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Morpho-physiological and anatomical responses to shade avoidance are exhibited in Surinam cherry seedlings

Respostas morfofisiológicas e anatômicas para evitar sombramento em mudas de pitangueira

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We aimed to evaluate the acclimation of *Eugenia uniflora* seedlings in response to irradiance gradient employing several morphological and photosynthetic traits. Seedlings were grown for 21 months under full sunlight (S0) and three artificial shade levels: 30% (S30), 50% (S50), and 80% (S80). Growth, biochemistry(pigments), photosynthetic, and anatomic parameters were assessed. Seedlings under S0 and S30 displayed the most significant growth, biomass accumulation, photosynthetic rate, and increased leaf thickness. S0 seedlings also had leaf anatomical traits associated with protection against total sunlight conditions, confirming irradiance tolerance. Nonetheless, the main attributes of shade acclimation in *E. uniflora* were revealed in S50 seedlings, where the increase in leaf area and the maintenance of gas exchange in this treatment achieved similar levels to seedlings under S0 and S30. Under S80, photosynthetic capacity, growth, and biomass accumulation were critically reduced. The plasticity index portrayed growth and photosynthetic traits as the most important variables that aid the adaptation of *E. uniflora* under different irradiance intensities. Conditions under S0 and S30 optimize the growth of Surinam cherry seedlings. Therefore, these conditions suit cultivating *E. uniflora* seedlings in nurseries and orchards. This study represents the first experimental approach to determining the optimal light intensity in *E. uniflora* seedlings.
Keywords: gas exchange, leaf anatomy, pigments.

O objetivo deste trabalho foi avaliar a aclimatação de mudas de *Eugenia uniflora* em resposta ao gradiente de irradiância, pela medição de características morfológicas e fotossintéticas. As mudas foram cultivadas durante 21 meses sob pleno sol (S0) e três níveis de sombra artificial: 30% (S30), 50% (S50) e 80% (S80). Foram avaliados parâmetros de crescimento, bioquímicos (pigmentos), fotossintéticos e anatômicos. Mudas sob S0 e S30 apresentaram maior crescimento, acúmulo de biomassa, taxa fotossintética e aumento da espessura foliar. Mudas em S0 também apresentaram características anatômicas foliares associadas à proteção contra condições de luz solar total, confirmando a tolerância à alta irradiância. Porém, os principais atributos de aclimatação à sombra em *E. uniflora* foram revelados nas mudas S50, onde o aumento da área foliar e a manutenção das trocas gasosas neste tratamento alcançaram níveis semelhantes às mudas sob S0 e S30. Sob S80, a capacidade fotossintética, o crescimento e o acúmulo de biomassa foram criticamente reduzidos. O índice de plasticidade retratou características de crescimento e fotossíntese como as variáveis mais importantes que auxiliam na adaptação de *E. uniflora* sob diferentes intensidades de irradiância. As condições S0 e S30 otimizam o crescimento de mudas de pitangueira. Portanto, essas condições são mais adequadas para o cultivo de mudas desta espécie em viveiros e pomares. Este estudo representa a primeira abordagem experimental para determinar a intensidade de luz ideal em mudas de *E. uniflora*.
Palavras-chaves: trocas gasosas, anatomia foliar, pigmentos.

1. INTRODUCTION

Eugenia uniflora L. (Myrtaceae), known as Surinam cherry or pitangueira, is native to the Atlantic Forest and Brazilian savannah ecosystems [1]. The species is commonly used as an ornamental tree in domestic orchards and is considered a light-demanding plant [2]; however, Surinam cherry trees can be found in shade and sun habitats [3]. Their fruits are appreciated for

fresh consumption and production of pulps, juices, ice cream, popsicles, jam, liquor, and wine [4]. As an antibacterial, anti-inflammatory, antioxidant, and analgesic, the leaf extract shows significant potential [5]. Furthermore, surinam cherry is used in traditional medicine against multiple human disorders such as sore throat, diarrhea, rheumatism, colic, headache, and hypertension [6].

Evidence suggests that *E. uniflora* seedlings exposed to various sunlight conditions can adjust their morphological and physiological characteristics to attain solar irradiation more efficiently [7-10]. These modifications are called acclimation or phenotypic plasticity, as a genotype's ability to produce diverse phenotypes under different environmental stresses [11]. The acclimation of plants under multiple light saturation levels is typically associated with leaf morphology and photosynthetic apparatus adjustments [12, 13]. However, according to Mielke et al. (2010) [2], in the case of *E. uniflora,* gas exchange attributes are an indicator of high plasticity under distinct shade environments. In order to clarify this statement, the phenotypic plasticity index (PPI) provides an in-depth understanding of traits' plasticity behavior under plant stress conditions [14]. PPI has been determined under pollution environments in Surinam Cherry, where in this case, anatomical features display the highest levels of plasticity [15]. In this sense, apparently, trait adjustment is context-dependent since some plant characteristics show unrestricted plasticity, avoiding expressing a stabilization through different regimes. Hence, irradiance knowledge and the effect of trait plasticity represent a potent tool in agriculture, optimizing field yields in cereals regarding shade avoidance [16] and increasing the biomass in fruits [17] and trees [18]. However, in Surinam cherry, the role of morpho-physiological and anatomical characteristics under different irradiance saturation levels in seedlings is partly understood.

