Morphophysiological aspects of *Erythroxylum pauferrense* Plowman seedlings submitted to shading

Aspectos morfofisiológicos de mudas de *Erythroxylum pauferrense* Plowman submetidas ao sombreamento

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ABSTRACT

Sunlight directly influences on the development of forest species by affecting the attributes of plant growth and physiology. Among these species, *Erythroxylum pauferrense* Plowman is considered a rare species, as its distribution is restricted only to the state of Paraíba. Therefore, studies are needed that seek to evaluate the adaptation, development and propagation of the species under different environmental conditions. This study aimed to evaluate morphophysiological aspects of *Erythroxylum pauferrense* seedlings submitted to different levels of shading. The experiment was carried out under greenhouse condition in a completely randomized design with five treatments (0, 30, 50, 70, and 90% shading) and eight replicates. Growth characteristics, morphofunctional attributes, gas exchange, chlorophyll (Chl) \( a \) fluorescence, and Chl content were evaluated. Data were submitted to analysis of variance followed by polynomial regression analysis. Results showed that plants grown under 30 to 50% shading showed higher plant height, stem diameter, absolute growth rate for plant height, leaf dry mass, stem dry mass, total dry mass, Dickson’s quality index, leaf area, and leaf area index, as well as net assimilation rate of CO\(_2\), stomatal conductance, transpiration, instantaneous water use efficiency, and Chl \( a, b \), and \( a+b \) contents. Therefore, levels from 30 to 50% shading are the most recommended for the production of *Erythroxylum pauferrense* seedlings. We recommend using *Erythroxylum pauferrense* to reforest the understory in disturbed areas in Brejos de Altitude and large-scale production of seedlings under intermediate levels of shading.

Keywords: Abiotic stress; Erythroxylaceae; Guarda-orvalho; Irradiance
RESUMO

A luz solar influencia diretamente no desenvolvimento das espécies florestais, afetando os atributos de crescimento e fisiologia das plantas. Dentre essas espécies, Erythroxylum pauferrense Plowman é considerada uma espécie rara, pois sua distribuição é restrita apenas ao estado da Paraíba. Diante disso, são necessários estudos que busquem avaliar a adaptação, desenvolvimento e propagação da espécie em diferentes condições ambientais. Este trabalho teve como objetivo avaliar os aspectos morfofisiológicos de mudas de Erythroxylum pauferrense submetidas a diferentes níveis de sombreamento. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado, com cinco tratamentos (0, 30, 50, 70 e 90% de sombreamento) e oito repetições. Foram avaliadas as características de crescimento, atributos morfofuncionais, trocas gásicas, fluorescência da clorofila (Chl) α e teor de Chl. Os dados foram submetidos à análise de variância seguida de análise de regressão polinomial. Os resultados mostraram que as plantas cultivadas sob 30 a 50% de sombreamento apresentaram maior altura da planta, diâmetro do caule, taxa de crescimento absoluto para altura da planta, massa seca da folha, massa seca do caule, massa seca total, índice de qualidade de Dickson, área foliar e índice de área foliar, bem como a taxa de assimilação líquida de CO₂, condutância estomática, transpiração, eficiência instantânea do uso da água e conteúdo de Chl α, b e α+b. Portanto, níveis de 30 a 50% de sombreamento são os mais recomendados para a produção de mudas de Erythroxylum pauferrense. Recomenda-se o uso de Erythroxylum pauferrense para reflorestar o sub-bosque em áreas perturbadas nos Brejos de Altitude e a produção em larga escala de mudas em níveis de 30 a 50% de sombreamento.

