

Morphophysiological dynamics of *Casearia sylvestris* seedlings under different natural irradiation conditions

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Abstract

Knowledge of the ability of species to acclimate to light environments allows for the most appropriate selection for the restoration of different sites. *Casearia sylvestris* Sw. is a species C3 with important functional characteristics, geographic distribution and described for its plasticity, and was used as a reference for the present investigation. Thus, this study aims to investigate the morphophysiological changes of species planted in pots under different conditions: High irradiance (full sun around 106175 ± 1.246 lux), moderate irradiance (moderate shade under 61775 ± 2.333 lux), and low irradiance (understory under 425 ± 57 lux). The sampling design was a totally random design. The survival rate was calculated by the total number of seedlings per treatment. For the other analyzes (variables) 5 random repetitions were used for the evaluations. We found that the plants altered their morphophysiological characteristics with varying light intensity. Under higher light availability, the species responded with increased photosynthetic rates, stomatal conductance, transpiration, survival (100%), reduced specific leaf area, pigment reduction, quantum yield of photosystem II. In the understory (425 ± 57 lux), the species modulates the photosynthetic apparatus, increasing chlorophyll concentration and specific leaf area, seeking to increase light capture, with a survival rate of 86%. The variables with the highest plasticity index were predominantly physiological (transpiration, stomatal conductance, photosynthesis, and photochemical quenching) and they contributed most to the acclimation process. It was confirmed by the plasticity index analysis, where the least plastic variables were specific leaf area and chlorophyll index total, water use efficiency, and photosystem II quantum efficiency. It is concluded that the plasticity of the species to light intensity is provided by aspects related to the photosynthetic capacity of plants, providing a higher rate of survival and growth in plants without water or nutritional restriction.

Keywords: Acclimatization, Chlorophyll fluorescence *a*, Ecophysiology, Gas exchange, Plasticity.

Introduction

The forest canopy cover changes in a stochastic way, and by anthropogenic actions (Moretti et al., 2019; Ballestreri et al., 2021). In the forest understorey, young plants can experience a wide variation in the amount of incident light, ranging from 166.3 lux to 3309.9 lux (Castello et al., 2017; Ballestreri et al., 2021). Changes in light incidence occurring in the understory can cause changes in the microclimate with variables such as soil moisture and air and soil temperature (Ballestreri et al., 2021). In this sense, it is necessary to correct management of the plants to be inserted and knowledge of the species ability to acclimate to full sun or shade under a dense canopy (Moretti et al., 2019; Yáñez et al., 2021; Zhang et al., 2022). Regarding light demand, acclimation is the response at the chloroplast, leaf,

and plant levels, occurring in days or months. Physiological responses can occur in minutes, such as stomatal closure under stress (Morales and Kaiser, 2020). Thus, studies are needed to corroborate information on the amount of light needed for more remarkable survival and efficiency of the forest species at the site. Seedlings usually have a greater ability to change the phenotype when compared to adult plants of the same species (Valladares et al., 2005).

The phenotypic plasticity is the ability of a genotype to respond to such changes, where species with high plasticity are more likely to survive and maintain growth in unfavorable and heterogeneous environments (Olguin et al., 2020), maintaining or maximizing carbon assimilation (Anjos et al., 2015). In addition, plants with high leaf plasticity

through modulation of the photosynthetic apparatus have a greater capacity to recover from the stress caused by excess light (Anjos et al., 2015; Lopes et al., 2019).

The increase in light availability is usually observed together with a greater stomatal conductance and increased CO₂ assimilation rate. In contrast, transpiration maximizes water loss (McAusland et al., 2016). Plants in full sun can experience photoinhibitory effects, perceived by the decrease in the quantum efficiency of photosystem II (F_v/F_m) (Olguin et al., 2020; Krause and Winter, 1996), where immediate post-planting (first 30 days) is crucial for plant establishment. There may also be an increase in specific leaf area (SLA) (Evans and Poorter, 2001; Lopes et al., 2019; Olguin et al., 2020) and chlorophyll content per unit of leaf mass. These changes are considered strategies that aim to increase light capture. On the other hand, there is a decrease in chlorophyll content in full sunlight as a mechanism to dissipate the excess energy absorbed by photosystems (Kitajima and Hogan, 2003; Hallik et al., 2012; Olguin et al., 2020).

Many forest species are described in the literature with multiple classifications regarding their light demand, as noted by (Reitz et al., 1988). Among these species, *Casearia sylvestris* Sw., popularly known as carvalinho, belongs to the Salicaceae family (Reitz et al., 1988), which has a wide geographic distribution, appearing from Mexico to Argentina and Uruguay. In Brazil, there are records from Amapá to Rio Grande do Sul and in phytogeographies such as Deciduous Seasonal Forest, Semideciduous Seasonal Forest, Ombrophylous Forest, Mixed Ombrophylous Forest and also in Riparian or Gallery Forest, Savanna, Restinga, Rupestrian or high altitude fields (Carvalho, 2007). *C. sylvestris* presents pharmacological principles, beekeeping potential, landscaped and to use joinery (Reitz et al., 1988; Carvalho, 2007), and for plantations to recover degraded environments, except for full sun conditions (Ballestreri et al., 2021). The species performs best in soils with good to regular drainage, clayey to sandy-clayey texture, and medium to high fertility (Carvalho, 2007).

C. sylvestris is described as a heliophile species and esciophile (Carvalho, 2007). Furthermore, there is no classification consensus in the literature about the ecological group, varying between pioneer (Bragion et al., 2019) to light-demanding climax (Scipioni et al., 2019). This lack of consensus, combined with its distinct ecophysiological characteristics and widespread natural occurrence, leaves gaps in information regarding the ecofunctional characteristics of the species in different light environments. Thus, the understanding of its biology can corroborate with forest restoration practices (Moretti et al., 2019; Ballestreri et al., 2021), with high phenotypic plasticity being desirable, as degraded environments are unstable and stressful (Olguin et al., 2020).

