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EFFECTS OF ARTIFICIAL SHADING AND PLANT DENSITY ON *Corymbia citriodora* SEEDLING CHARACTERISTICS

Frederico Westphalen, RS
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Artigo de Conclusão de Curso apresentado ao Curso de Graduação em Engenharia Florestal Universidade Federal de Santa Maria (UFSM) *Campus* Frederico Westphalen, como requisito parcial para obtenção do título de **Engenheiro Florestal.**

Orientador: Profº Dr°. Braulio Otomar Caron

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Aprovado em 08 de setembro de 2020:

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Frederico Westphalen, RS
2020

DEDICATÓRIA

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“Quando tudo nos parece dar errado, acontecem coisas boas que não teriam acontecido se tudo tivesse dado certo”.

(Renato Russo)

Effects of artificial shading and plant density on *Corymbia citriodora* seedling characteristics

Claiton Nardini[1], Jaqueline Sgarbossa[2], Liliane Bárbara Tibolla[3], Matheus Milani Pretto[4], Elvis Felipe Elli[5], Davi de Oliveira[6], Denise Schmidt[7], Braulio Otomar Caron[8]

ABSTRACT

In forestry, alternative seedling production systems that lead to increased growth rates and efficiency in natural resource use are needed. The objective of this work was to evaluate the influence of colored shade mesh and plant density on dry biomass, leaf area index, chlorophyll content, and radiation use efficiency of *C. citriodora* seedlings. The study was conducted in a greenhouse at the Federal University of Santa Maria, campus Frederico Westphalen-RS. The experimental design was completely randomized and arranged in a 2 x 3 factorial scheme with two plant densities (high density, 402 plants/m² and medium density, 201 plants/m²) and three shade conditions (red or blue mesh with 30% solar radiation restriction or no mesh). The variables evaluated were dry biomass, leaf area index, chlorophyll content (a, b, and total), radiation use efficiency, and solar radiation interception. The use of colored shade mesh and plant density appears to alter microclimate conditions, affecting the efficiency of radiation use and dry biomass of *C. citriodora* seedlings. The leaf area index, however, is affected only by plant density. For seedling production, the red mesh provided the most similar results to the treatment without mesh. Additionally, high plant density yielded the best results for dry biomass and leaf area index for all shade treatments.

Keywords: Leaf area index; temperature; radiation use efficiency

Na silvicultura, são necessários sistemas alternativos de produção de mudas que aumentem as taxas de crescimento e a eficiência no uso dos recursos naturais. O objetivo deste trabalho foi avaliar a influência da malha de tonalidade colorida e da densidade de plantas na biomassa seca, índice de área foliar, teor de clorofila e eficiência de uso de radiação de mudas de *C. citriodora*. O estudo foi realizado em casa de vegetação na Universidade Federal de Santa Maria, campus Frederico Westphalen-RS. O delineamento experimental foi inteiramente casualizado e arranjado em esquema fatorial 2 x 3 com duas densidades de plantas (alta densidade, 402 plantas / m² e densidade média, 201 plantas / m²) e três condições de sombreamento (malha vermelha ou azul com 30% de radiação solar restrição ou sem malha). As variáveis avaliadas foram biomassa seca, índice de área foliar, teor de clorofila (a, be total), eficiência do uso da radiação e interceptação da radiação solar. O uso de malhas coloridas e densidade de plantas altera as condições do microclima, afetando a eficiência do uso da radiação e da biomassa seca de mudas de *C. citriodora*. O índice de área foliar, entretanto, é afetado apenas pela densidade das plantas. Para a produção de mudas, a tela vermelha proporcionou resultados mais semelhantes ao tratamento sem tela. Além disso, a alta densidade de plantas proporcionou os melhores resultados para biomassa seca e índice de área foliar para todos os tratamentos de sombra.