Palavras-chave: Estresse abiótico; Erythroxylaceae; Guarda-orvalho; Irradiância

1 INTRODUCTION

Erythroxylum pauferrense Plowman is a shrub species belonging to the Erythroxylaceae family. Endemic to the Northeast region of Brazil, where it is popularly known as “guarda-orvalho”, the species is restricted to Paraíba state, in the municipality of Areia. Currently, the species is listed on the Lista Vermelha da Flora do Brasil as an endangered species (“EN” category) (CENTRO NACIONAL DE CONSERVAÇÃO DA FLORA, 2021). This is a consequence of its habitat destruction caused by deforestation that has reached small subpopulations and declined the number of adult individuals, which thus have turned it a rare species. Occurring within isolated fragments of humid forests, the “Brejos de Altitude” located in forest remnants of the Atlantic Forest, Erythroxylum pauferrense has great ecological importance, mainly for the conservation
of the region's genetic resources. In addition, its fruits are a food source for the local fauna that acts in the dispersion of seeds in disturbed areas, such as the Brejos de Altitude (RIBEIRO et al., 2019).

Understory forest species are influenced by several abiotic factors, such as light, temperature, and water availability. A deviation and fluctuation in one of these factors can impair the vigor, growth, and development of the plants (KOCHHAR; GUJRAL, 2020). Luminosity is one of the main factors interfering in plant development by affecting most of the physiological and morphological processes. Sunlight intensity significantly regulates primary production, affecting plant height, leaf area, chloroplast structure, and the content of chlorophyll $a$ and $b$ (GRIME, 2020). In addition, changes in irradiance levels directly affect photosynthesis and efficiency of energy absorption and transfer in the photosynthetic apparatus, it may change the plant gas exchange response, such as the net assimilation rate of $\text{CO}_2$, transpiration, and stomatal opening (RIBEIRO et al., 2020a).

Sunlight is not only a source of energy for photosynthesis, it also promotes signals responsible for regulating plant development through receptors sensitive to the quality, intensity, and polarization of light (ALBUQUERQUE; EVANGELISTA; ALBUQUERQUE NETO, 2015). According to Perrella et al. (2020), plants adapted to different conditions of luminosity can quickly and efficiently adjust their biomass production and physiological development patterns. Furthermore, different forest species respond differently to light intensity (PINTO et al., 2016; RIBEIRO et al., 2020a).

Given the ecological importance of *Erythroxylum pauferrense*, it is crucial to study the adaptation, development, and propagation of this species under different environmental conditions. It is also important to optimize management techniques for the production of seedlings. Therefore, this study aimed to evaluate morphophysiological aspects in *Erythroxylum pauferrense* seedlings submitted to different levels of shading.
2 MATERIAL AND METHODS

The experiment was carried out from May to December 2018 under greenhouse conditions at the Plant Ecology Laboratory, Department of Phytotechnics and Environmental Sciences, Center for Agrarian Sciences, Federal University of Paraíba, Campus II, Areia, Paraíba, Brazil. During the experiment, the average temperature and air relative humidity were 28.3°C and 54.5%, respectively. Climatic data were measured using a portable digital thermo-hygrometer (MT-241A, Minipa).

_Erythroxylum pauferrense_ seeds were obtained from fruits collected from mother plants located at Mata do Pau-Ferro State Ecological Park, municipality of Areia, Paraíba state, Brazil. Seeds were manually separated from the fruit pulp then washed in running water for five minutes. Subsequently, seeds were sown in 5 dm³ plastic pots filled with a substrate composed of soil mixed with vermiculite at the 3:1 volume ratio. The soil used as the substrate was collected at 20 cm depth at the same location where mother plants grow (Table 1). The soil analysis were carried out at the Soil Chemistry and Fertility Laboratory of the Department of Soils and Rural Engineering, at the Federal University of Paraíba.

Table 1 – Chemical attributes of the soil used as substrate in the experiment

<table>
<thead>
<tr>
<th>pH (H₂O)</th>
<th>P (mg dm⁻³)</th>
<th>K⁺ (cmol·dm⁻³)</th>
<th>Na⁺</th>
<th>H⁺ + Al³⁺</th>
<th>Al³⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>SB</th>
<th>CEC</th>
<th>V</th>
<th>SOM (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>33</td>
<td>32.31</td>
<td>0.1</td>
<td>8.09</td>
<td>0.4</td>
<td>2.0</td>
<td>1.5</td>
<td>3.68</td>
<td>11.77</td>
<td>31.3</td>
<td>34.97</td>
</tr>
</tbody>
</table>

Source: Authors (2021)

In where: SB: sum of bases; CEC: cation exchange capacity; V: base saturation; SOM: soil organic matter.