Light saturation levels and flooding intensities have been evaluated in *E. uniflora* [2, 9, 19], demonstrating their capacity to overcome flooding conditions, partly due to complete sunlight tolerance. In these studies, researchers used different sunlight levels in *E. uniflora* seedlings: 70-75% [2, 19] and 95% [9] of shade and employed full sunlight as a control treatment. Nevertheless, the in-depth evaluation of morphological, physiological, and anatomical responses in various light regimes (including under 50% shade level) has not been assessed in *E. uniflora*. In orchards and Surinam Cherry nurseries, the minimum level of shade to capture the maximum fitness currently needs to be determined. Estimating the threshold light intensity in *E. uniflora* seedlings is critical because, once planted, more vigorous seedlings will be healthier and more able to adapt to environmental changes beyond performance [20-22].

Surinam cherry has been detected in the open canopy, forest borders, deep shade forests, or entire sunlight orchards [4], indicating its wide range of light environment distribution. Hence, we hypothesize that *E. uniflora* has phenotypic plasticity to allow acclimation under different light intensities. The acclimation here was accessed through a set of ecophysiological traits linked to changes in light supply.

2. MATERIALS AND METHODS

2.1 Plant material and treatments establishment

Seeds were collected from a Surinam cherry tree in Coronel Vivida, Paraná, Brazil (25°57'36" S, 52°35'22" W, 630 m.a.s.l). Plants were cultivated in pots (2.0 L) in a nursery with 50% shade for 70 days. After that, seedlings were transplanted in pots (40.0 L of capacity) for 21 months (treatment evaluation). Plots were occupied with a mixture of soil (62.5%), commercial substrate (31.5%), and vermiculite (6.0%), and submitted to full sun (S0: 1247.3 PPFD) and three artificial shade levels: 30% (S30: 704.4 PPFD), 50% (S50: 607.3 PPFD) and 80% (S80: 253.1 PPFD) of solar irradiation intercepted by black screens, with 12 replications of each treatment. Two NPK fertilization formulations (8-28-16) with 20 g per plant at 12 and 18 months after transplantation were employed. Regular (daily) irrigation and monthly manual weed control were performed. The total assessment was evaluated at the end of the experiment (21 months after transplantation) during the spring season (November). Non-destructive traits,

photosynthetic, and gas exchange traits were measured a day before destructive parameters (dry mass, biochemical, and anatomical leaf traits). In the case of leave analysis (biochemical, photosynthetic, gas exchange and anatomical parameters), fully expanded leaves that did not overlap with other leaves from the upper third of each plant were randomly selected.

2.2 Growth traits

In order to test the hypothesis that *E. uniflora* seedlings have growth plasticity under several light intensities, growth evaluation was performed by the difference in height and diameter measured at the experiment's initial and final. The leaf area was measured employing the LI-3100 meter (Li-Cor, Inc.) in 100 leaves of four plants for each treatment. In addition, the total number of leaves per plant was counted. Roots and shoots were oven-dried at 60 ºC until they reached a constant mass, obtaining root and shoot dry mass. Dickson's quality index (DQI) was calculated as:

$$DQI = [\text{total dry mass}/(RSD + RSR)] \quad \text{Equation 1}$$

RSD represents the ratio between shoot height and stem diameter, and RSR is the ratio between shoot and root dry mass [23]. A larger DQI value represents a better seedling vigor, representing a balance between shoot and root biomass [24].

2.3 Biochemical traits

To test photosynthetic concentrations under irradiance levels, chlorophyll *a*, chlorophyll *b*, and carotenoids from two leaves of 12 plants per treatment were analyzed. Hence, one disc of 0.6 cm diameter was removed from each leaf and immersed in 5.0 mL of dimethyl sulfoxide; then, the leaf was placed in water at 65 °C until 18 hours, when disks were translucent, under darkness conditions. Consequently, the absorbance lectures were measured on a UV/VIS spectrophotometer (Shimadzu model UV-1800) at 480 nm (carotenoids), 649.1 nm (chlorophyll *a*), and 665.1 nm (chlorophyll *b*), respectively. Subsequently, the concentrations of each pigment were calculated [25].

2.4 Photosynthetic and gas exchange traits

Regarding gas exchange adjustments in *E. uniflora*, we calculated these parameters using the infrared gas analyzer (IRGA) model LC-pro (ADC BioScientific Ltda., UK) between 9 a.m. and 11 a.m. on a sunny day. Three leaves fully expanded were evaluated in five plants per treatment. Net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s), intracellular CO_2 concentration (Ci), and photosynthetically active radiation (PAR) were evaluated. The instantaneous carboxylation efficiency (EiC) was calculated through the relationship between A/Ci.

Finally, chlorophyll *a* fluorescence was determined using a fluorometer (Multi-Mode Chlorophyll Fluorometer®, Model OS5p). The evaluations were performed between 9:00 a.m. and 11:00 a.m. on three fully expanded laminae leaves of five plants per treatment. In this time-lapse, one pulse of light saturation was exposed to each sample. Initial fluorescence (F'), maximum fluorescence (Fm), the maximum quantum yield of photosystem II [$Y_{(II)}$], and electron transport rate (ETR) were obtained.

