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ORIGINAL ARTICLE – Silvticulture

Effects of Artificial Shading and Plant Density on *Corymbia Citriodora* Seedling Characteristics

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Abstract

The objective of this work was to evaluate the influence of colored shade mesh and plant density on the morphological characteristics of *Corymbia citriodora* seedlings. The highest radiation efficiency was found in the blue mesh, 7.01 g MJ^{-1} and 5.72 g MJ^{-1} for average and high density respectively. For dry matter and leaf area index at high density, the treatment without mesh followed by the red mesh was the best. The chlorophyll index did not differ between treatments, with mean values of 206.4, 59.1 and 262.0 for chlorophyll a, b and total, respectively. Using principal component analysis, we found the total variance to be 70%. The use of colored shade mesh and plant density altered the radiation use efficiency and dry matter accumulation. Leaf area index was affected only by plant density. The red mesh resulted in the most similar values to the treatment without mesh.

Keywords: Leaf area index, air temperature, radiation use efficiency.

1. INTRODUCTION

The growing demand for forest products requires an increase in the production of seedlings of species with a high potential output and relatively short rotation period, in order to establish productive and sustainable forest systems (Eloy et al., 2013). Among the tree species that are attractive to the industry, *Corymbia citriodora* (Hook) is distinctive, being characterized by fast growth, adaptability to different climates, silvicultural quality, and extraction of essential oil (Morais et al., 2019).

Growth conditions such as plant density, substrate type, water availability, water quality, and intensity of solar radiation are fundamental for developing high-quality seedlings. In particular, solar radiation plays a crucial role in plant growth and development since it is the primary source of energy for photosynthesis (Albuquerque et al., 2016) and dry matter accumulation. Plants that grow under restricted solar radiation may become more efficient (Coelho et al., 2014a) to compensate for the reduced levels of solar radiation. For example, as important components of plant acclimatization, proportions of chloroplast pigments may be adjusted (Martins et al., 2010).

Artificial nursery shading which modifies the quality and intensity of solar radiation may help produce more uniform seedlings (Caron et al., 2010). The use of colored shade mesh is therefore an alternative system for seedling production, changing the spectrum of solar radiation the seedlings are exposed to and fractioning direct radiation into diffuse radiation (Corrêa et al., 2012). Cultivation of seedlings under colored shade mesh causes photomorphogenic changes in plants as wavelengths are selectively transmitted, influencing plant growth, development, and physiological function (Tsormpatsidis et al., 2008).

Little is known about the effects of colored shade mesh (blue and red) and plant density on the radiation use, plant growth, and morphological characteristics of tree seedlings. In this study, we hypothesized that colored mesh and plant density modifies the efficiency of radiation use in *C. citriodora* plants, affecting their characteristics through microclimate changes and plant interactions. Thus, the objective of this study was to evaluate the influence of colored shade mesh and plant density on the morphological characteristics of *Corymbia citriodora* seedlings.

2. MATERIALS AND METHODS

2.1. Study area and experimental design

This study was conducted from May to September 2018 in a greenhouse at the city of Frederico Westphalen, on geographical coordinates of 27° 22" S, 53° 25" W and altitude of 480m. The characteristic climate type of the region is Cfa, according to the Köppen climate classification (Alvares et al., 2013): the three coldest months of the year have temperatures ranging from –3 to 18 °C, with an average annual temperature of approximately 22 °C, and rainfall occurring year-round.

The experimental design was completely randomized and arranged in a 2 X 3 factorial scheme. Two plant densities were used: high density (402 plants/m²) with all tray cells occupied (96 plants) and medium density (201 plants/m²) with 50% of the planting tray occupied (48 plants). Three shade options were used inside the greenhouse: red shade mesh with 30% solar radiation restriction, blue shade mesh with 30% solar radiation restriction, and no mesh, with three repetitions. All treatments were organized in order to have the same environmental conditions inside the greenhouse. Each treatment was applied to two trays (with the same spacing) for the duration of the study. At each evaluation, 3 plants were removed per treatment, corresponding to 1.6% and 3.1% of the plants from the high and medium density trays, respectively.

C. citrodora seeds were obtained from the Forestry Science and Research Institute. On 22 May, 2018, seeds were sown directly into 90 cm³ conical shaped tubes with six open-bottom polypropylene striations filled with an organic substrate (Tecnomax®). During the study, irrigation was performed daily, manually, and always while maintaining the substrate field capacity for all treatments.

