vieira, S.C. Bertolli, and A.C. Pacheco. 2016. Photosynthetic behavior, growth and essential oil production of *Melissa officinalis* L. cultivated under colored shade net. *Chilean J. Agric. Res.* 76(1), 123-128. Doi: 10.4067/S0718-58392016000100017
- Quesada, C.A., J. Lloyd, L.O. Anderson, N.M. Fyllas, M. Schwarz, and C.I. Czimczik. 2011. Soils of Amazonia with particular reference to the RAINFOR sites. *Biogeosciences* 8(6), 11415-11440. Doi: 10.5194/bg-8-1415-2011

- Ribeiro, A.S.R., M.S. Ribeiro, S.K.V. Bertolucci, W.J.M. Bittencourt, A.A. Carvalho, W.N. Tostes, E. Alves, and J.E.B.P. Pinto. 2018. Colored shade nets induced changes in growth, anatomy and essential oil of *Pogostemon cablin*. *An. Acad. Bras. Ciênc.* 90(2), 1823-1835. Doi: 10.1590/0001-3765201820170299
- Santos, H.G., P.K.T. Jacomine, L.H.C. Anjos, V.A. Oliveira, J.F. Lumbreras, M.R. Coelho, J.A. Almeida, J.C. Araújo Filho, J.B. Oliveira, and T.J.F. Cunha. 2018. Sistema brasileiro de classificação de solos. 5<sup>th</sup> ed. Embrapa Solos, Rio de Janeiro, Brazil.
- Sarto, M.V.M., D. Bessegio, C.A. Rosolem, and J.R.W. Sarto. 2018. Safflower root and shoot growth affected by soil compaction. *Bragantia* 77(2), 348-355. Doi: 10.1590/1678-4499.2017191
- Silva, D.F., R. Pio, J.D.R. Soares, P.V. Nogueira, P.M. Peche, and F. Villa. 2016. The production of *Physalis* spp. seedlings grown under different-colored shade nets. *Acta Sci. Agron.* 38(2), 257-263. Doi: 10.4025/actasciagron.v38i2.27893
- Soares, J.D.R., G.M.G. Dias, R.A.L. Silva, M. Pasqual, C.R.G. Labory, S.A. Asmar, and J.D. Ramos. 2017. Photosynthetic pigments content and chloroplast characteristics of tamarind leaves in response to different colored shading nets. *Aust. J. Crop. Sci.* 11(3), 296-299. Doi: 10.21475/ajcs.17.11.03.p7906
- Souza, J.P., N.M.J. Melo, A.D. Halfeld, and J.N. Reis. 2017. Shading effects on leaf life span and functional traits in the widespread species *Enterolobium contortisiliquum* (Vell.) Morong. *Acta Sci. Biol. Sci.* 39(1), 113-122. Doi: 10.4025/actascibiols.v39i1.33400
- Souza, N.H., M.E. Marchetti, T.O. Carnevali, D.D. Ramos, S.P.Q. Scalón, and E.F. Silva. 2013. Estudo nutricional de canafístula (I): crescimento e qualidade de mudas em resposta a adubação com nitrogênio e fósforo. *Rev. Árvore.* 37(4), 717-724. Doi: 10.1590/S0100-67622013000400015
- Taiz, L., E. Zeiger, I. Møller, and A. Murphy. 2017. Fisiologia e desenvolvimento vegetal. 6<sup>th</sup> ed. Artmed, Porto Alegre, Brazil.
- Xing, D. and Y. Wu. 2014. Effect of phosphorus deficiency on photosynthetic inorganic carbon assimilation of three climber plant species. *Bot. Stud.* 55, 60. Doi: 10.1186/s40529-014-0060-8