2.5 Anatomical leaf traits

To evaluate the physical structure modifications in leaves under different irradiance regimes, three leaves were collected from five plants per treatment, which were fixed in FAA_{50} [formaldehyde, acetic acid, ethanol (50%); 1: 1: 18, vol] for one day (24 hrs), bathed in 50% ethanol and conserved in 70% ethanol [26]. 0.5 cm^2 fragments of the samples were cut in the inner

part of the leaf, dehydrated with ethanol-graded series (80%, 90%, and 95%, respectively), and then immersed in methacrylate (Historesina, Leica Instruments). Cross-sections (with 8.0 µm thickness) were obtained and stained in toluidine blue (0.12%); finally, the fragments were fixed on a slide and coverslip using glass varnish. A photomicroscope (Zeiss Axiolab) and a digital camera (Sony Cybershot 7.2mb) were used for image digitalization. The abaxial and adaxial epidermis, spongy and palisade parenchyma, and limbus thickness were measured in nine measurements per sample.

2.6 Phenotypic plasticity index and statistical analysis

The phenotypic plasticity index (PPI) was calculated for each trait according to Valladares et al. (2005) [14] formula, based on the maximum and minimal means of the four light treatments (Equation 2).

$$PPI = [M-m]/m \qquad \text{Equation 2}$$

Where M was the maximum mean and m was the minimum average of the light treatments. In our case, it was only considered to be plastic when the treatment effect was significant [27]. After that, each plasticity index were grouped by the principal type of variable (biochemistry, growth, photosynthetic and gas exchange, leaf anatomy). PPI varies from zero to one, where low values demonstrate low plasticity in the parameter assesed.

The data analysis was subjected to employing the normality test (Shapiro Wilk) and homogeneity of variance (Bartlett). The box-Cox transformation was required when necessary, followed by ANOVA in a completely random design, and the Scott-Knott test was applied. All the measurements were processed using the R program [28].

3. RESULTS AND DISCUSSION

3.1 Growth traits

Surinam cherry seedlings submitted to shade conditions showed more significant height growth than plants under full sunlight. Nonetheless, the diameter of the stem was greater in seedlings under S0 and S30. Therefore, these results explain seedlings' etiolation under deep shade (S50 and S80), increasing their stem height and, at the same time, dropping their diameter. PhyB, also known as light receptor, is the molecule thar allows plants to detect low red/far-red light wavelengths when they are shaded [12, 29], which in turn causes plants to produce auxin [30] and gaining height in order to access to sunlight.

The dry shoot mass under S30 and the root dry mass under S0 and S30 were higher than in the other treatments (Figure 1). The total dry mass was more elevated in S0 and S30 and decreased gradually under shade intensity. According to the results evaluated by Mielke and Schaffer (2010) [2], the biomass rose due to the partial effect of a high-light environment in *E. uniflora* seedlings. Similar morphological results were also established in *Acca sewolliana* [10] and *Araucaria angustifolia* [8], demonstrating that all these plants are sun-demanding species.

Leaf area decreased at S0 and increased at S50 (Figure 1). The total number of leaves was higher in seedlings at S80. Under S80, leaf growth was limited. The Dickson quality index was more significant in the seedlings under S0 and S30 because of the higher growth (height and diameter) and dry mass (shoot and root) in these treatments. Thus, total dry mass represented an essential trait for shade tolerance plant species detection, considering stem height indicated an etiolation result of phototropism response and shade avoidance [30]. Due to the optimal growth under full sunlight and low shade regimes (S30), *E. uniflora* may be sowed early during restoration programs in subtropical forests. Hence, it might be considered a potent pioneer and early secondary species in successional stages [9].