Palavras-chaves: Índice de área foliar; temperatura; eficiência de uso de radiação

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Abstract

In forestry, alternative seedling production systems that lead to increased growth rates and efficiency in natural resource use are needed. The objective of this work was to evaluate the influence of colored shade mesh and plant density on dry biomass, leaf area index, chlorophyll content, and radiation use efficiency of *C. citriodora* seedlings. The study was conducted in a greenhouse at the Federal University of Santa Maria, campus Frederico Westphalen-RS. The experimental design was completely randomized and arranged in a 2 x 3 factorial scheme with two plant densities (high density, 402 plants/m² and medium density, 201 plants/m²) and three shade conditions (red or blue mesh with 30% solar radiation restriction or no mesh). The variables evaluated were dry biomass, leaf area index, chlorophyll content (a, b, and total), radiation use efficiency, and solar radiation interception. The use of colored shade mesh and plant density appears to alter microclimate conditions, affecting the efficiency of radiation use and dry biomass of *C. citriodora* seedlings. The leaf area index, however, is affected only by plant density. For seedling production, the red mesh provided the most similar results to the treatment without mesh. Additionally, high plant density yielded the best results for dry biomass and leaf area index for all shade treatments.

Keywords: Leaf area index; 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). Fast-growing species are ideal for this, particularly those with suitable physical, chemical, mechanical, and anatomical wood properties (Medeiros et al., 2016). 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 workability of wood for rural buildings 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 (Sanquetta et al., 2014). Plants that grow under restricted solar radiation may become more efficient in their radiation use, possessing morphoanatomical adaptations (Coelho et al., 2014) to compensate for the reduced levels of solar radiation. For example, as important components of plant acclimatization, proportions of chloroplast pigments (chlorophyll and carotenoids) 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). Therefore, plants may respond variably under different seedling production systems.

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 dry biomass, leaf area index (LAI), chlorophyll content, and efficiency of radiation use of *C. citriodora* seedlings.

2. MATERIAL AND METHODS

2.1. Study area and experimental design

This study was conducted between May to September 2018 in a greenhouse belonging to the Agroclimatology and Plant Biometrics Laboratory of the Federal University of Santa Maria (Frederico Westphalen campus, Rio Grande do Sul, Brazil). Specifically, the greenhouse was located at 27° 22” S, 53° 25” W, at an altitude of 480 m. 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 medium annual temperature of approximately 22 °C, and rainfall occurring year-round.

The greenhouse comprised plastic film lining 150 μm thick, 3.5 m high, 10 m wide, and 20 m long. The curtains were adjusted daily to control ventilation and temperature.

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 or blue shade mesh with 30% solar radiation restriction, or no mesh. Each treatment was applied to two trays (with the same spacing) for the duration of the study. At each evaluation point, 3% and 6% of plants from high and medium density trays, respectively, were removed.

Corymbia citriodora seeds from seed production areas (SPAs) in Piracicaba, São Paulo, Brazil, were obtained from the Forestry Science and Research Institute. On 22 May, 2018, seeds were sown directly into 90 cm^3 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. When necessary, fungicide (Priori Xtra) was applied in order to control diseases.

2.2. Solar radiation transmissivity and air temperature

The transmissivity of solar radiation through the interior 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 (the combination of direct and diffuse solar radiation). Measurements were recorded daily at 9, 10, 14, 15, and 16 h. The transmissivity of solar radiation was calculated based on the following equation:

$$T=\frac{I}{I_0} \times 100 \qquad \text{Eq. 1}$$

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

The medium daily 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 DDc,the following equation was used:

$$DDc = \sum \left(Tavg - Tb\right) \quad \text{Eq. 2}$$

Where: DDc = degrees days accumulated; Tmed = medium 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 (MJ m^{-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 medium accumulated dry biomass and the intercepted photosynthetically active radiation, according to equation 3, described by (Monteith & Moss, 1977).

$$DBP = RUE * PARi \quad \text{Eq. 3}$$

Where: DBP = dry biomass production in g m^{-2} and PARi = photosynthetically active radiation intercepted in MJ m^{-2}.