2.2. Solar radiation transmissivity and air temperature

The transmissivity of solar radiation through the inside of the greenhouse and subsequently through the shade mesh structure was measured at each plant growth assessment. The incident solar radiation (W m^{-2}) was measured outdoors, inside the greenhouse, and below each mesh structure. This was completed using a portable sensor pyranometer (LICOR PY32164) coupled to a data logger (LICOR 140), which determined the global solar radiation. Measurements were recorded daily at 9, 10, 14, 15, and 16h. The transmissivity of solar radiation was calculated based on the following equation:

$$T = \frac{I}{I_0} \times 100 \tag{1}$$

Where T = transmissivity of solar radiation, I = solar radiation incident under the canopy of *C. citriodora* meshes and seedlings; I_0 = global solar radiation incident inside the greenhouse and on the meshes.

The average daily air temperature was calculated using the maximum and minimum temperatures. The maximum and minimum air temperature data were manually collected at a daily time-step using a thermo-hygrometer (Th-02). Values were measured inside the greenhouse and within each mesh structure in order to standardize the measurement of these variables.

To determine the cumulative degree days (CDD), the lower basal temperature (BT) of 8.7 °C for eucalyptus species was used, according to the findings of Freitas et al. (2017). For the sum of the CDD, the following equation was used:

$$CDD = \sum(Tavg - Tb) \tag{2}$$

Where: CDD = cumulative degree days; Tavg = average air temperature; Tb = base temperature.

2.3. Radiation use efficiency and leaf area index

To determine the radiation use efficiency (RUE) of *C. citriodora* seedlings, incident global solar radiation (MJm^{-2}) data were collected from the National Meteorological Institute (INMET) automatic weather station, located approximately 200 m from the experiment. Corrections to this data were made according to the transmissivity values.

The RUE was determined by assessing the relationship between the average accumulated dry matter and the intercepted photosynthetically active radiation (IPAR), according to the methodology proposed by Monteith and Moss, 1977. The incident photosynthetically active radiation (PAR) was estimated to be 45% of the global solar radiation (Assis and Mendez, 1989). The estimate of IPAR was determined using the model described by Varlet-Grancher et al. (1989). The extinction coefficients used for the study were those found by Schwerz et al. (2019): values of 0.18 and 0.13 for high and medium densities, respectively.

The leaf area index (LAI) was determined by the ratio of the total leaf area (LA) and the surface area (AS) explored by each plant evaluated in each treatment. LA was assessed using the methodology of Jadoski et al. (2012). All leaves were removed from the plant and consequently digitized on a printer together with a reference scale. ImageJ® software (Ferreira and Rasb, 2012), was then used to measure the LA.

2.4. Plant collection

Growth evaluations were performed every 15 days; at each evaluation, three plants were collected from each treatment. The first plant collection was performed when the plants had two pairs of leaves, corresponding to 44 days after emergence (DAE). The last collection was performed when the plants were ≥ 25 cm tall with a stem diameter ≥ 2.5 mm, when they were ready for planting (Sturion et al., 2000) and corresponding to 155 DAE, totaling 30 plants evaluated throughout the study.

After collection, the plants were taken to the laboratory and separated into leaves, stem, root, and senescent leaves. Leaves were considered senescent when 50% of their area was dead or compromised. After the samples were separated, they were placed in paper bags and dried at 60 ºC until they reached a constant mass. The sum of the dry matter of each plant component was taken to be the total dry matter of the plant.

Based on the dry matter, LA and LAI results, the crop growth analysis was performed. The calculated variables were: absolute growth rate (AGR), relative growth rate (RGR), net assimilation rate (NAR), biological productivy (BP), leaf weight ratio (RWL), leaf area ratio (LAR) and specific leaf area (SLA), according to the methodology proposed by Benincasa (2003).

2.5. Chlorophyll index

Chlorophyll a (Chl A), b (Chl B) and total (Chl Total) content in *C. citriodora* plants was measured using a portable chlorophyll meter (ChlorofiLOG - CFL1030). For chlorophyll ratio values, calculations were by chlorophyll division (Chl A / Chl B). This measurement was taken when the plants were ready for planting, that is, at the end of the seedling cycle, in order to quantify the effects of the shade mesh. For this evaluation, three plants were selected from each treatment, and the measurement was performed on the third leaf from the top of the plant.

2.6. Statistical analysis

The data was statistically analyzed using the R software (R Core Team, 2019), using the ExpDes package (Ferreira et al., 2018). The data were initially submitted to variance analysis (ANOVA) to determine the effects of possible interactions. When there was significance, it was verified by the F tests ($p < 0.05$). The were compared by the Tukey test ($p < 0.05$). The normality of the residue and the homoscedasticity of the residual variances were performed by the Shapiro-Wilk and Bartlett tests, respectively, where no violation of the assumptions was verified.