Five seeds were sown per pot, and a thinning was performed at 30 days after emergence (DAE), leaving in the pot plants with 5 cm in height on average. Then, the pots were transferred to different shading levels used as treatments, and plant evaluations started 60 DAE. Shading levels were determined using a digital lux meter (MLM-1011,
Minipa), calculating the irradiance under shading and comparing proportionally to the condition under full sun (0% shading). Moreover, during the experimental period, plants were daily irrigated using the gravimetric method, by weighing and rearranging pots, aiming to keep substrate at 80% field capacity.

The experimental design was completely randomized, with five treatments (0%, 30%, 50%, 70%, and 90% shading) and eight replicates (one plant each).

For growth analysis, plant height (cm), stem diameter (mm), and number of leaves were evaluated at 60, 90, 120, 150, 180, and 210 DAE. Also, absolute and relative growth rates for plant height and stem diameter were calculated according to Benincasa (2003) by the following Equations (1), (2), (3) and (4):

\[ AGR_{\text{plant height}} = \frac{(Ph_2 - Ph_1)}{(t_2 - t_1)} \]  \tag{1}

\[ RGR_{\text{plant height}} = \frac{(\ln Ph_2 - \ln Ph_1)}{(t_2 - t_1)} \]  \tag{2}

\[ AGR_{\text{stem diameter}} = \frac{(Sd_2 - Sd_1)}{(t_2 - t_1)} \]  \tag{3}

\[ RGR_{\text{stem diameter}} = \frac{(\ln Sd_2 - \ln Sd_1)}{(t_2 - t_1)} \]  \tag{4}

Where: AGR is the absolute growth rate (cm dia\(^{-1}\)); RGR is the relative growth rate (cm cm\(^{-1}\) dia\(^{-1}\)); Ph1 and Ph2 are the plant heights (g) at times 1 and 2, respectively; Sd1 and Sd2 are the stem diameters (mm) at times 1 and 2, respectively; and ln is the natural logarithm.

At the end of the experiment (210 DAE), plants were removed from the pots and subdivided into leaves, stems, and roots. Roots were washed in running water
for cleaning and removal of residues, and the length (cm) of the main root was then measured using a millimetric ruler. Leaves, stems and roots were stored in Kraft paper bags and dried in a forced-air circulation oven (TE-394/2-MP, Tecnal, Piracicaba, São Paulo, Brazil) at 65°C over 72 h. Then, dry mass of leaves (LDM), stems (SDM), and roots (RDM), and total dry mass (TDM) were measured and results were expressed as g per plant.

The ratio between shoot (stem plus leaves) dry mass and root dry mass (SHDM/RDM) was calculated. Moreover, Dickson's quality index (DQI) was calculated by the formula (DICKSON; LEAF; HOSNER, 1960) in Equation (5):

$$DQI = \frac{TDM}{\frac{SHDM + PH}{RDM + SD}}$$

In where: TDM is the total dry mass; SDM is the shoot dry mass; RDM is the root dry mass; PH is the plant height; and SD is the stem diameter.

To determining leaf area (cm²), leaves of each plant were digitized using a scanner (P-215II, Canon), and images were then processed and analyzed in ImageJ® software for estimating leaf area. From the leaf area data, specific leaf area (cm² g⁻¹), leaf area ratio (cm² g⁻¹), and specific leaf weight (g cm⁻²) were calculated using equations proposed by Benincasa (2003).