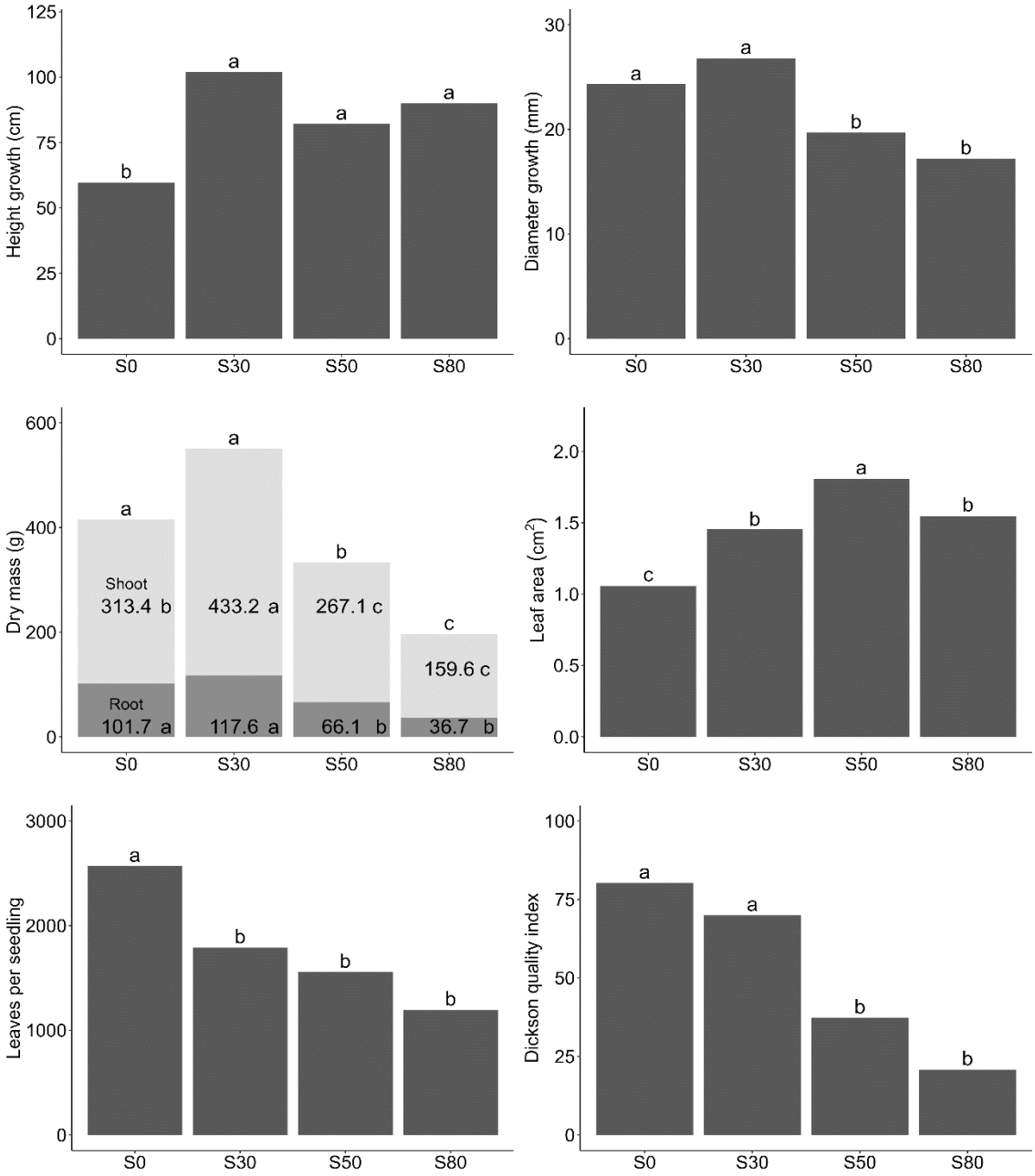

Figure 1. Growth parameters of Eugenia uniflora *seedlings under irradiance gradient in full sun (S0) and 30% (S30), 50% (S50), and 80% (S80) shade. Leaf area data were transformed by BoxCox. Bars represent the averages of each treatment and contain different letters that differ from each other by the Scott-Knott test (p≤0.05).*

3.2 Biochemical traits

The photosynthetic pigments, chlorophyll *a*, and total chlorophyll content observed in the plants under the S0 were lower than in the three shade treatments (Figure 2). The chlorophyll *b* content and carotenoids did not differ among the light saturation levels. Similar results in chlorophyll *a* and total chlorophyll content were observed in *E. uniflora* [2] under full sun against 70% shade.

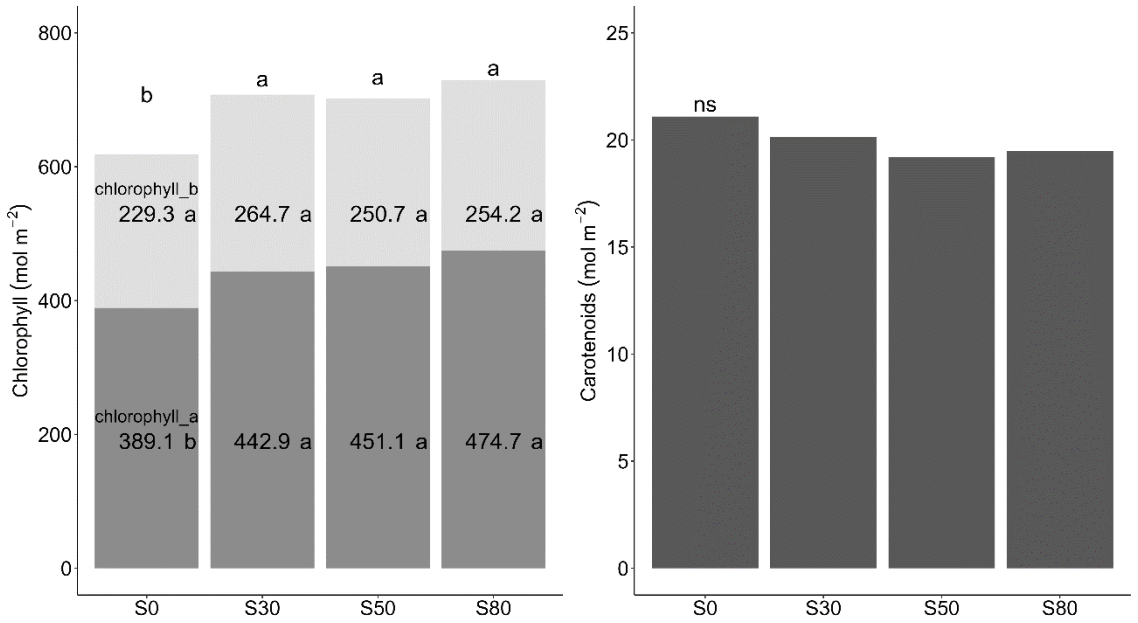

Figure 2. Photosynthetic molecules content of Eugenia uniflora *leaves under irradiance gradient, in full sun (S0) and 30% (S30), 50% (S50), and 80% (S80) shade. Bars represent the averages of each treatment and contain different letters that differ from each other by the Scott-Knott test ($p \leq 0.05$).*

Previous studies portrayed that minimum radiance induces higher chlorophyll pigment content per mass unit [11], being part of the shade stress response. Various tree species displayed different chlorophyll levels under shade conditions [7, 10, 31]. Otherwise, although carotenoids are crucial in photoprotection under high-light regimes, some plants do not change these contents under light stress [31]. Our results suggest that Surinam cherry has a likely low plasticity in the pigments content during different shade levels.