The incident photosynthetically active radiation (PAR) was estimated to be 45% of the global solar radiation (Assis & Mendez, 1989). The estimate of IPAR was determined using the model described by Varlet-Grancher et al. (1989). 4:

$$PARi=0{,}95*(PARinc)*(1-e^{(-k*LAI)}) \quad \text{Eq. 4}$$

Where: PARi corresponds to photosynthetically active radiation intercepted at MJ m^{-2}; PARinc, refers to the photosynthetically active radiation incident on MJ m^{-2}; k, refers the extinction coefficient. 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, for Australian cedar seedlings.

The leaf area index was determined from the total leaf area and the surfase area explored by each plant, according to the following equation 5:

$$LAI=LA/AS \quad \text{Eq. 5}$$

Where: LAI is the leaf area index; LA refers to the total leaf area of the plant in m^{-2}; AS is the area of surface area explored by the plant.

Leaf area 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 was then used to measure the leaf area. This software captures the image of the leaves through a color contrast and compares this image to the reference scale, thus calculating the total area of *C. citriodora* leaves.

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.

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 mass of each plant component was taken to be the total dry mass of the plant.

2.5.Chlorophyll contents

Chlorophyll a and b content in *C. citriodora* plants was measured using a portable chlorophyll meter (ChlorofiLOG - CFL1030). 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. The total chlorophyll variable was determined by summing the chlorophyll a and chlorophyll b (a + b) content.

2.6.Statistical analysis

Data were statistically analyzed using SISVAR software (Version 5.6; Ferreira, 2011). The data were initially submitted to analysis of variance (ANOVA) to determine the effects of possible interactions. When there was significance, it was verified by F tests ($p < 0.05$). Means were compared using the Tukey test ($p < 0.05$).

3. RESULTS

3.1.Meteorological conditions

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

Insert Figure 1

The medium global solar radiation flux was 11.74 MJ m^2 day^{-1} (range 1.37–25.15 MJ m^2 day^{-1}; without mesh), 9.78 MJ m^2 day^{-1} (range 1.14–20.95 MJ m^2 day^{-1}; red mesh), and 5.13 MJ m^2 day^{-1} (range 0.59–10.99 MJ m^2 day^{-1}; blue mesh). Total cumulative solar radiation was 1,621 MJ m^2, 1,350 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.

Insert Figure 2

3.2.Radiation use efficiency

The use of different shade mesh colors and cultivation without mesh led to variation in the RUE values. The highest εb 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 3). However, the lowest εb 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.

Insert Figure 3

3.3.Plant Growth

Figure 4 shows the values of dry biomass and LAI as a function of CDD during the production cycle of *C. citriodora* seedlings, to which quadratic polynomial equations were fitted. Greater dry biomass was observed for the high density treatment with no mesh: 1.58 g per plant with 1,469.45 °C CDD (Figure 4A). Regarding the shaded treatments, the highest value for dry biomass was found for high density planting under red mesh: 1.03 g of dry mass with 1,423.40 °C CDD (Figure 4C). Similarly, for medium density planting, the highest value was also found for red mesh: 0.87 g dry mass with 1,423.40 °C CDD (Figure 4C).

Insert Figure 4

The LAI values showed the same pattern as dry biomass; the highest values were obtained without shade (3.27 g with 1,469.45 °C CDD; Figure 4D), followed by red mesh

(3.08 g with 1,423.40 °C CDD; Figure 4F). For medium density planting, the highest value of LAI was found in the red mesh treatment (0.97 g with 1,423.40 °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.

3.4. Chlorophyll content

According to ANOVA, there was no mesh x density interaction and no significant effect of treatments for the chlorophyll a, chlorophyll b, and total chlorophyll variables (Figure 5). The overall medium for chlorophyll a was 206.43 (ranging from 186.50 to 234.11), for chlorophyll b the medium was 59.08 (ranging from 49.67 to 66.14), and the total chlorophyll medium was 262.03 (ranging from 221.56 to 299.56). It can therefore be inferred that greenhouse-grown *C. citriodora* plants present no changes in chlorophyll content when grown under colored mesh.