For morphofunctional evaluation of leaves, ten leaf discs (1 cm² in diameter) were collected per plant. The discs were hydrated with distilled water in Petri dishes for 24 h until tissue turgidity. After hydration, leaf thickness (LT, mm) was measured using a digital caliper (± 0.01 mm) and mass of turgid discs using a digital scale (0.0001 g). Then, the turgid discs were placed in Kraft paper bags and dried in a forced circulation oven at 65°C for 72 h to determine dry mass as g. With area, dry mass, and turgid mass values of discs, leaf mass per unit area (LMA, g m⁻²) was calculated as the ratio between dry mass and disk area (POORTER et al., 2009), and succulence (SUC, g m⁻²) as
the difference between turgid mass and dry mass divided by disc area (KLUGE; TING, 1978). Also, density (DEN, mg mm\(^{-3}\)) was calculated using the formula (DEN = LMA / LT).

For gas exchange analyzes, net assimilation rate of CO\(_2\) (\(A\), µmol m\(^{-2}\)s\(^{-1}\)), stomatal conductance (\(gs\), mol m\(^{-2}\) s\(^{-1}\)), transpiration rate (\(E\), mmol m\(^{-2}\) s\(^{-1}\)), internal concentration of CO\(_2\) (\(Ci\), µmol mol\(^{-1}\)), and leaf temperature (°C) were measured at 210 DAE. Subsequently, instantaneous water use efficiency \([WUE = A/E\), (µmol m\(^{-2}\) s\(^{-1}\)) / (mmol m\(^{-2}\) s\(^{-1}\))], intrinsic water use efficiency \([iwUE = A/gs\), (µmol m\(^{-2}\) s\(^{-1}\)) / (mol m\(^{-2}\) s\(^{-1}\))\], and instantaneous carboxylation efficiency \([ICE = A/Ci\), (µmol m\(^{-2}\) s\(^{-1}\)) / (µmol mol\(^{-1}\))\] were calculated. A leaf chamber (6 cm\(^2\)) coupled with a natural irradiance sensor was used, with air humidity between 50 and 60%, air flow of 300 µmol s\(^{-1}\), and atmospheric CO\(_2\) concentration of 400 µmol mol\(^{-1}\). The analyzes were performed using an infrared gas analyzer (IRGA, LI-6400XT, LI-COR) on healthy and fully expanded leaves, located in the middle portion of the plant canopy, on a sunny day (without cloudiness) between 11 and 12 a.m. (RIBEIRO et al., 2020b).

Chlorophyll (Chl) \(a\) fluorescence was evaluated at 210 DAE. Using the measures of initial fluorescence (\(F_0\)) and maximum fluorescence (\(F_m\)), variable fluorescence (\(F_v = F_m - F_0\)), maximum quantum yield of PSII (\(F_v / F_m\)), and \(F_v / F_0\) ratio were calculated. Measurements were performed on healthy leaves located at the middle third of the plant canopy using a portable modulated fluorimeter (OS-30p, Sciences Inc., Hudson, USA). Leaves were dark-adapted before analyzes using leaf clips for 30 min.

Chl \(a\), Chl \(b\), Chl \(a+b\), and Chl \(a/b\) were measured at 210 DAE using a portable chlorophyll meter (CFL 1030, ClorfiLOG\(^{®}\)). Measurements were performed on four leaves at the middle third of the plant canopy to calculate an average, and results were expressed as FCI (Falker Chlorophyll Index).

Data were submitted to one-way analysis of variance followed by polynomial regression analyzes using SAS University software.
3 RESULTS AND DISCUSSION

Shading significantly influenced plant height, stem diameter, number of leaves, and absolute growth rate for plant height, showing quadratic responses depending on the evaluation day (Figure 1). At 150 DAE, plant height, stem diameter and number of leaves were higher under shading, maintaining this response until the end of the experiment (210 DAE), when the higher values were observed under 40 to 55% shading (Figure 1).