For the RUE calculations, a linear regression between the cumulative dry matter intercepted solar radiation was fitted (Monteith & Moss, 1977). For the same amount of intercepted solar radiation, different production of dry matter can occur. For the relationship between dry matter and LAI and CDD, polynomial quadratic equations were adjusted.

Principal component analysis (PCA) was used to reduce the dimensionality of the data into a few principal components, allowing the interpretation of the relationships between variables and treatments. The Bartlett sphericity test was performed to test the null hypothesis (H_0), in order to verify if the variables were correlated or if the correlation matrix was an identity matrix (Raykov and Marcoulides, 2008). The data were also submitted to the Kaiser-Meyer-Olkin (KMO) sample adequacy test, in which a KMO value of 0.39 was observed. Thus, the use of the PCA technique was appropriate based on the Bartlett test, while the result from the KMO test suggests the use of the technique may cause some loss of information.

Two PCs were selected considering all variables, and two-dimensional, ordering graphs were made, in which the axes were designated as main component 1 (PC1) and main component 2 (PC2).

3. RESULTS

3.1. Meteorological conditions

According to the statistical analysis, there was no significant difference in temperature between the different treatments. The average daily temperature was 20.2 °C, with an overall temperature range of 10.4 °C to 29.3 °C (Figure 1a).

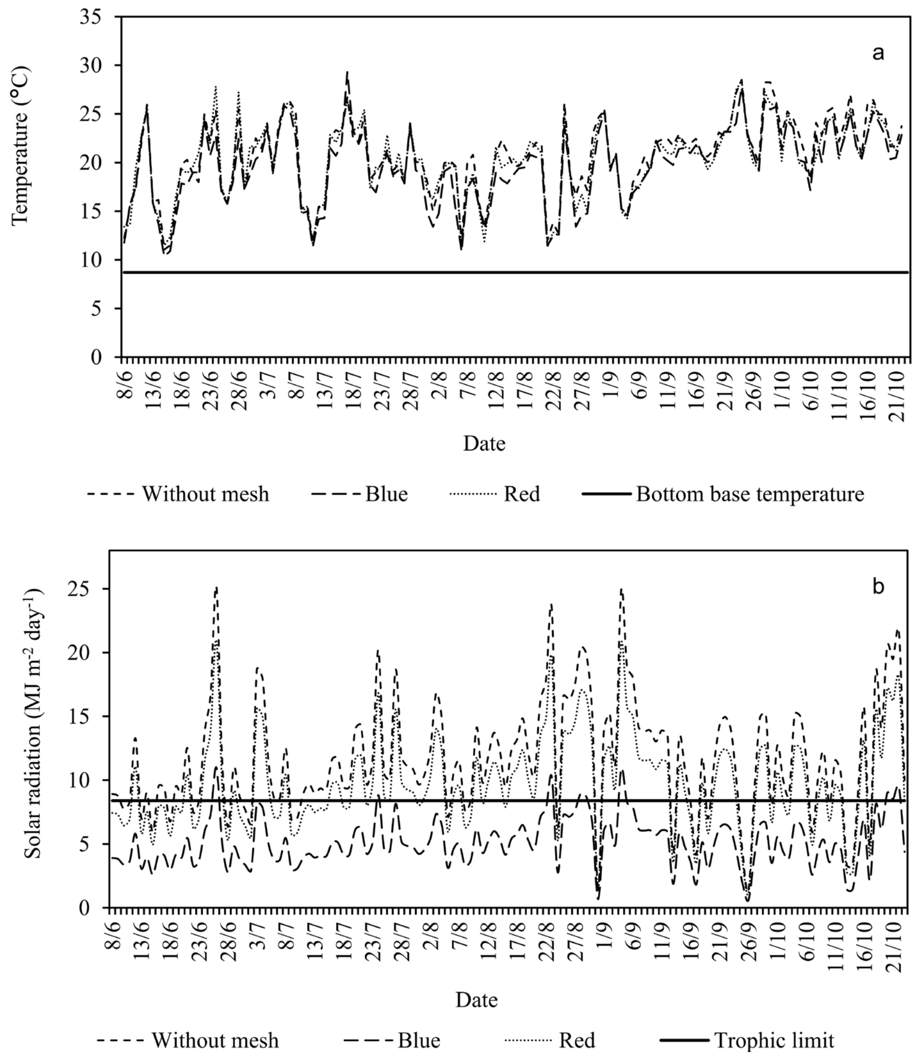

Figure 1. Average air temperature (a) and incident global solar radiation (b) values throughout the study. Horizontal lines indicate inferior base temperature (a) and trophic Limit (b) for *Corymbia citriodora*.