Insert Figure 5

4. DISCUSSION

4.1. Meteorological conditions

According to Monteiro, (2009), the ideal temperature for the growth and proper development of *C. citriodora* is between 20 and 23 °C. The medium value found in this study was within this temperature range. However, the lowest base temperature for *C. citriodora* is 8.7 °C, which is the minimum temperature required by this species to maintain plant growth and development. The base temperature in this study for this species was therefore higher. Mesh use can be important in preventing plants from being subjected to extreme temperatures; they also help with temperature control and provide better absorption of nutrients and development of the root system (Costa et al., 2012).

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 (in both quality and intensity). 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 biomass. 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 m² was considered this limit (Nisen et al., 1990), (Figure 1B). This explains the lower values of dry biomass 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 (Bamberg et al., 2012, Sanquetta et al., 2014). Therefore, increasing plant density is an interesting alternative strategy for obtaining seedlings with a higher leaf area index.

The study period (between winter and spring) may have been a limiting factor regarding dry biomass 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 biomass 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 content

The increase in chlorophyll content 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 Coelhoe et al. (2014a), while studying physiological and anatomical adaptations of *Melissa officinalis* L. (Lamiaceae) and *Vigna unguiculata* submitted to different shading levels, respectively.

The use of colored shade mesh in seedling production implies the accumulated degree-days 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.

5. CONCLUSIONS

The use of colored shade mesh and plant density tends to alter microclimate conditions, affecting the efficiency of radiation use and dry biomass of *C. citriodora* seedlings. The leaf area index, however, is affected only by plant density. During seedling production, red mesh resulted in the most similar values to the treatment without mesh. Additionally, high density planting resulted in the best values for dry biomass and leaf area index for all treatments.

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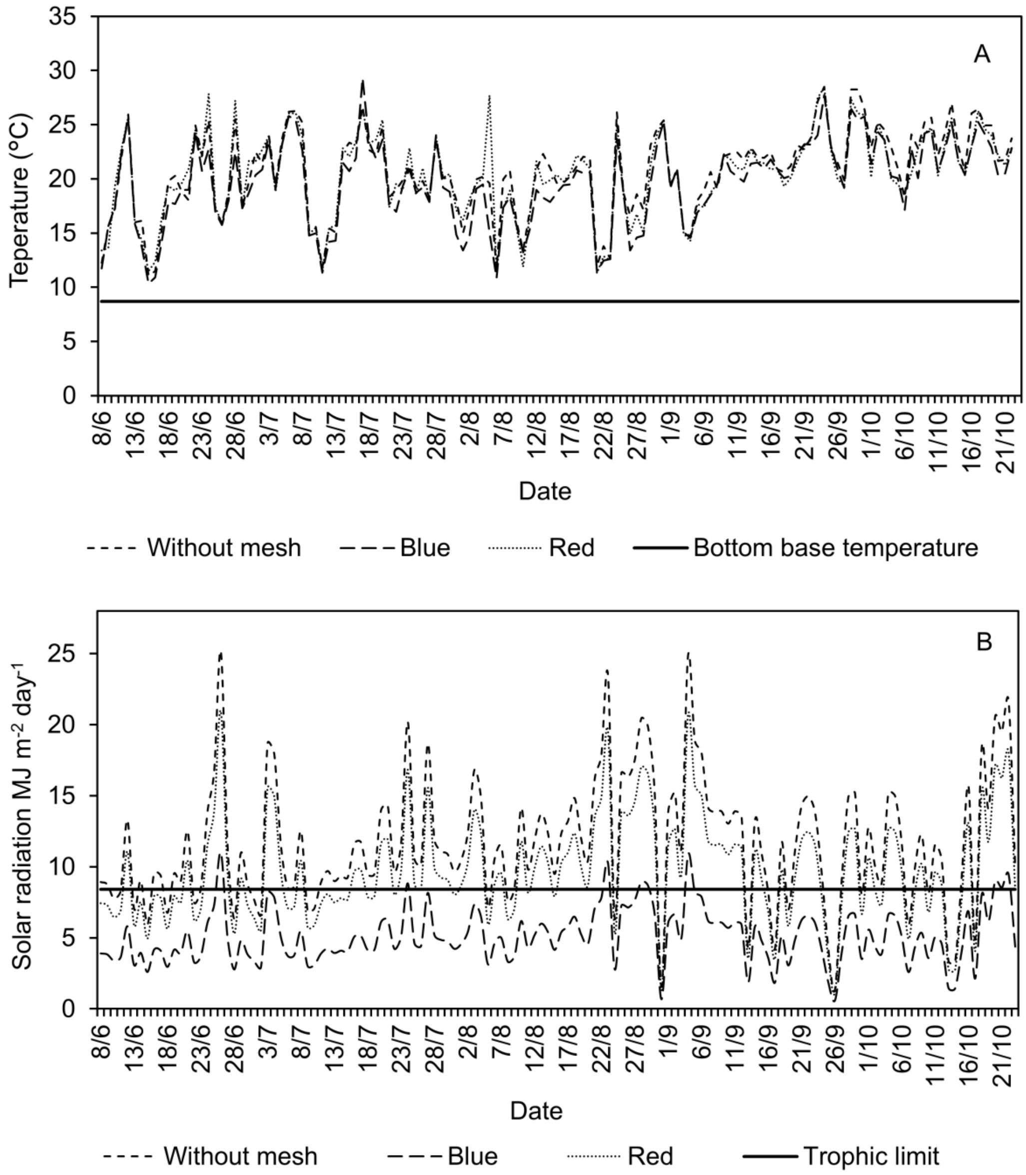