Figure 1 – Plant height (A), stem diameter (B), number of leaves (C), and absolute growth rate for plant height (\(\text{AGR}_{\text{plant height}}\)) (D) in Erythroxylum pauferrense plants as a function of evaluation time and shading levels

Source: Authors (2021)
An upward trend was observed in *Tabebuia aurea* growth under different shading conditions (PINTO *et al*., 2016). Under intermediate luminosity (50% shading approximately), plants grown more in height, stem diameter and number of leaves (Figure 1A-C). Also, in a study with *Bertholletia excelsa*, the plants that grew more in shoot (height and stem diameter) had a higher number of leaves (ALBUQUERQUE; EVANGELISTA; ALBUQUERQUE NETO, 2015). Henrique *et al*. (2011), analyzing coffee (*Coffea arabica*) seedlings, stated that higher shoot growth improves the plant's ability to capture photosynthetically active radiation, directly influencing its vigor.

Regarding growth rates, there was a significant effect only on AGR for plant height (cm day\(^{-1}\)) over the days after emergency (Figure 1D). Such a result is consistent with the increase in plant height under 40% shading, indicating that AGR increases as the plant grows. According to Benincasa (2003), AGR is an important index to evaluate plant growth over the experimental period.

Shading significantly affected plant growth, regarding the dry mass of leaves (LDM), stems (SDM) and roots (RDM), and total dry mass (TDM) (Figure 2 A-D). The maximum biomass accumulation in the leaves, stems, and roots occurred under 38, 41, and 40% shading, respectively, which thus led to maximum total biomass accumulation under 44% shading (Fig 2A-D). In the present study, *Erythroxylum pauferrense* seedlings submitted to environments that simulate a clearing (without end of light and shade), had characteristics that indicate the success of planting in these environmental conditions of intermediate light. On the other hand, plants under full sun (0% shading) and 90% shading showed the lowest biomass accumulation in all organs. This fact shows that the minimum (0%) and maximum (90%) luminosity tested can negatively affect the photoassimilates production by plants, reducing the performance of the photosynthetic apparatus (REIS *et al*., 2016). It occurs because high irradiance can harm plants, by causing high absorption of photons and less assimilation, leading to photoinhibition or plant death (TAIZ *et al*., 2017; KOCHHAR; GUJRAL, 2020). In contrast, low irradiance provides inadequate amounts of energy for plants, thus limiting the photosynthetic performance during the photochemical phase (TAIZ *et al*., 2017; GRIME, 2020).
Figure 2 – Dry mass of leaves (LDM) (A), stems (SDM) (B) and roots (RDM (C), and total dry mass (TDM) (D) in Erythroxylum pauferrense plants as a function of shading levels.

Main root length and root volume linearly decreased as shading level increased. Shading also affected the ratio between shoot dry mass and root dry mass (SHDM/RDM) and Dickson’s quality index (DQI) (Figure 3A-D). Root length was 39% higher in plants under full sun compared to 90% shading (Figure 3A). Similarly, root volume was higher under maximum luminosity (5.3 cm³) than under maximum shading (1.3 cm³) (Figure 3B).
A conjoint analysis of root length and volume showed that under maximum light (0% shading) in experimental conditions, *Erythroxylum pauferrense* plants prioritized root growth (in length and volume) over the shoot. This root expansion under full sun
may be a response of the plant to cope with water loss through evapotranspiration, which reduces water availability in the root zone. To stimulate root growth, plants increase the translocation of photoassimilates to the organ and thus improve its ability to explore denser layers of soil in search of water (MOTA; SCALON; MUSSURY, 2013).

SHDM/RDM increased under up to 50% shading, then declined by 21% under up to 90% shading (Figure 3C). This behavior indicates that *Erythroxylum pauferrense* plants change dry matter partition between shoot and root depending on shading conditions.

Assessing the quality of *Erythroxylum pauferrense* seedlings, DQI was higher in plants cultivated under 49% shading (Figure 3D). Such a result indicates that this shading condition is similar to that found in the natural environment of the species, thus being the most suitable for its growth pattern. Also, in the present study, DQI varied significantly between the lowest and highest shading levels. 0, 70, and 90% shading is not recommended for the production of *Erythroxylum pauferrense* seedlings because the DQI values were lower than 0.20, the minimum recommended value for obtaining good quality seedlings (HUNT, 1990).