The average global solar radiation flux was 11.7 MJ m^2 day^{-1} (range 1.4–25.2 MJ m^2 day^{-1}; without mesh), 9.8 MJ m^2 day^{-1} (range 1.1–20.9 MJ m^2 day^{-1}; red mesh), and 5.1 MJ m^2 day^{-1} (range 0.6–10.9 MJ m^2 day^{-1}; blue mesh). Total cumulative solar radiation was 1621 MJ m^2, 1350 MJ m^2, and 708 MJ m^2, for no red, and blue mesh, respectively (Figure 1b).

Transmissivity of solar radiation values for each treatment differed significantly (Figure 2). The transmissivity inside the greenhouse was considered 100%, the red mesh had a transmissivity of 83.3%, while the blue mesh showed 43.7% transmissivity.

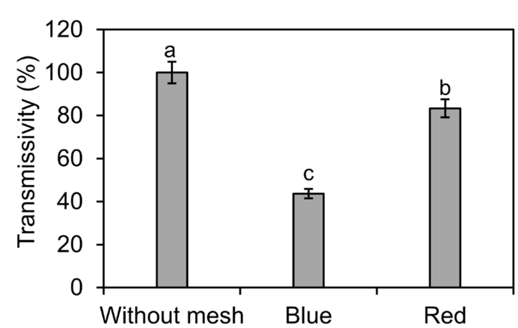

Figure 2. Solar radiation transmissivity (%) for the treatments Red mesh, blue mesh and no mesh. Different letters indicate significant differences (P<0.05) according to Tukey test.

3.2. Radiation use efficiency

The use of different shade mesh colors and cultivation without mesh led to variation in the RUE values (Figure 3). The highest RUE values were found for plants in the blue mesh structure at medium and high densities 7.01 g MJ^{-1} and 5.72 g MJ^{-1} respectively (Figure 3b). However, the lowest RUE values (2.75 g MJ^{-1} and 3.26 g MJ^{-1}) were found in high density treatments without mesh and under red mesh, respectively (Figure 3c).

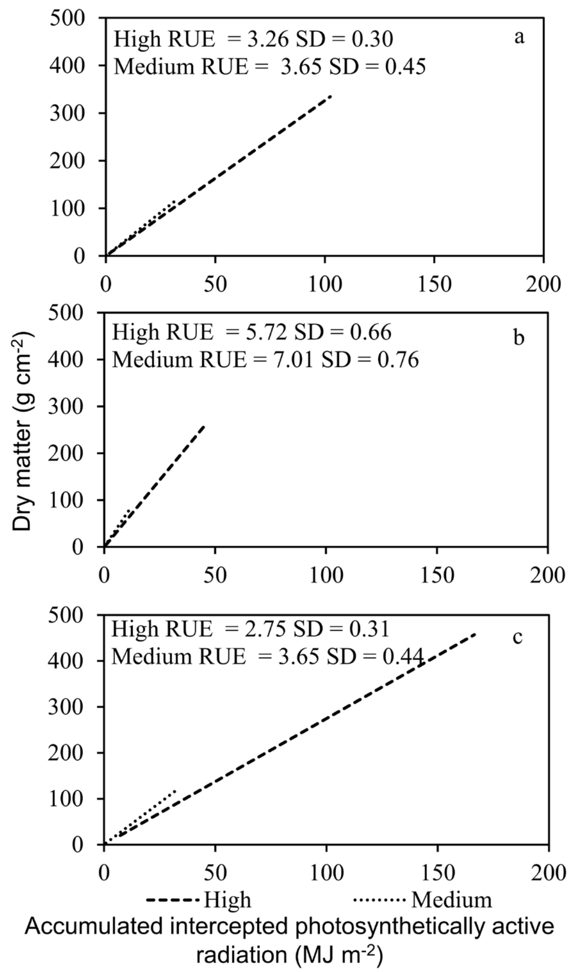

Figure 3. Radiation use efficiency (RUE, g MJ^{-1}) of *Corymbia citriodora* seedlings produced under colored (blue and red) mesh and without mesh, under high and medium density. Red mesh (a), blue mesh (b) and no mesh (c). SD is the standard deviation of radiation use efficiency.

3.3. Plant Growth

Figure 4 shows the values of dry matter and LAI as a function of CDD during the production cycle of *C. citriodora* seedlings, to which quadratic polynomial equations were fitted. Greater dry matter was observed for the high density treatment with no mesh 1.6 g $plant^{-1}$ with 1,469.5 °C CDD (Figure 4a). Regarding the shaded treatments, the highest value for dry matter was found for high density planting under red mesh 1.1 g $plant^{-1}$ of dry matter with 1,423.4 °C CDD (Figure 4c). Similarly, for medium density planting, the highest value was also found for red mesh: 0.9 g $plant^{-1}$ dry matter with 1,423.4 °C CDD (Figure 4c).