Studies evaluating morphophysiological and growth characteristics of young plants are necessary to understand the species' ability to survive and grow in different environments in tropical forests (Moretti et al., 2019). Studies that evaluate the morphophysiological and growth characteristics of young plants are necessary to understand the species' ability to survive and grow in different environments in tropical forests (Moretti et al., 2019). Thus, in this study, we aimed to investigate the morphophysiological changes in young plants of *C. sylvestris* that

occurred during the process of initial acclimation to irradiance, evaluating the photosynthetic plasticity of the species in contrasting light environments.

Results

Analysis of survival and growth

The highest survival rates were observed in the HI and MI environments, both with 100% survival. In contrast, the LI environment showed an 86% survival rate in the same period. Plants submitted to the HI environment followed by MI showed higher initial stem diameter growth (Fig 1a) compared to plants kept in LI. The robustness quotient, the ratio of height to stem diameter (H/D), was higher in LI and lower and not different in HI and MI (Fig 1b).

No differences in height growth were observed across the environments. The calculation of daily increment (Table 1) showed that the absolute growth rate in stem diameter was higher in HI, followed by MI. However, the increment of AGR_H in different light environments showed no differences.

Specific leaf area

Plants kept in the LI environment showed larger specific leaf areas (SLA) throughout the evaluated period (10th, 20th, and 30th day) so that at the end of 30 days, the SLA was about 60% higher than when compared to the other environments (Fig 2). On days 20 and 30, it could be seen that the SLA in plants in the HI and MI environments remained similar to each other.

Gas exchange and chlorophyll fluorescence a

The photosynthetic rate (A), transpiration (E) and stomatal conductance (gs) remained high throughout the evaluated period in *C. sylvestris* plants in environments with higher luminosity (MI, and HI) compared to plants in the LI environment. On the 10th day in LI, there was already a marked reduction of these parameters, remaining low during the entire evaluation period (Table 2). Water use efficiency (WUE) was higher in the MI and LI treatments, and from 20th day, the highest averages for MI and HI. By 30th day, the HI treatment had the highest mean. 20th day photosystem II quantum yield (F_v/F_m) was highest in plants in the LI subjected plants, with an overall mean of 0.78 ± 0.05 , followed by the MI treatment, which had an overall mean of 0.69 ± 0.01 . The lowest means were associated with the highest irradiance environment (HI), with an overall mean of 0.58 ± 0.09 . By the 10th day of evaluation, a decline in F_v/F_m could already be observed for plants subjected to HI (Fig 3a). The highest values of quenching photochemistry (qP) were associated with the HI and MI treatments compared to LI (Fig 3b).

Falker chlorophyll index

In general, the MI environment increased the Falker chlorophyll a index (FCIa) compared to other light environments. At 30th day, plants under MI and LI remained similarly superior to the HI environment (Table 3). Similarly, plants grown under LI and MI during the whole period showed the highest average FCIb. The total FCI was lower in plants under full sun (HI), with a tendency to increase after the 20th day of exposure.

Phenotypic plasticity

In the initial acclimation period of *C. sylvestris*, the physiological attributes (Table 4) were the most sensitive to the applied treatments, solidifying the low plasticity of the species at different shading levels. On the 10th day of experimentation, the most plastic variables were *gs*, *A*, *qP*, and *E*, with values greater than 0.6. On the 20th day, the *gs*, *A*, and *E* variables remained constant. On day 30, the only variable considered plastic was *gs*. At day 20 and day 30, a lower PPI was also observed for the variable *FCl_a*.

In general, there was a more significant divergence in plants conducted in LI when compared to those acclimated to HI and MI. This is perceptible in the groups in different positions in the two-dimensional space of the ordinations, indicating different responses of these treatments concerning the acclimatization process (Fig 4).

The diagrams formed by the two axes in the Principal Component Analysis (PCA) explained most of the variation in the original matrices (about 82%). Component 1, represented by the y axis, explained 54.8%, and the variables: *E* (47%), *gs* (54%), and *A* (48%) were the ones that most contributed to express the existing variation. The component 2, represented by the x-axis, presented a value of 27.1%, with the variable *FCl_b* (63%) that contributed the most to explaining the existing variation (Table 5).

Discussion

The higher irradiance intensities (HI and MI) in the early establishment phase of *C. sylvestris* provided an increase in *D* and *AGR_D* and *LGI* and survival. According to Bragion et al. (2019), this behavior is characteristic of pioneer species, which initially develop better under higher light conditions. On the other hand, this species is often found within Atlantic Forest fragments, requiring partial shade (Venzke, 2018), which the present study does not dispute. However, we do not recognize it as the best performance environment. The better performance in these MI and HI environments may be associated with the elevated leaf gas exchange, which is stimulated by increased carbohydrate production in response to photosynthetic rates (Aguiar et al., 2011). In addition, increased shoot diameter under higher light levels, typical of the heliophytic species group (Carvalho, 2007), which associated with higher photosynthetic rates, may have favored plant growth (Aguiar et al., 2011; Müller and Munné-Bosch, 2021). Other forest species (e.g. *Caesalpinia echinata* Lam. and *Bertholletia excelsa* Bonpl.) also show higher growth and survival when submitted to full sun and medium overstory environments (Aguiar et al., 2011; Souza et al., 2017). Similarly, the robustness quotient (*H/D*) was favored in environments with higher light, since the HI (6.5) and MI (7.2) environments did not indicate etiolation of the seedlings, i.e., excessive increase in seedling height, since the values for *C. sylvestris* seedlings were around 5.4 and 8.1, values considered adequate for shipping (Carneiro, 1995), except for the understory (LI) environment where the ratio was 8.3. The lower this ratio is better, which means that the seedling is more robust than usual (Ritchie et al., 2010; Honorio et al., 2019), as well as the greater the ability of the plants to survive and establish in the field (Honorio et al., 2019), consequently reducing the need for replanting (Gomes et al., 2013). In contrast, the higher ratio indicates