The 49% shading provided a larger leaf area (201.7 cm²), increasing 60.3% in relation to full sun condition (Figure 4A). Increased leaf area in shaded environments may be related to a plant acclimatization mechanism. Plants raise the allocation of biomass for leaf growth, which induces morphophysiological changes under low radiation, in order to improve the efficiency of their photosynthetic process (ALMEIDA et al., 2015).

For specific leaf area (SLA), leaf area ratio (LAR), and specific leaf weight (SLW), there were no significant differences among shading levels, which average values were 251.44 cm² g⁻¹, 94.66 cm² g⁻¹, and 0.0041 g cm⁻², respectively. On the other hand, leaf mass per unit area (LMA) and leaf thickness (LT) decreased with increasing levels of shading. Plants showed superior values when exposed to full sun (0% shading) than under shading levels, with values of 328.56 g m⁻² and 0.1351 mm, respectively (Figure 4B-C).
Leaf thickness is of fundamental importance in the development and photosynthetic process of plants, being associated with their strategies for acquisition and use of resources (TOUNEKTI et al., 2017). According to Gratani (2014), thicker
leaves are generally found in environments with high sunlight intensity, as observed in the present study. Also, this author stated that LMA is positively correlated with leaf thickness, and negatively with density. Other studies have shown high LMA is typical of slow-growing and stress-tolerant plants, while low LMA occurs in fast-growing plants (KUNSTLER et al., 2016).

Shading did not affect succulence (SUC) and density (DEN) of leaves, which were 408.43 g m\(^{-2}\) and 0.2255 mg mm\(^{-3}\) on average, respectively.

Gas exchange variables, except by leaf temperature, changed significantly according to shading levels (Figure 5 and 6). The net assimilation rate of CO\(_2\) (A) and stomatal conductance (gs) responded similarly to shading levels, with maximum values in environments under 51% and 40% shading, respectively. Then, A declined by 56.7% (Figure 5A), and gs by 40.0% (Figure 5B), relative to the environment under full sun. Low values for both variables were found under 0 and 90% shading.

Similar results were observed for A and gs, when the highest values were found under intermediate shading conditions (51% and 41%, respectively), affirming a strong relationship between these variables. Probably, 30 and 50% shading conditions improved the capture of CO\(_2\) by leaves, thus directly influencing the plant photosynthetic processes. On the other hand, the conditions of full sunlight or extreme shading possibly induced stomatal closure, to prevent damages to the photosynthetic apparatus and anatomical changes that limit plant photosynthesis (ALMEIDA et al., 2018).

Transpiration (E) responded similarly to A and gs, showing a quadratic adjustment to the shading level increase (Figure 5C). Plants under 42% shading showed the highest A, 39.1% higher than under full sun (Figure 5C). This behavior is a defense mechanism against excessive luminosity; plants limit stomatal opening to prevent water loss, thereby reducing the transpiration rate (LIMA et al., 2016).

The internal concentration of CO\(_2\) (Ci) also responded similarly to other gas exchange variables under increased shading levels. Plants under 40% shading showed the highest Ci, a 16.9% increase compared to plants under full sun (Figure 5D). Low Ci
under full luminosity conditions is according to decrease in $A$, which may be favored by resistance to $\text{CO}_2$ diffusion over the leaf surface. As stated by Dalastra et al. (2014), plants under optimal growth conditions have high concentration and assimilation of $\text{CO}_2$, while low concentration may limit photosynthesis.