The LAI values showed the same pattern as dry matter; the highest values were obtained without shade (3.4 with 1,469.5 °C CDD; Figure 4d), followed by red mesh (3.1 with 1,423.4 °C CDD; Figure 4f). For medium density planting, the highest value of LAI was found in the red mesh treatment (0.9 with 1,423.4 °C CDD; Figure 4f).

In terms of the relationship between LAI and CDD, we observed that even in the blue mesh treatment (with lower CDD), the high density planting tended to present with the same trends as the red and no mesh treatments, which had higher values of CDD. For plants at medium density, the lowest LAI was found, with the same growth tendency in relation to CDD.

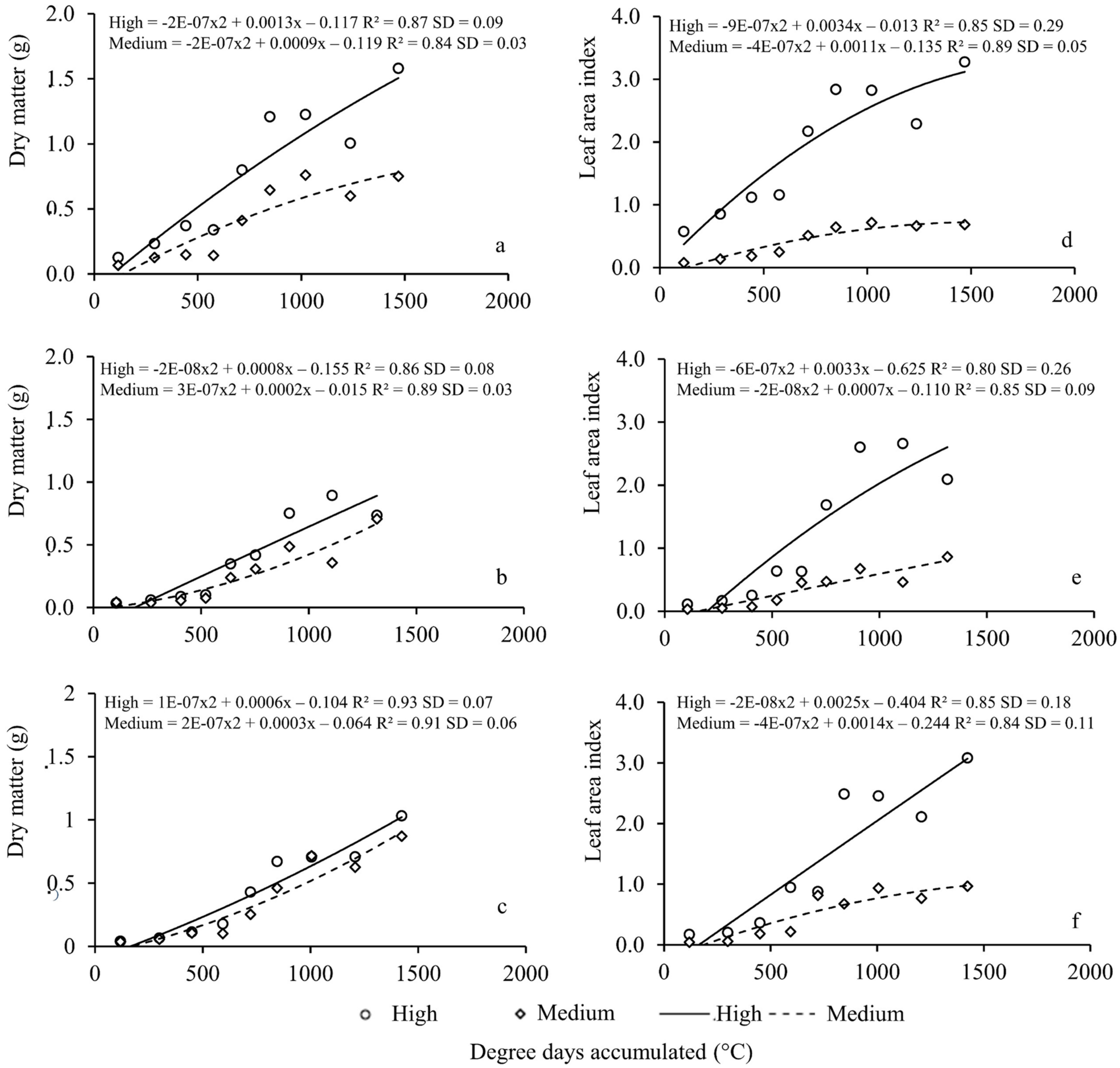

Figure 4. Dry matter for treatments without mesh (a), Blue mash (b) and red mash (c) and leaf area index for treatments without mesh (d), Blue mash (e) and Red mash (f) as a function of the accumulated degree days of *Corymbia citriodora* seedlings.