Figure - 1 Mean temperature (A) and incident global solar radiation (B) values throughout the study. Horizontal lines indicate inferior base temperature (A) and trophic Limit (B) for *C. citriodora*. Frederico Westphalen, Rio Grande do Sul, Brazil, 2020.

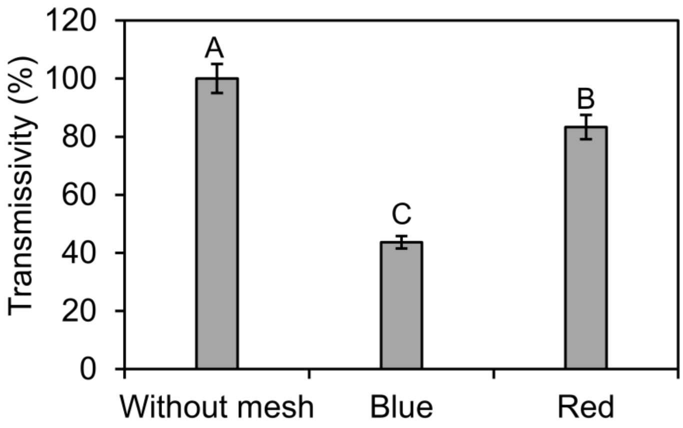

Figure - 2 Solar radiation transmissivity (%) for the treatments without mesh, red mesh and blue mesh. Frederico Westphalen, Rio Grande do Sul, Brazil, 2020.