3.2 Photosynthetic and gas exchange traits

In terms of light use, the net CO_2 assimilation rate and the instantaneous carboxylation efficiency were lower in S80 due to the PAR restriction, limiting photosynthesis (Figure 3). On the contrary, seedlings under S50 might acclimate the photosynthetic apparatus, demonstrated by the most significant increase in transpiration rate and stomatal conductance. The lower transpiration rates in S0, S30, and S80 were associated with decreased stomatal conductance (g_s). Under S0 and S30, the reduced stomatal conductance indicates that higher irradiance connected directly with more elevated temperature caused stomatal closure to avoid water loss [11]; however, in S80 weather were probably under low temperature in comparison with the other irradiance treatments and the evaporative demand was not enough, reducing g_s. Similar results in stomatal conductance between S0 and S30 in *E. uniflora* were previously determined [2].

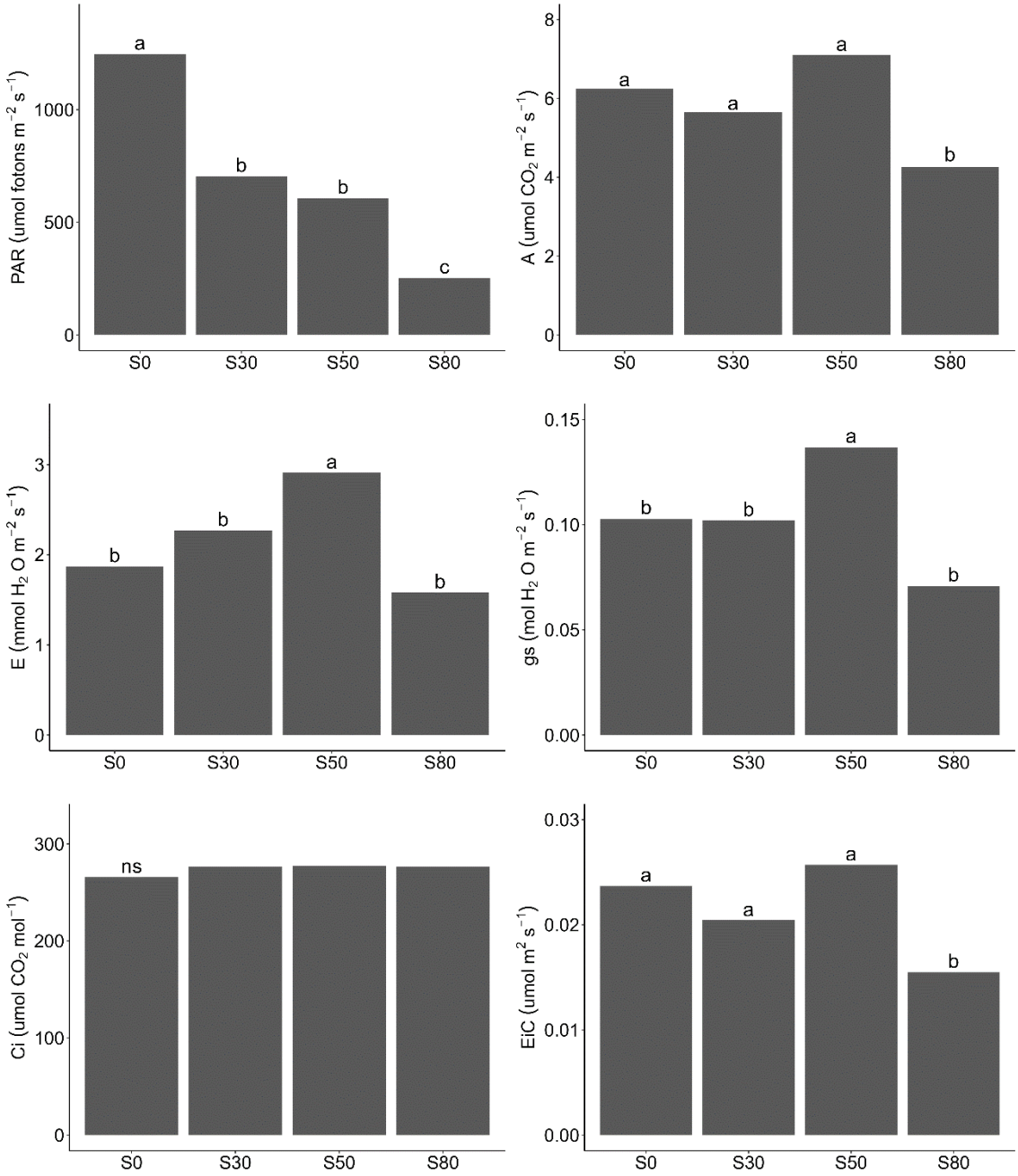

Figure 3. Gas exchange parameters of Eugenia uniflora *leaves under irradiance gradient in full sun (S0) and 30% (S30), 50% (S50), and 80% (S80) shade. Ci data were transformed by BoxCox. Bars represent the averages of each treatment and contain different letters that differ from each other by the Scott-Knott test ($p \leq 0.05$). Photosynthetically active radiation (PAR); net CO_2 assimilation rate (A); transpiration rate (E); stomatal conductance (g_s); intercellular CO_2 concentration (Ci); and carboxylation efficiency (EiC).*

In the case of chlorophyll fluorescence, there are divergent results compared to previous studies (Figure 4). Savacinski et al. (2023) [9] showed a significant carboxylation efficiency in Surinam cherry at 95% radiance limitation compared to control seedlings after 12 days of treatment establishment. In addition, Mielke and Schaffer (2011) [19], indicated that stomatal conductance increases during the 11 days after a 70-75% shade regime in *E. cauliflora* seedlings. In this sense, the importance of measurements during the initial days of shade establishment may be considered for further studies.