3.4. Chlorophyll index

According to ANOVA, there was no mesh density interaction and no significant effect of treatments for the Chl A, Chl B, and Chl Total variables (Figure 5). The overall average for Chl A was 206.4 (ranging from 186.5 to 234.1), for Chl B the average was 59.1 (ranging from 49.7 to 66.1), and the Chl Total average was 262.0 (ranging from 221.6 to 299.6). It can therefore be inferred that greenhouse-grown *C. citriodora* plants presented no changes in chlorophyll index when grown under colored mesh.

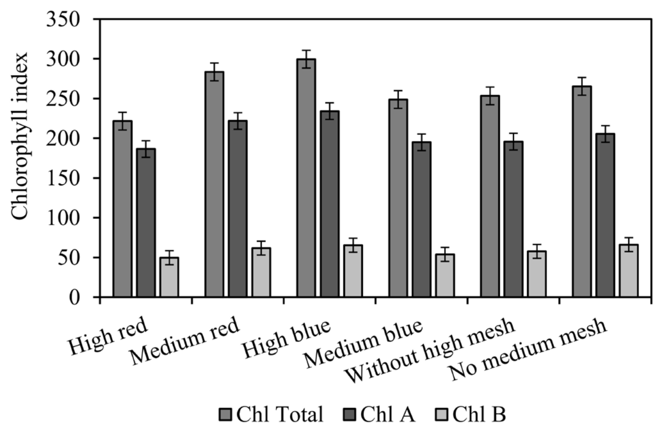

Figure 5. Chlorophyll index of *Corymbia citriodora* seedlings produced in 30% colored, red, Blue and non-meshed meshes inside the greenhouse at high and medium density.

3.5. Principal component analysis

PCA showed that the variances contained in the original variables can be explained by the first two principal components, with PC1 corresponding to 41.0% and PC2 to 29.0% of the accumulated variance, respectively, totaling 70% of the variance (Figure 6). The PCA results also showed that the cultivation conditions altered growth and physiological characteristics of the plants due to different shading grids and plant densities. The cultivation conditions RH, WH and WM were each arranged in a quadrant, while BH, BM and RM were arranged in another quadrant, indicating differences between them.

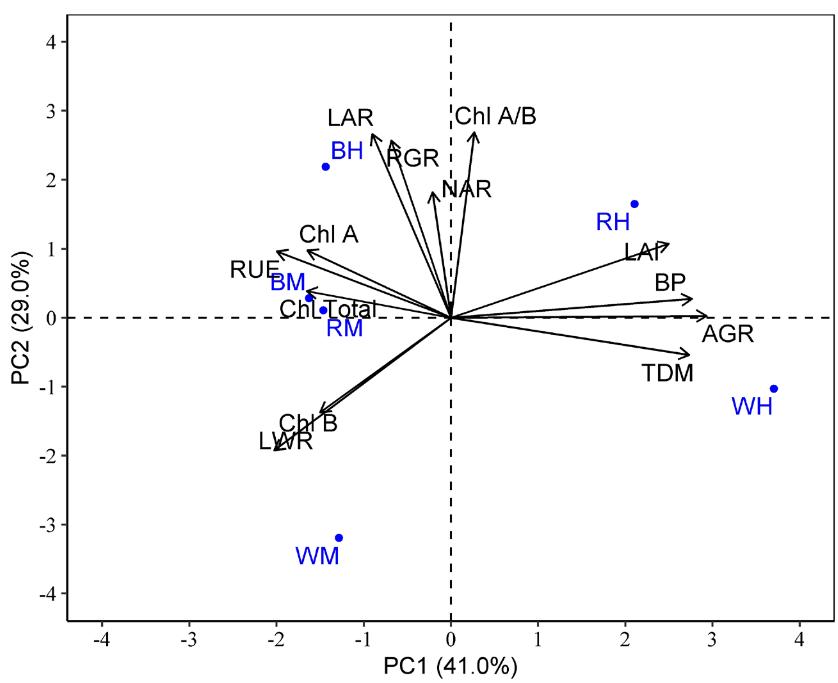

Figure 6. Principal component analysis (PCA) for analysis of variables for the leaf area ratio (LAR), leaf weight reason (LWR), leaf area index (LAI), relative growth rate (RGR), net assiilation rate (NAR), radiation use efficiency (RUE), total dry matter (TDM), absolute growth rate (AGR), biological productivy (BP), chlorophyll a (Chl A), chlorophyll b (Chl B), chlorophyll total (Chl Total), chlorophyll a/b (Chl A/B) and in the treatments: high density blue mesh (BH), high density red mesh (RH), high density no mesh (WH), medium density blue mesh (BM), medium density red mesh (RM), medium density no mesh (WM).