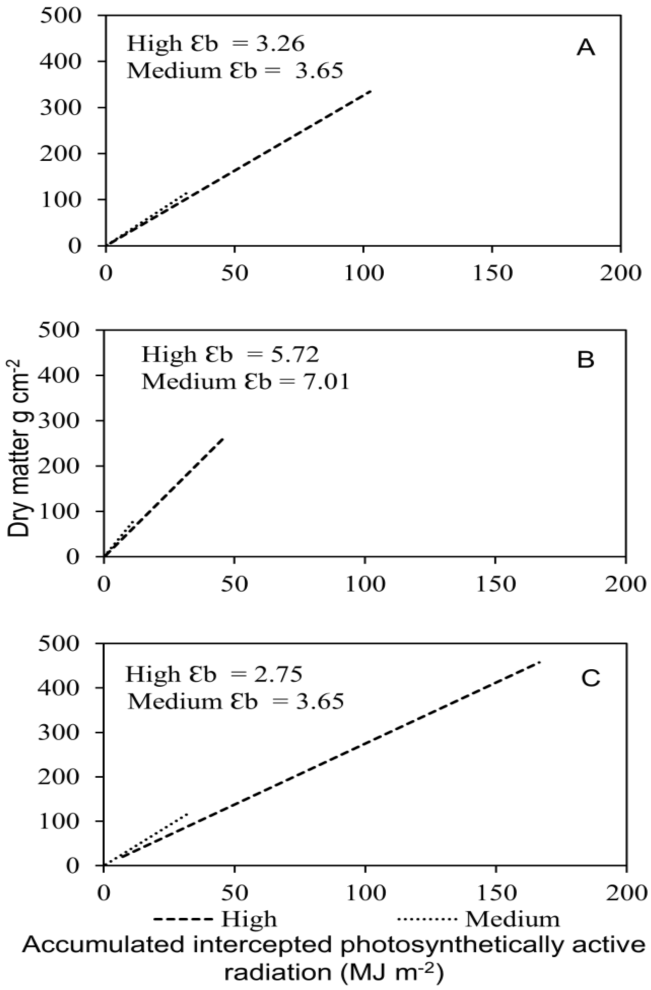

Figure - 3 Radiation use efficiency (RUE, g MJ^{-1}) of *C. citriodora* seedlings produced under colored (blue and red) mesh and without mesh, under high and medium density. Blue mesh (A), red mesh (B) and no mesh (C). Frederico Westphalen, Rio Grande do Sul, Brazil, 2020.