that photoassimilates go directly to shoot (height) and less to stem diameter (Ritchie et al., 2010). It may be considered a plant strategy to increase the chances of reaching the canopy by increasing light capture. On the other hand, it may make the plant more susceptible to tilting after transplanting to the field. Shading provided different morphoanatomical responses to *C. sylvestris* plants. After only 30 days of exposure, we found that SLA was altered. Plants under LI showed higher SLA values. Modifications of this variable may indicate a change in the size or even the thickness of the leaves. Since this was a short-term experiment (30 days), this alteration possibly must have been due to a decrease in leaf thickness, not necessarily an increase in leaf area. However, it is not possible to affirm this, since no leaf anatomical evaluation was performed. Under higher light intensities, plants are expected to have smaller leaf areas when compared to plants exposed to lower irradiance levels, due to the lower need for greater absorption of light radiation, as this resource is widely available to plants, and also to avoid photo-inhibitory effects and photodamage, as leaves are protected by upper layers of parenchymal cells (Lopes et al., 2019), thus increasing light diffusion. This is a strategy to avoid excessive light absorption and reduce transpiration, since the unit area is possibly smaller (Taiz et al., 2017). In addition, the formation of thicker palisade tissue may reflect an increase in chloroplasts and photosynthetic enzymes, improving photosynthetic capacity per unit area (Evans and Poorter, 2001).

During the process of acclimation to different light availability, adjustments in carbon balance may occur in plants (Sanchez et al., 2009) so that higher *A* values in higher irradiance environments may be related to a higher amount of rubisco and electron carriers (Jiang et al., 2011). Therefore, under low irradiance, net photosynthesis is lower than higher light conditions (Magalhães et al., 2009), although species respond differently depending on genetic characteristics and location. Thus, net assimilation is associated with species growth (Guimaraes et al., 2018). For *C. sylvestris* the best photosynthetic performance was observed in HI and MI environments, as verified by the highest in *D* and survival.

At the same time, the higher *E* and *gs* in these environments (HI and MI), may have contributed to increased CO₂ influx, stimulating rubisco activity, thus contributing to the increase in photosynthetic rate in these environments with higher sunlight exposure (HI and MI). Importantly, there was no restriction in stomatal conductance (*gs*), as this was accompanied by an increase in *E*, indicating that even at higher temperatures, the oak plants were hydrated and with good nutritional management. These results are also related to higher water use efficiency (McAusland et al., 2016; Taiz et al., 2017). This contrasts with what was found in the study developed by Ballestreri et al. (2021), in which high irradiance environments in heavily anthropized areas did not provide adequate results for this species. Less effective conditions for the performance of this species also occurred in the understory environment (LI), because in this case, lower photosynthetic rates, lower growth, and survival rates were found despite the mild microclimate and high quantum yield.

Table 1. Absolute growth rates in stem diameter (AGR_D) and height (AGR_H) for *C. sylvestris* plants at different shading levels. High irradiance (HI), moderate irradiance (MI), and low irradiance (LI). Legend: Values are means \pm standard deviations (n = 10). The different letters indicate significant difference according to Tukey's (5%).

| Treatment | AGR_SD (mm/day) | AGR_H (cm/day) |
|-----------|----------------------|---------------------|
| HI | 0.044 \pm 0.016 A | 0.119 \pm 0.128 A |
| MI | 0.025 \pm 0.020 AB | 0.074 \pm 0.101 A |
| LI | 0.003 \pm 0.015 B | 0.080 \pm 0.118 A |

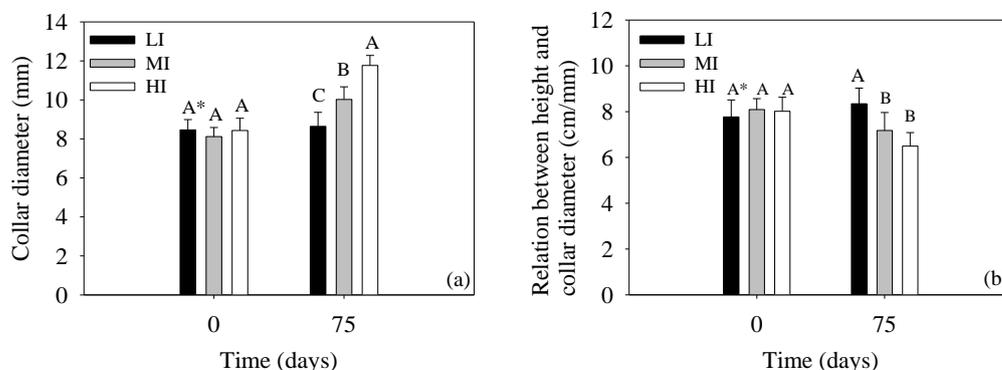


Fig 1. Growth of *C. sylvestris* seedlings under different levels of shading. Stem diameter (a); Relation between height and stem diameter (b); Low irradiance (LI); Moderate irradiance (MI); High irradiance (HI). Vertical lines represent the standard error (n=10). The different letters indicate significant difference according to Tukey's (5%).