When analyzing the contribution of the variables, we found that the variables with the highest contributions to PC1 were AGR (18.3%), BP, (16.2%), TDM (15.9%), LAI (13.2%), LWR (8.7%) and RUE (8.5%), via WH and RH, while for PC2 they were Chl A/B (21.7%), LAR (21.2%), RGR (19.8%) and LWR (11.1%), via WM and BH. In addition, the vectors in different directions of the variables Chl A, RUE and ChlTotal, LAR, RGR and NAR, indicate an inverse relationship with the variables AGR and TDM. An inverse relationship is also observed for Chl A/B, LAI and BP, with Chl B and LWR.

As for LAI and BP, a positive relationship was observed, as well as AGR and TDM in function of RH and WH for PC1. However, for PC2 the variables LAI, BP, AGR show little relationship and TDM observed a negative relationship in function of WH. There is a negative relationship in PC1 for Chl A, Chl Total, Chl B, RUE and LWR as a function of BM, RM and WM. For PC2, there is little relationship in Chl A, Chl Total and RUE, but it was observed that in ChlB and LWR this relationship is negative as a function of WM.

4. DISCUSSION

4.1. Meteorological conditions

For optimal growth and development of *C. citriodora* seedlings, a temperature range of 20 to 23 °C is required (Monteiro, 2009). The general average value found in this study (20.2 °C) was within this range. The same occurred for the minimum temperature (10.4 °C), which was also above the lowest base temperature of *C. citriodora*, which is 8.7 °C. Thus, we can infer that the temperature was not a limiting factor in any treatment for the growth and development of seedlings. Although the temperature did not present a limiting factor for the plants and it did not differ between treatments, Costa et al. (2012) mention that the use of meshes can alleviate impacts of extreme temperatures, in addition to providing better nutrient absorption and root development.

The variations between the transmissivity values observed in Figure 2 may be related to the shade mesh colors. Oren-Shamir et al. (2001) observed that the use of colored mesh tends to manipulate the light spectrum, resulting in differential solar radiation. Thus, under shade mesh, solar radiation tends to change spectrum and wavelength, consequently reducing its incidence. Mesh provides an environment that restricts available solar radiation.

Solar radiation increases photoassimilate production, plant growth and development, and consequently the increase of dry matter. However, this only occurs when the amount of solar radiation available is greater than the trophic limit (Reis et al., 2013). In this study, 8.4 MJ m^2 was considered as this global radiation limit incident (Nisen et al., 1990), (Figure 1b). This explains the lower values of dry matter and LAI for high and medium density treatments under blue mesh; under this particular mesh, the radiation remained below the trophic limit during most of the study period.

According to Áscoli et al. (2015), photosynthetically active radiation (without shade mesh) occupies a wavelength spectrum between 400 and 700 nm. Thus, under colored mesh, transmittance spectra tend to exhibit changes in photosynthetically active radiation. For the blue mesh, the transmittance peak is in the blue-green region (400–540 nm); however, for the red mesh, transmittance is greater than 590 nm (Oren-Shamir et al., 2001). Thus, the wavelength spectrum under colored mesh remains within the required values for photosynthesis.

4.2. Growth characteristics of C. citrodrora under different shading meshes

The higher RUE for plants grown under blue mesh at both medium and high densities (Figure 3) may be due to the lower values of transmissivity observed in this treatment. Specifically, these plants suffered a greater restriction of solar radiation, which led to them becoming more efficient at utilizing the available radiation. According to Nardini et al. (2019), this radiation restriction during plant growth can be compensated for by diffuse radiation penetrating the shading mesh and the plant canopy.

The LAI results found in this study may be related to the different plant densities (Figure 4). When working with a large plant population per unit area, intraspecific competition stimulates leaf growth in order to increase leaf area and thus intercept larger amounts of solar radiation for photosynthesis. Similar results were observed by (Junior et al., 2020). Therefore, increasing plant density is an interesting alternative strategy for obtaining seedlings with a higher LAI. In addition, it reduces the time and space required for seedling production (Trautenmüller et al., 2017).