Figure 5 – Net assimilation rate of $\text{CO}_2$ ($A$) (A), stomatal conductance ($gs$) (B), transpiration rate ($E$) (C), and internal $\text{CO}_2$ concentration ($Ci$) (D) in *Erythroxylum pauferrense* plants as a function of shading levels

Source: Authors (2021)
Instantaneous water use efficiency (\textit{WUE}) showed a quadratic adjustment as the shading level increased, with a maximum at 61\% shading (Figure 6A). \textit{WUE} is the ratio between \( A \) and \( E \), to bring a relation between the amount of \( \text{CO}_2 \) assimilated and water lost (LIU et al., 2019). According to Peters et al. (2018), plants lose water as they assimilate \( \text{CO}_2 \), and saving water reduces the capture of this gas. Thus, plants under full sun were 47.4\% less water use efficient (\textit{WUE}) than plants under 61\% shading. It may be due to plants under the same sunlight conditions having also shown lower \( A \) and \( E \).

Figure 6 – Instantaneous water use efficiency (\textit{WUE}) (A), intrinsic water use efficiency (\text{iWUE}) (B), and instantaneous carboxylation efficiency (\text{iCE}) (C) in \textit{Erythroxylum pauferrense} plants as a function of shading levels

\begin{align*}
\text{WUE} &= 0.9156 + 0.0245x - 0.0005x^2 \quad R^2 = 0.84 \\
\text{iWUE} &= 32.208 + 1.3942x - 0.0130x^2 \quad R^2 = 0.80 \\
\text{iCE} &= 0.0067 + 0.0004x - 0.0378x^2 \quad R^2 = 0.90
\end{align*}

Source: Authors (2021)
Regarding intrinsic water use efficiency (iWUE), this variable ranged significantly according to shading levels. Maximum was found under 54% shading, which corresponded to a 53.4% increase compared to full sun conditions (Figure 6B). Such an increase was due to higher $A$ and $gs$ values, which enhance the iWUE in plants, as reported by Wieser et al. (2018).

Similar to iWUE, instantaneous carboxylation efficiency (ICE) also showed quadratic adjustment to increase in shading levels (Figure 6C). Maximum was obtained under 50% shading, in which the ICE was 68.5% higher compared to full sun (Figure 6C). This parameter allows evaluating non-stomatal factors affecting photosynthetic rate in plants. Therefore, such behavior indicates that non-stomatal factors were also affected by maximum exposure to sunlight, thus limiting the ATP and NADPH from the electron transport chain of photosystem II (PSII) (Silva et al., 2015).

For chlorophyll fluorescence analysis, shading did not affect initial fluorescence ($F_0$), maximum fluorescence ($F_m$), variable fluorescence ($F_m - F_0$), maximum quantum yield of PSII ($F_v / F_m$), and the $F_v / F_0$ ratio, which were 97.8, 340.5, 242.7, 0.70, and 2.54 electrons quantum$^{-1}$ on average, respectively.

On the other hand, the contents of chlorophyll $a$, $b$, and $a+b$ was significantly affected by shading levels (Figure 7A-C), with the exception for Chl $a/b$. The maximum content of Chl $a$, $b$, and $a+b$ were found under 57, 56, and 57% shading, respectively, which corresponded to increases of 31, 48, and 35% compared to plants under full sun (Figure 7A-C). The increase in Chl contents under 50% shading may be due to plants synthesizing more Chl per reaction center, producing thicker leaves, and presenting a larger leaf area as an adaptation mechanism to this shading condition, in order to favor the capture of light (Taiz et al., 2017; Lambers; Oliveira, 2019).
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Figure 7 – Chlorophyll a (A), b (B) and a+b (C) contents in *Erythroxylum pauperrense* plants as a function of shading levels

![Figure 7 - Chlorophyll contents](image)

Source: Authors (2021)

### 4 CONCLUSIONS

Levels from 30 to 50% shading are the most recommended for the production of *Erythroxylum pauperrense* seedlings under shading environment because positively affected most of the morphophysiological variables evaluated;
Levels of 0 or 90% shading are not recommended for the production of *Erythroxylum pauferrense* seedlings, because reduce performance and cause stress in plants;

We recommend using *Erythroxylum pauferrense* to reforest the understory in disturbed areas in Brejos de Altitude and large-scale production of seedlings under intermediate levels of shading.

**ACKNOWLEDGEMENTS**

This research was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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