Table 2. Gas exchange of *C. sylvestris* under different shading levels, high irradiance (HI), moderate irradiance (MI), and low irradiance (LI). Photosynthesis (A); transpiration (E); Stomatal conductance (gs); water use efficiency (WUE). Values are means \pm standard deviations (n = 10). The different letters indicate significant difference according to Tukey's (5%).

| Variables | Treatments | Time (days) | | |
|---|------------|--------------------|---------------------|---------------------|
| | | 10 | 20 | 30 |
| A ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | HI | 4.04 \pm 0.52 B | 5.22 \pm 0.70 A | 6.06 \pm 1.21 A |
| | MI | 8 \pm 1.62 A | 4.98 \pm 0.81 A | 6.84 \pm 1.30 A |
| | LI | 2.43 \pm 0.52 C | 1.48 \pm 0.24 B | 3.24 \pm 0.58 B |
| E ($\text{mmol m}^{-2} \text{s}^{-1}$) | HI | 0.96 \pm 0.25 A | 0.88 \pm 0.21 A | 0.92 \pm 0.28 B |
| | MI | 0.85 \pm 0.15 A | 0.74 \pm 0.08 A | 1.29 \pm 0.26 A |
| | LI | 0.33 \pm 0.07 B | 0.33 \pm 0.06 B | 0.68 \pm 0.10 C |
| gs ($\text{mmol m}^{-2} \text{s}^{-1}$) | HI | 42.87 \pm 8.50 B | 63.43 \pm 10.96 A | 53.79 \pm 10.43 B |
| | MI | 94.43 \pm 20.98A | 57.05 \pm 10.58 A | 74.42 \pm 17.10 A |
| | LI | 27.20 \pm 5.15 C | 20.30 \pm 4.00 B | 29.19 \pm 4.53 C |
| WUE ($\text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) | HI | 4.69 \pm 0.86 B | 6.41 \pm 1.20 A | 6.12 \pm 1.27 A |
| | MI | 8.48 \pm 1.12 A | 6.53 \pm 1.18 A | 4.88 \pm 0.68 B |
| | LI | 7.73 \pm 1.46 A | 4.60 \pm 0.79 B | 4.79 \pm 0.61 B |

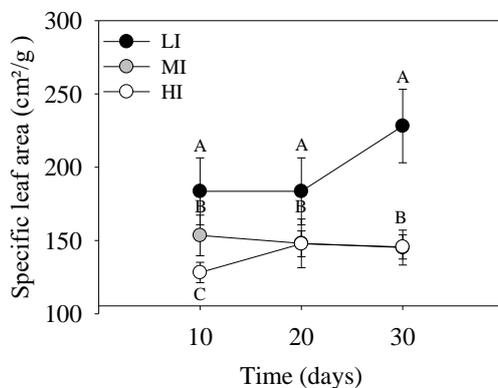


Fig 2. Specific leaf area of *C. sylvestris* under different levels of shading. Low irradiance (LI); Moderate irradiance (MI); High irradiance (HI). Vertical lines represent the standard error (n=10). The different letters indicate significant difference according to Tukey's (5%).

Table 3. Falker Chlorophyll Index (FCI) in young *C. sylvestris* plants under different shading levels. Values are means \pm standard deviations ($n = 10$). Means followed by the same letter do not differ from each other, by Tukey's test at 5% probability of error.

| Variables | Time (days) | | | |
|-----------|-------------|-------------------|------------------|------------------|
| | Treatments | 10 | 20 | 30 |
| FCIa | HI | 34.2 \pm 3.4 C | 39.8 \pm 1.8 B | 38.8 \pm 1.6 B |
| | MI | 40.7 \pm 2.0 A | 42.8 \pm 1.8 A | 42.6 \pm 1.7 A |
| | LI | 37.6 \pm 0.9 B | 38.1 \pm 1.6 B | 43.8 \pm 1.2 A |
| FCIb | HI | 16.8 \pm 2.1 B | 16.2 \pm 2.5 B | 15.4 \pm 2.4 B |
| | MI | 27.3 \pm 3.6 A | 25.3 \pm 2.4 A | 25.6 \pm 4.0 A |
| | LI | 30.30 \pm 2.6 A | 24.7 \pm 2.4 A | 25.8 \pm 2.8 A |
| FCItotal | HI | 51.0 \pm 5.1 B | 56.0 \pm 4.1 A | 54.2 \pm 3.9 B |
| | MI | 68.0 \pm 4.2 A | 68.1 \pm 4.0 A | 68.2 \pm 5.2 A |
| | LI | 67.8 \pm 2.6 A | 62.8 \pm 2.7 B | 69.5 \pm 3.2A |

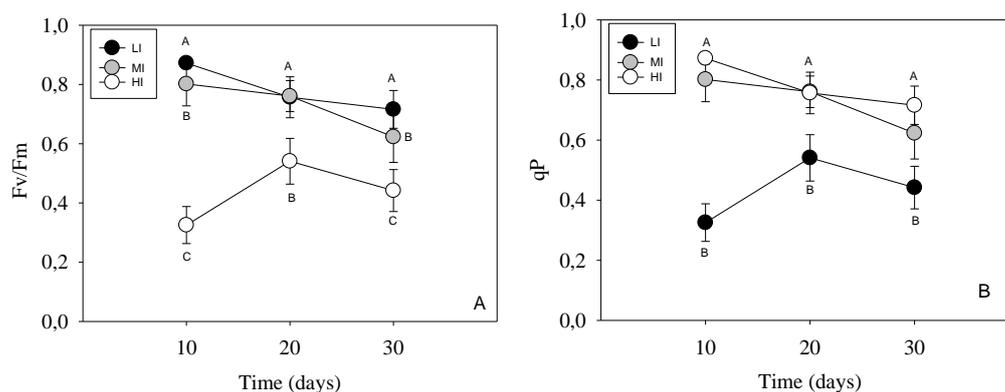


Fig 3. Quantum yield of photosystem II (a) and photochemical quenching (b) of *C. sylvestris* seedlings. Low irradiance (LI); Moderate irradiance (MI); High irradiance (HI). Vertical lines represent the standard error ($n=10$). The different letters indicate significant difference according to Tukey's (5%).