The study period may have been a limiting factor regarding dry matter and LAI values, despite only the blue mesh presenting values below the trophic limit of the species (8.4 MJ m^2 day^{-1}). At this time of year, lower temperatures and lower daily radiation values are obtained. According to Freitas et al. (2017), the growth of *C. citriodora* seedlings requires a greater accumulation of solar radiation for leaf emission and, consequently, demands more energy for dry matter and LAI production when compared to other species.

The LAI values found in this study were higher than those found by Sanquetta et al. (2014), who studied seedling production as a function of environment and plant density and found LAI values between 0.41 and 0.71. Therefore, the production of seedlings with or without colored mesh does not appear to affect the LAI.

4.3. Chlorophyll index

The increase in chlorophyll index in relation to shading varies between species (Amarante et al., 2009). In this study, we observed no difference between treatments (Figure 5). This result may be related to the absorption characteristics of chlorophyll; it strongly absorbs energy in the blue and red region of the spectrum (Taiz et al., 2017), the spectra used to compose the treatments. Therefore, the use of red and blue colored mesh may have been responsible for the lack of change in chlorophyll compared to plants grown without mesh. These results are consistent with those observed by Brant et al. (2011) and Coelho et al. (2014b), while studying physiological and anatomical adaptations of *Melissa officinalis* L. (Lamiaceae) and *Vigna unguiculata* submitted to different shading levels, respectively.

According to Yu et al. (2020), there is often an increase in chlorophyll in plants exposed to a rate of 75% of incident solar radiation, below this exposure there is a decrease in chlorophyll levels. In this way, the non-differentiation of Chl A, Chl B and Chl Total, can also be influenced by the shading performed by the greenhouse plastic + colored mesh in the treatments with mesh and without mesh with the greenhouse plastic. The study period should also be highlighted, as it has a lower intensity of solar radiation compared to other periods of the year.

The growth and development of seedlings were modified by colored shading meshes and plant densities. For the blue mesh, it can be observed that, regardless of density, it presented lower values of average daily global solar radiation. This results in higher RUE, as they are two proportional variables. However, higher RUE was inversely proportional to higher LAI and dry matter. On the other hand, seedlings without mesh in high density showed the lowest RUE but had the highest daily global radiation, LAI and dry matter between treatments.

The use of colored shade mesh in seedling production implies the CDD of the production environment, as well as the alteration to solar radiation quality, providing modifications in the RUE of *C. citriodora* plants. Thus, the production of seedlings under colored mesh, primarily red mesh, leads to similar results as obtained without mesh. However, further studies are needed on this subject in order to improve cultivation practices and RUE.

4.4. Principal component analysis

The use of PCA was important to simplify the understanding between the interactions between the different components of the seedling production systems. However, according to the results obtained in this study, it was possible to observe some information about the response in the production of *C. citriodora* seedlings submitted to treatments with the use of colored meshes and without shading mesh in high and medium density.

Based on the results obtained in the present study, the main findings were: The development of *C. citriodora* was strongly related to high density. The LAI is strongly correlated with seedlings under high density and red mesh. Our results suggest that seedlings grown at high density under red mesh or without mesh tend to have higher LAI and dry matter, consequently higher growth rates. This can be observed by PC1, which corresponded to 41.0% of the accumulated variance of the treatments without mesh and red mesh for high density.

A study by Caron et al. (2012) showed that in *Eucalyptus grandis* plants, high density promotes faster occupation of the leaf area in the spaces between the seedlings during the growth period, corroborating what was observed in the development of *C. citriodora* in the present work. In the same way, it could explain the answer in the RH variable where it shows an increase in LAI. But, in addition to the high density, this fact can be explained by the quality that the red mesh makes available to the plants. Because the quality available is present in the visible spectrum where there is greater stimulation and the main absorptions of some photosynthetic pigments in this range (Taiz et al., 2017).

5. CONCLUSIONS

The use of colored shade mesh and plant density altered the radiation use efficiency and dry matter accumulation. Leaf area index was affected only by plant density. The red mesh resulted in the most similar values to the treatment without mesh. Better quality seedlings were obtained under high plant density.

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Axel Bruno Mariotto: Supervision (Supporting); Validation (Supporting); Visualization (Supporting).

Matheus Milani Pretto: Methodology (Supporting); Validation (Supporting); Visualization (Supporting).

Elvis Felipe Elli: Conceptualization (Supporting); Investigation (Supporting); Supervision (Supporting); Visualization (Supporting).

Davi de Oliveira: Supervision (Supporting); Visualization (Supporting).

Denise Schmidt: Funding acquisition (Lead); Resources (Lead); Supervision (Supporting); Visualization (Supporting)

Braulio Otomar Caron: Conceptualization (Supporting); Funding acquisition (Supporting); Investigation (Supporting); Supervision (Lead); Visualization (Supporting).

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