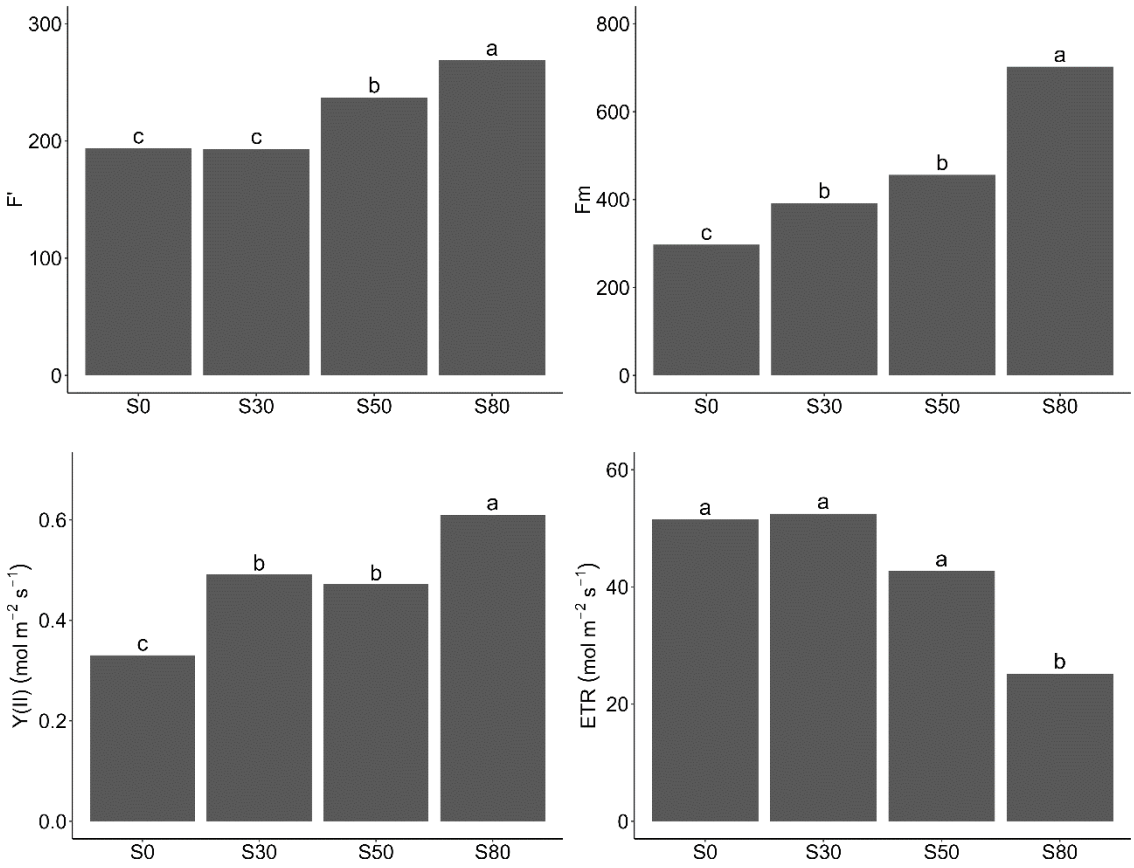

Figure 4. Fluorescence of chlorophyll parameters from Eugenia uniflora *leaves under irradiance gradient in full sun (S0) and 30% (S30), 50% (S50), and 80% (S80) shade. Bars represent the averages of each treatment and contain different letters that differ from each other by the Scott-Knott test (p≤0.05). Initial fluorescence (F'); maximum fluorescence (Fm); effective quantum yield of photosystem II (Y(II)); and electron transport rate (ETR).*

The reduction of the net CO_2 assimilation rate after 21 months in seedlings under S80 indicates severe limitations in the photochemical phase of photosynthesis, leading to a restriction of ATP and NADPH production [13]. In this sense, the electron transport rate (ETR) during steady state was lower in seedlings under S80, even though there was a maximum quantum yield of photosystem II [$Y_{(II)}$]. Hence, although the PSII was efficient, substantial limitations in incident irradiation occurred, needing to be increased to maintain the ETR at the level of the other treatments. This was also evidenced by the lowest instantaneous carboxylation efficiency (EiC), demonstrating that the seedlings under S80 intensity could not assimilate a large amount of CO_2. Consequently, this molecule was accumulated in intercellular space (Ci) [13]. Moreover, this treatment had more significant chlorophyll fluorescence (F' and Fm), demonstrating higher energy dissipation and less use of light energy for photosynthesis [32]. In contrast, the lower $Y_{(II)}$ in the S0 shows that a large part of the absorbed light has been unconverted into photochemical energy. This, coupled with higher ETR and lower chlorophyll fluorescence in S0, suggests that the photosynthetic apparatus of *E. uniflora* can deal with full sunlight energy, and also might be a prevention strategy in the case of high irradiation stress environments [33].