Table 4. Phenotypic plasticity index (PPI) in young *C. sylvestris* plants under different shading levels. Values in bold represent values closest to 1.

| Variable | PPI_10 days | PPI_20 days | PP_30 day | |
|---------------|---|-------------|-----------|-----|
| Physiological | Stomatal conductance (<i>gs</i>) | 0.7 | 0.7 | 0.6 |
| | Water use efficiency (WUE) | 0.4 | 0.3 | 0.2 |
| | Photosynthesis (A) | 0.7 | 0.7 | 0.5 |
| | Falker chlorophyll index <i>a</i> | 0.2 | 0.1 | 0.1 |
| | Falker chlorophyll index <i>b</i> | 0.4 | 0.4 | 0.4 |
| | Falker Chlorophyll Index <i>Total</i> | 0.2 | 0.2 | 0.2 |
| | <i>Photochemical quenching (qP)</i> | 0.6 | 0.3 | 0.4 |
| | Quantum yield of photosystem II (F_v/F_m) | 0.4 | 0.3 | 0.3 |
| | Transpiration (E) | 0.7 | 0.6 | 0.5 |
| Morphological | Specific leaf area (SLA) | 0.3 | 0.2 | 0.4 |

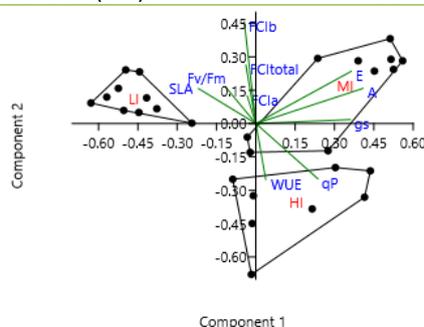


Fig 4. Principal component analysis performed at 30 days in different shades. Legend: Stomatal conductance (*gs*); Water use efficiency (WUE); Photosynthesis (A); Falker chlorophyll index *a* (FCIa); Falker chlorophyll index *b* (FCIb); Falker Chlorophyll Index *Total* (FCItotal); Photochemical quenching (*qP*); Quantum yield of photosystem II (F_v/F_m); Transpiration (E); Specific leaf area (SLA); Low irradiance (LI); Moderate irradiance (MI); High irradiance (HI).

Table 5. Correlations of variables and two ordination axes produced by the Principal Component Analysis (PCA). Values in bold represent values above 0.40.

| Variables | Component 1 | Component 2 |
|---|-------------|-------------|
| Transpiration (E) | 0.47 | 0.34 |
| Stomatal conductance (gs) | 0.54 | 0.23 |
| Photosynthesis (A) | 0.48 | 0.06 |
| Water use efficiency (WUE) | 0.05 | -0.29 |
| <i>Photochemical quenching</i> (qP) | 0.32 | -0.26 |
| Quantum yield of photosystem II (F_v/F_m) | -0.19 | 0.28 |
| Falker chlorophyll index <i>a</i> | -0.05 | 0.17 |
| Falker chlorophyll index <i>b</i> | -0.10 | 0.63 |
| Falker Chlorophyll Index <i>Total</i> | -0.07 | 0.36 |
| Specific leaf area (SLA) | -0.31 | 0.20 |

Table 6. Initial characterization of plants under 50% shading in February 2020.

| Measured variables | Average values |
|--|---|
| Photosynthesis (A) | 7.59 ± 0.76 $\mu\text{mol m}^{-2} \text{s}^{-1}$ |
| Transpiration (E) | 1.32 ± 0.16 $\text{mmol m}^{-2} \text{s}^{-1}$ |
| Stomatal conductance (gs) | 91.57 ± 8.52 $\text{mmol m}^{-2} \text{s}^{-1}$ |
| Water use efficiency (WUE) | 5.83 ± 0.81 $\text{mmol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$ |
| Quantum yield of the photosystem II (F_v/F_m) | 0.6983 ± 0.0624 |
| Photochemical quenching (qP) | 0.6582 ± 0.0987 |
| Falker Chlorophyll Index a (FCl _a) | 40.9 ± 1.2 |
| Falker Chlorophyll Index b (FCl _b) | 31 ± 3.3 |
| Falker Chlorophyll Index total (FCl _{total}) | 71.9 ± 3.3 |
| Specific leaf area (SLA) | 173.10 ± 47.3 cm^2/g |

Legend: Values are means ± standard deviations (n = 10).