3.3 Anatomical leaf traits

The leaf blade thickness and each leaf structure were thinner under S50 and S80 compared to other treatments (Figure 5). Anatomically, *E. uniflora* leaves have a uniseriate epidermis, with adaxial cells larger than abaxial cells. The cuticle thickness on the adaxial surface is visible, being

inconspicuous under S50 and S80. Under S0, intensely stained compounds were observed, while under S50 and S80, the epidermal cells were hyaline (Figure 6). We also detected secretory cavities on both sides of the epidermis in all Surinam cherry leaves. The mesophyll is dorsiventral, consisting of two layers of palisade parenchyma (except under S80, in which only one layer of this tissue was observed) and several layers of spongy parenchyma. In response to high shade intensity (80%), palisade and spongy parenchyma presented a floppy arrangement, likely reflecting an increase in intercellular spaces. Variation in the thickness of palisade parenchyma has been reported previously in *E. uniflora*, even during environmental pollution conditions [15].

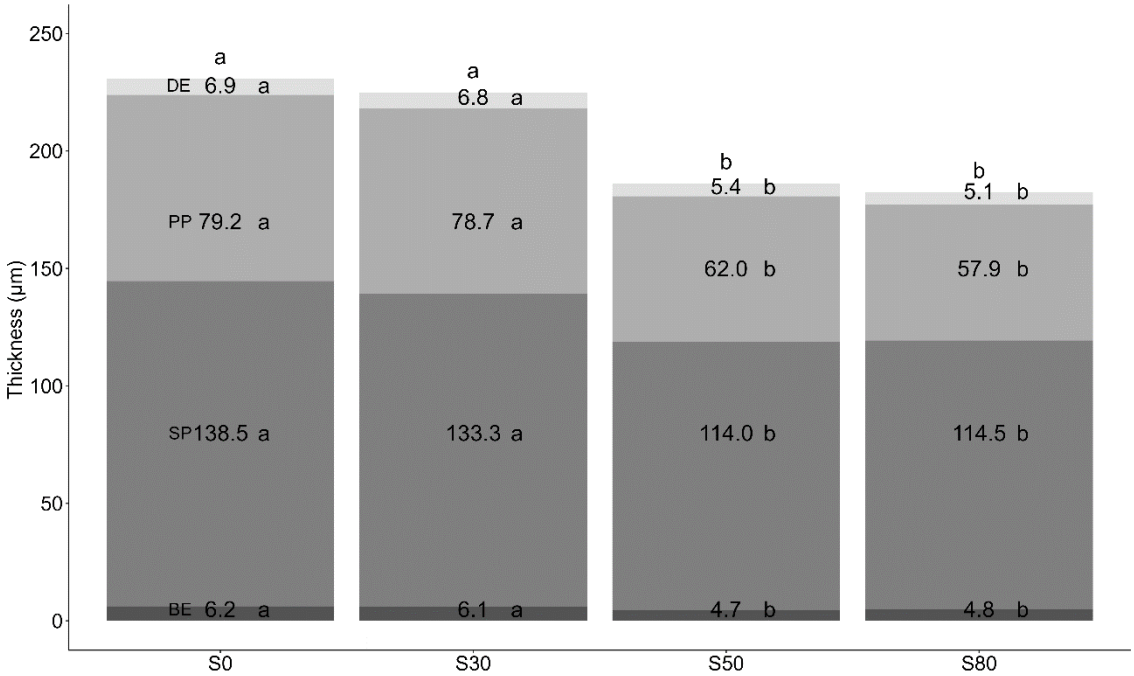

Figure 5. The thickness of foliar portions and structural leaf anatomy of Eugenia uniflora *leaves under full sun (S0) and 30% (S30), 50% (S50), and 80% (S80) shade conditions. Averages followed by different letters differ by the Scott-Knott test (p≤0.05). BE abaxial epidermis; SP: spongy parenchyma; PP: palisade parenchyma; DE: adaxial epidermis).*

The leaves exposed to S50 and S80 shade had lower layers of palisade parenchyma, an increase in intercellular spaces, less cuticle thickness, and reduced leaf thickness (Figure 6). The thinner leaves in plants grown under shade allowed a more efficient light distribution through the mesophyll [34]. The proportion of tissues and intercellular spaces changes are associated with regulating light diffusion and gases inside the leaves [35]. Besides, the thinner leaves may have contributed to diffuse light getting through the canopy and making it available to leaves in the basal part of the plant. This effect reduces respiration and improves the net CO_2 assimilation in the whole plant [36]. Under S50, larger leaves might enhance photosynthesis, providing a large surface area associated with the smaller thickness. On the other hand, the greater cuticle thickness in the leaves under S0 and S30 may protect the plant against water loss, minimizing heating and other damages caused by a possible irradiance excess [34].

It has been well-established that in comparison with sun leaves, shaded ones have higher leaf area, and chlorophyll content per mass unit and less thick, possessing fewer proteins, including Rubisco, in the chloroplasts, and a greater amount of antenna complex, which allows them to maximize the total light absorption available [37-39]. Hence, this response allows plants to deal with intermediate or restricted light conditions [35].