Table 7. Characterization of light treatments from February to March 2020.

| Treatments | LUX | Incident radiation (%) | Environments | LUX | References |
|--------------------------|----------------|------------------------|---------------------|--------|---------------------------|
| Low irradiance (LI) | 425 ± 57 | 0.4 | Understory forest | 484.9 | Castello et al. (2017) |
| Moderate irradiance (MI) | 61775 ± 2.333 | 58 | Enrichment Planting | 64800 | Ballestreri et al. (2021) |
| High irradiance (HI) | 106175 ± 1.246 | 100 | Clearing | 103734 | Ballestreri et al. (2021) |

Legend: Values are means ± standard deviations

Table 8. Characterization of the soil used in pots to plant *C. sylvestris* seedlings, submitted to different shading levels. Where: pH: hydrogen ion potential; P-Mehlich – phosphorus extracted by the Mehlich-1 method; K – potassium; Ca – calcium; Mg – magnesium; MO – organic matter; SE – sum of exchangeable bases; H+Al – potential acidity; CTC pH 7.0 – cation exchange capacity.

| pH | P (mehlich) mg dm ⁻³ | K mg dm ⁻³ | Ca Cmolc.dm ⁻³ solo | Mg Cmolc.dm ⁻³ solo | M.O. % | SB % | H + Al Cmolc.dm ⁻³ solo | CTC (pH7) |
|-----|------------------------------------|--------------------------|-----------------------------------|-----------------------------------|-----------|---------|---------------------------------------|-----------|
| 4.9 | 20.3 | 40.0 | 1.7 | 1.0 | 0.7 | 20.9 | 10.9 | 13.8 |

Table 9. Temperature and relative humidity characterization in the light treatments from February to March 2020.

| Treatments | Temperature (°C) | | | Relative humidity (%) | | |
|--------------------------|------------------|---------|---------|-----------------------|---------|---------|
| | Maximum | Minimum | Average | Maximum | Minimum | Average |
| Low irradiance (LI) | 29.0 | 10.6 | 23.2 | 95.8 | 24.8 | 68.9 |
| Moderate irradiance (MI) | 32.3 | 13.2 | 26.5 | 90.6 | 17.2 | 59.9 |
| High irradiance (HI) | 32.7 | 12.7 | 27.2 | 93.0 | 6.1 | 55.6 |

The reduction in F_v/F_m in the first days of AI exposure may indicate that photoinhibition occurred (Anjos et al., 2015) due to the drop in *A* and F_v/F_m , possibly in response to photodamage occurring in the *D1* protein of the PSII reaction center, which may be a consequence of limitation of electron transport on the donor or acceptor side of PSII

(Carmo Araújo and Deminicis, 2009). However, there was a tendency for these attributes to recover after this initial period, due to the acclimation that occurred due to the high irradiance.

Furthermore, this reduction in photosynthetic rates did not negatively influence plant growth, since under AI, plants in

general showed higher growth and survival rates. On the other hand, in the LI environment, without stress and healthy conditions, F_v/F_m was in the appropriate range (0.75-0.85) (Bolhar-Nordenkamp et al., 1989). It did not provide an increase in A, but a decline at day 10, which was expressed in low growth rates. In this context, it is possible to state that conditions with high and moderate irradiance do not negatively interfere with the performance and initial growth of *C. sylvestris*, emphasizing that other factors could significantly influence photosynthesis and growth, such as water and nutrients, factors that in nursery conditions, were adequately supplied.

In general, there was a higher proportion of open PSII reaction centers in HI environments followed by MI (Fig 3b) and a sharp drop in the treatment under LI. This means that more energy was consumed for photosynthesis in treatments under higher light conditions (Hazrati et al., 2016). Falker's chlorophyll index indicated an increase in chlorophyll content in restricted light (LI) environments as a strategy to optimize light capture by PSII light-harvesting complexes, seeking to improve light energy uptake and conversion (Valladares and Niinemets, 2008; Jin-hui et al., 2013). On the other hand, under high irradiance, the rate of chlorophyll degradation by light is generally higher and is considered a plant strategy to dissipate excess energy and thus avoid oxidative damage to photosystem II (Valladares and Niinemets, 2008). Similar results were found in young plants of *Swietenia macrophylla* King, where chlorophyll content was lower in full sun (Gonçalves et al., 2012).

With increasing light availability, *C. sylvestris* responds with higher photosynthetic rates, stomatal conductance, transpiration, reduced specific leaf area, reduced pigment and photosystem II quantum yield. However, under low light conditions, the species modulates the photosynthetic apparatus, increasing the Falker chlorophyll index and increasing leaf specific area as a strategy to increase light capture. However, even in these modulations, plants exposed to low light conditions did not show adequate growth, relative to the other treatments, indicating the low plasticity of the species in this environment. It is not recommended to plant this species in very shady locations. The physiological indicator variables that contributed most to the acclimation process were transpiration, stomatal conductance, photosynthesis and the Falker chlorophyll b index, as verified in the PCA (Fig. 4).

Some studies with native Brazilian species subjected to contrasting light environments under nursery conditions have obtained similar results. The forest essential *Hymenaea courbaril* L. (late secondary) also did not recover photochemical efficiency after 30 days under high sun exposure (Anjos et al., 2012). Species considered to have moderate plasticity such as *Pseudobombax grandiflorum* (Cav.) A. Robyns (early secondary), *Hymenaea courbaril* L. and *Joannesia princeps* Vell. LC (late secondary) were indicated to compose enrichment plantings while avoiding high light intensity (Anjos et al., 2015). Other species considered to have high physiological plasticity are useful for planting in different light conditions, since they recovered the parameters of the acclimated condition quickly and with satisfactory performance, such as *Bertholletia excelsa* Humn. & Bonpl. (Climax) (Lopes et al., 2019), *Schinus terebinthifolius* Raddi (pioneer) (Anjos et al., 2012), *Swietenia macrophylla* King (shade tolerant climax)

(Gonçalves et al., 2012) and *Adesmia bijuga* Phil. (Yáñez et al., 2021).

In the case of *C. sylvestris*, the photosynthetic apparatus is more efficient and suitable when subjected to medium to high light environments to achieve higher levels of carbon assimilation and growth at the early seedling conductance stage. Although the species has a wide geographical occurrence, occupying different phyto-physiognomies and with different successional classifications, it is possible to describe that, under adequate availability of water and nutrients, the species is moderately plastic, and its planting is not recommended in very shaded locations. Just like those found in the forest understory. Finally, we highlight the importance of similar studies that consider the initial growth of species for the successful formation of forest stands, and especially those with emphasis on the recovery of areas, where the environment presents multiple restrictions.

Materials and methods

Location of study

The research carried out at the Silviculture and Forest Nursery Laboratory, located at the Federal University of Santa Maria (UFSM) (29°43' 13.8" S and 53°43' 17.76" W; 91 m). The climate is described according to the Köppen classification, subtropical, of the "Cfa" type, with average annual precipitation between 1400 and 1760 mm, with rainfall distributed throughout all months of the year (Alvares et al., 2013). The precipitation during the experiment period was 98.2 mm, and the average temperature was $23.3 \pm 2.5^\circ\text{C}$, with a maximum of 30.5°C and a minimum of 17.6°C (BDMET-INMET, 2020).

Seedling production

Seed collection occurred in the remnants of Deciduous Seasonal Forest in the central Rio Grande do Sul (29° 45' 02.18" S and 53° 42' 17.32" W; 107 m). In December 2018, after processing the fruits, the seeds were sown in perforated plastic trays containing sand at the base and commercial substrate based on Sphagnum peat, organic compost and carbonized rice husk (2:1:1), mixed with 6 g L^{-1} of controlled release fertilizer (NPK 15-8-12) in the surface layer and placed in a greenhouse to allow the emergence of the seedlings. Irrigation in this phase was approximately 6.4 mm d^{-1} , distributed in four daily applications. Germination was slow, occurring after about 60 days. In May 2019, when the plant had two to three pairs of leaves (height approximately 8 cm), sub-cultivation was carried out in conical polypropylene tube containers with a volume of 180 cm^3 , containing a peat-based substrate to retain moisture, using the same organic compost in the plastic trays, with five to six months of release, as described by the manufacturer, and kept in a shade house (50% shading) where irrigation consisted of approximately 11.4 mm d^{-1} distributed in six applications per day.

In October 2019, the soil used in the pots was air-dried and sieved through a 2 mm mesh sieve. First, the Soil Analysis Laboratory at UFSM determined the chemical composition (Table 6). Subsequently, to raise its pH to 5.5, dolomitic limestone (PRNT 75%) was applied, as recommended by the genus *Eucalyptus* (Sociedade Brasileira de Ciência do solo 2016). After 30 days of application, the soil got a dose of 6 g L^{-1} controlled-release fertilizer NPK (18-05-09). According to

the manufacturer's technical specifications, the release of nutrients occurs gradually, between five and six months.

On December 2019, at approximately 9 months of age, the seedlings were transferred to plastic pots with a capacity of 3 L, containing soil as substrate (Table 6). The seedlings remained in a shade house (50% shading) with the same irrigation regime as in the previous phase. Visual evaluation of the seedlings occurred in February 2020, in order to select them regarding their phytosanitary status, and uniformity of growth for height and stem diameter evaluations. From the total number of plants produced, 200 potted seedlings, 35 plants were selected per treatment.

In this period and condition in a shaded state (50% shade), the first data collections on the seedlings produced were performed, where the following characteristics were evaluated: diameter, height, number of leaves, specific leaf area, gas exchange, chlorophyll a fluorescence, Falker chlorophyll index, in order to characterize the initial state of the plants (Table 7).

Characterization of the treatments

To simulate the different light environments that plants can experience in the forest environment in the initial phase of growth (Castello et al., 2017; Ballestreri et al., 2021), a pre-selection of light environments was made: full sun or gaps, moderate shade (enrichment plantation, for example), and understory environment, respectively designated: High irradiance (106175 ± 1.246 lux), moderate irradiance (61775 ± 2.333 lux), and Low irradiance (425 ± 57 lux), respectively HI, MI, and LI (Table 8). In HI, the plants were kept in full sun; in MI, shading was obtained by a nylon screen suitable for greenhouses; in LI, shading was obtained in an area adjacent to the others, within a forest remnant.

Light intensity measurements in the environments were performed on days with full sun, with the Digital Luxmeter MLM - 1011 (Minipa®), in the period between 12:00 and 14:00 h, during the summer of 2020 (January to March 2020). After the first measurement (February 2020), the plants were then divided into each light environment, HI, MI and LI, totaling 35 seedlings per treatment, for each analysis, 5 seedlings were used for each treatment.

Environmental condition assessments

Temperature and relative humidity monitoring in each treatment occurred from January to March 2020. The outdoor Temperature and Humidity DataLogger (Asko®, model AK174) automatically obtained daily records, every half hour, during the entire period of morphophysiological data collection (Table 9).

Substrate temperature was measured using a SoilControl soil thermometer, obtaining the following values, HI = $34.9 \pm 1.3^\circ\text{C}$; MI = $33.2 \pm 1.0^\circ\text{C}$, and LI = $24.2 \pm 0.3^\circ\text{C}$. Soil moisture was measured weekly using an ML3 sensor coupled to the HH2 Meter Delta-T, where the following values were verified, HI = $0.19 \pm 0.04 \text{ m}^3/\text{m}^3$; MI = $0.18 \pm 0.02 \text{ m}^3/\text{m}^3$, and LI = $0.20 \pm 0.02 \text{ m}^3/\text{m}^3$. Both assessments were made at 3:00 pm. Irrigation was done daily to avoid the effects of water stress as needed for each treatment to maintain soil moisture close to 60% of field capacity in all three treatments

Survival and growth analysis

Survival rate (%) was calculated by the ratio of the total number of seedlings (35 plants per treatment) to the number of living plants after 75 days from the start of treatments (for each treatment). Growth assessment took place at the beginning of the experiment, still in the 50% shade house (time zero) and after 75 days. Height (H - from stem base to apical bud), stem diameter (D) provided the growth assessment. A millimeter ruler and a digital caliper measured height, determined D, and subsequently calculated the relationship between height and collar diameter (H/D). Absolute growth rate (AGR) in height (AGR-H) and stem diameter (AGR-D) were also measured (Bugbee, 1996).