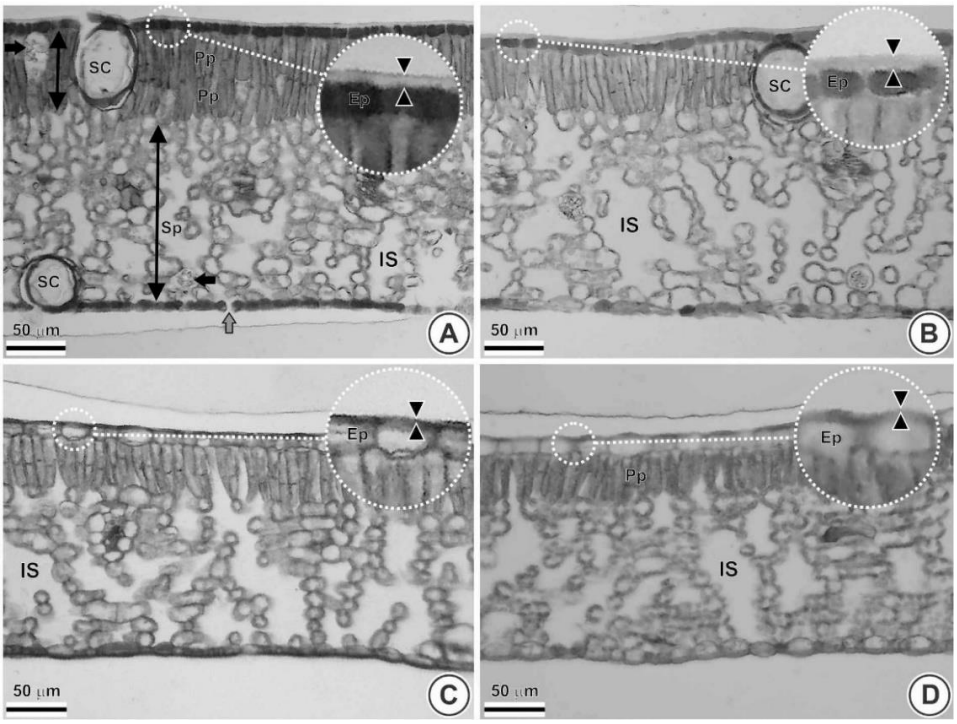

Figure 6. The thickness of foliar portions and structural leaf anatomy of Eugenia uniflora *leaves under the full sun (S0) and 30% (S30), 50% (S50), and 80% (S80) shade. Full sun (A), 30% (B), 50% (C) and 80% (D) shade. Ep (epidermis); Pp (palisade parenchyma); Sp (Spongy parenchyma); SC (Secretory cavities); IS (intercellular spaces); arrowhead (stomatas)*

3.4 Phenotypic plasticity index

Higher phenotypic plasticity exhibited by *E. uniflora* seedlings under different irradiance intensities was mostly related to morphological, photosynthesis, and gas exchange traits (Table 1). These results suggest that Surinam cherry seedlings exhibit a partial plastic response, where distinct sets of variables displayed different shade tolerance, being low for anatomical and biochemical plant characteristics. The weak plasticity for pigment traits is in concordance with a previous study in holly (*Ilex aquifolium*) [14] under shade conditions. Conversely, the anatomical features of the Surinam cherry displayed the highest levels of plasticity under air pollution stress [15]. Another case was *Myrcia Amazonia*, a *Myrtaceae* species, which demonstrated a significant PPI in chlorophyll content under contrasting Brazilian seasons [40]. This underscores the species-specific and stress-dependent nature of phenotypic plasticity in plant features. The phenotypic response of *E. uniflora* seedlings was higher in morphological growth variables, a characteristic commonly associated with shade-tolerant trees [14]. Certain traits with significant plastic behavior are likely under epigenetic control [41], while others may be predominantly governed by genetic factors. For instance, the low plasticity observed in stomatal speed in woody species under different light conditions reflects a strong genetic regulation [42]. This variation in plasticity emphasizes the intricate interplay between species-specific traits, environmental stressors, and the mechanisms that govern plant responses.

Table 1. Mean phenotypic plasticity index (PPI) in Eugenia uniflora *seedlings subjected to different light environment in four variable groups.*

Type of variable	**PPI**
Growth (morphological)	0.42 ± 0.03 a
Photosynthesis and gas exchange	0.39 ± 0.02 a
Leaf anatomy	0.20 ± 0.10 b
Biochemical (pigments)	0.14 ± 0.10 b

Averages followed by different letters differ by the Scott-Knott test ($p \leq 0.05$).

Our findings highlight the evidence that *E. uniflora* has an intermediate tolerance shade, as demonstrated mainly by changes in growth, photosynthetic and gas exchange parameters [36, 37]. These valuable results demonstrate that seedling production in nurseries and plantings in orchards or reforestation should be carried out up to S30. Moreover, morphoanatomical barriers were evident over total irradiance on the leaves, adaptanding them against this condition. These results explain why Surinam cherry is more naturally observed at the edges of forest fragments and open areas, but also in the forest understory of the Atlantic Forest and Cerrado Brazilian Biome [4]. Thus, our initial hypothesis that *E. uniflora* has sufficient plasticity to acclimate to shade was confirmed, except for deep shade intensity (80%).

4. CONCLUSIONS

This study proves that *Eugenia uniflora* is a sun-demanding species that achieves higher biomass accumulation, photosynthetic rates, and leaf thickness when grown under full sunlight or low shade regimes. While seedlings under medium irradiance demonstrated some efficiency with morphological and physiological modifications, they ultimately displayed a biomass reduction. This decline was particularly evident in plants under high shade conditions. Anatomical and biochemical traits that displayed low plasticity were probably stronger regulated by genes. Therefore, for optimal production of *E. uniflora* seedlings in nurseries and cultivation of intercropped other tree species, it is recommended to grow them under full sun or until 30% shade. By understanding the light requirements of these plant trees, agroforestry and reforestation programs can improve management practices and ensure better productivity in *E. uniflora* cultivation.

5. ACKNOWLEDGMENTS

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