Specific leaf area

The specific leaf area (SLA) was determined from ten leaf discs of known area (0.283 cm^2) removed from each plant, placed in paper bags, and submitted to drying in an oven at $\pm 65^\circ\text{C}$ until obtaining the constant dry weight. SLA is the ratio between leaf area (cm^2) and dry leaf mass (g) (Lopes et al., 2019). SLA evaluation took place at the beginning of the experiment (time zero) and 10, 20, and 30 days in each environment. Measurements were performed on fully expanded mature leaves, in 10 plants per treatment, at the beginning of the experiment (time zero), and 10, 20, and 30 days in each environment.

Determination of physiological attributes

The punctual measurements of gas exchange were obtained in the period of 08:00 to 12:00h on sunny days, at the beginning of the experiment (in the shade house- 50% shading) and on the 10th, 20th, and 30th day in each environment, using the Portable Photosynthesis System - IRGA (Infra-Red Gas Analyzer) model AGA300 (TARGAS-1, PP SYSTEMS, USA), at the saturating photon flux density used (PPFD) was $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Ballestreri et al., 2021). The flux during the measurements was around $400 \mu\text{mol s}^{-1}$. The CO_2 concentration inside the measurement chamber was around $400 \mu\text{mol mol}^{-1}$. The photosynthetic rate (A), stomatal conductance (gs), and transpiration (E) were obtained from measurements taken from leaves located in the middle third of the plants, in good phytosanitary conditions and fully expanded, in 10 plants per treatment. Through the ratio between the net assimilation rate (A) and the transpiration rate (E) the water use efficiency (WUE) was obtained.

Chlorophyll a fluorescence

The chlorophyll a fluorescence was monitored using a portable fluorometer (Junior-Pam Chlorophyll Fluorometer Walz Mess-und-Regeltechnik) on fully expanded leaves of 10 seedlings per treatment at the beginning of the experiment (in the shade house- 50% shading) and the 10th, 20th, and 30th day in each environment. The selected leaves were acclimated for 30 minutes in the dark to measure the initial fluorescence (F_0) and later exposed to pulses of saturated light at a wavelength induced by red light (peak at 650 nm) for 0.6 s, determining the maximum fluorescence (F_m), the variable fluorescence (F_v) is given by the difference between F_0 and F_m . These variables provide the maximum photochemical efficiency of photosystem II (F_v/F_m) with $F_v =$

$F_m - F_0$, and the photochemical quenching (qP) was calculated $(F_m' - F_0' \text{act.}) / (F_m' - F_0' \text{calc.})$.

Falker Chlorophyll Index

The Falker chlorophyll index (FCI) was the tool to assess chlorophyll *a* (FCI_a) and chlorophyll *b* (FCI_b). For this purpose, the portable optical chlorophyllometer ClorofiLOG (Falker®, model: CFL 3010), was used on both sides of the medium cradle, using a fully expanded and in good phytosanitary condition per plant, in 10 plants per treatment, at the beginning of the experiment (zero time) and on the 10th, 20th and leaves number day in each environment. The readings were taken between 08:00 and 12:00 h, obtaining the average per plant.

Phenotypic Plasticity Index

The phenotypic plasticity index (PPI) objective was to ascertain the degree of plasticity of the different variables studied. The index ranges from 0 to 1, where $PPI \geq 0.6$ are considered plastic variables and $PPI < 0.6$ non-plastic variables. The calculation is the difference between the maximum mean value and the minimum mean value in each treatment, divided by the maximum mean value of the variable of the same treatment, according to the formula (Valladares et al., 2005).

$$PPI = \frac{(\text{maximum average} - \text{minimum average})}{\text{maximum average}}$$

Statistical analysis

The experimental design was completely random, represented by 35 seedlings per treatment, considering three levels of light: High irradiance (HI), Moderate irradiance (MI) and Low irradiance (LI). Survival rate was calculated by the total number of seedlings per treatment. While for the other analyzes (variables) 5 repetitions were used, being randomized for the evaluations. For each of these 5 repetitions 2 seedlings were used each (pseudo-replications) (Hurlbert, 1984). The Shapiro-Wilk and Bartlett tests checked the data for either normality of the errors and homogeneity of variances, respectively, and transformed the data when they did not satisfy the assumptions. Subsequently, the variables were subjected to one-way ANOVA, considering only light environments as one factor. Tukey's test ($p < 0.05$) compared means when factors proved significant. Analyses were processed using Sigma Plot version 11.0 software (Systat Software, San Jose, CA). As a complementary approach, principal component analysis (PCA) evaluated the combination of morphophysiological traits of the species in different light environments. This study has only two dimensions, two axes labeled component 1 and component 2. PAST software version 4.02 performed the analysis. The measured plants were chosen at random to avoid auto-correlation in all cases.

Conclusions

C. sylvestris performs better under conditions of medium to high light intensity, where it converges to the greater initial growth of seedling conduction and survival in this initial period of growth. Consequently, the species is tolerant to high irradiance in the initial growth phase but does not tolerate high shading. In addition, the initial process of acclimatization of the species is associated with changes in

physiological characteristics (transpiration, stomatal conductance, and CO₂ assimilation).

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