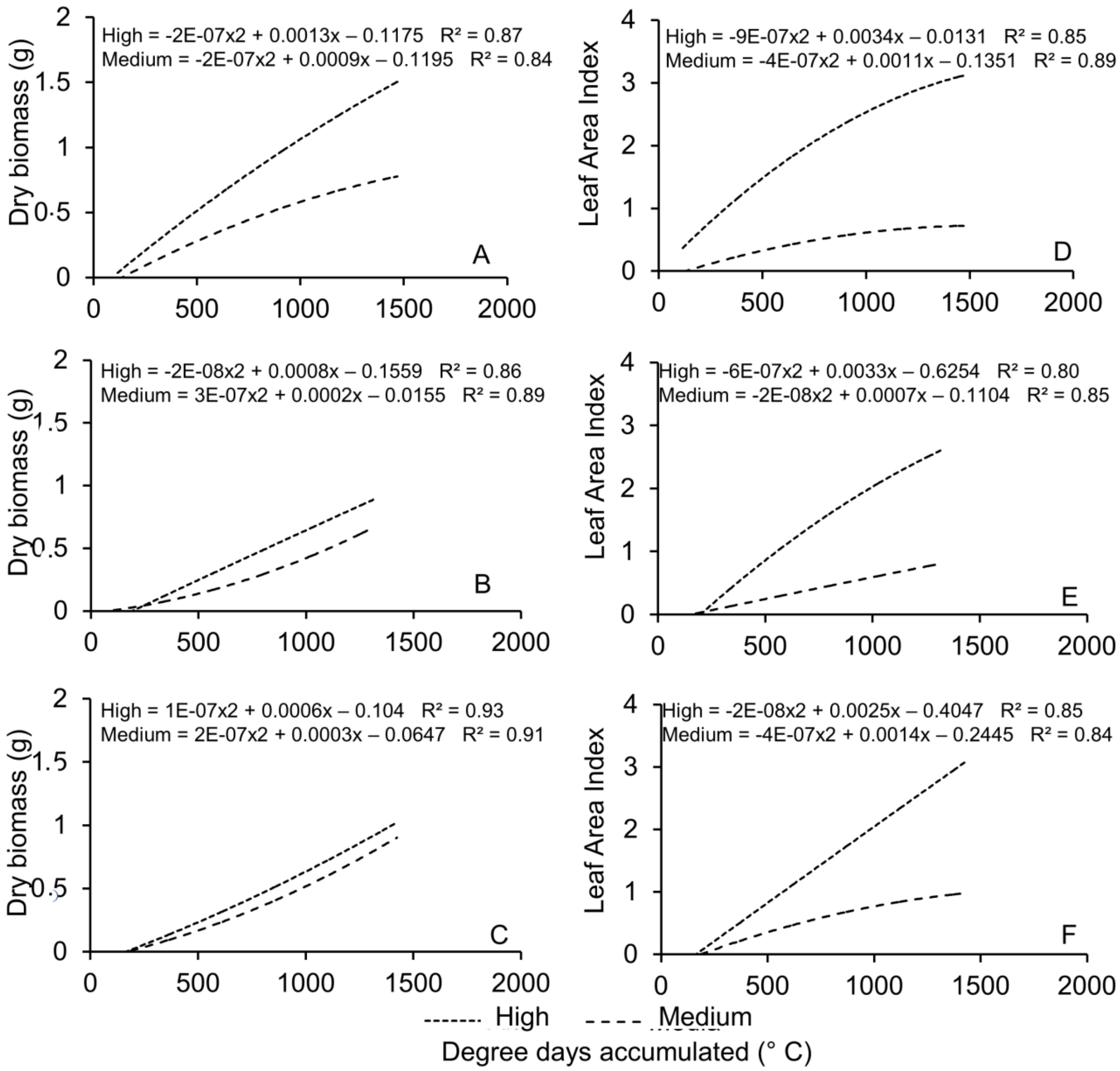

Figure - 4 Dry biomass for treatments without mesh (A), Blue mash (B) and red mash (C) and leaf area index for treatments without mesh (D), Blue mash (E) and Red mash (F) as a function of the accumulated degree days of *C. citriodora* seedlings. Frederico Westphalen, Rio Grande do Sul, Brazil, 2020.

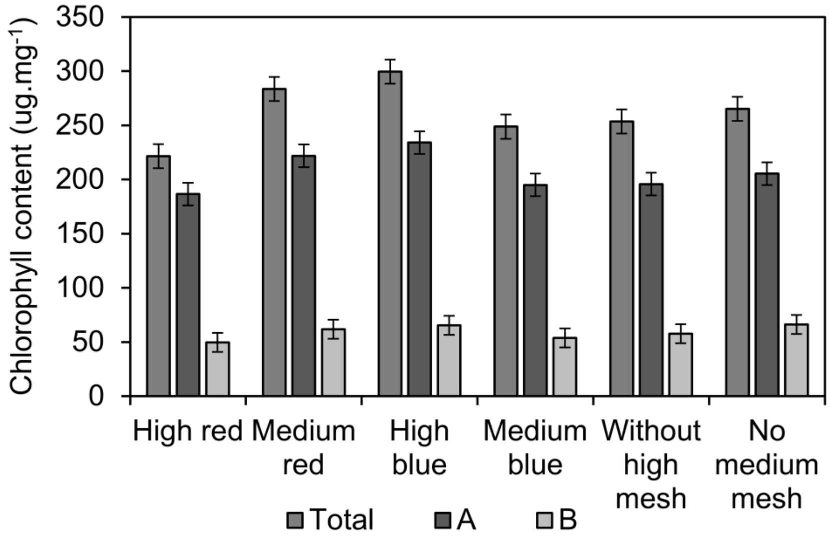

Figure - 5 Chlorophyll content of *C. citriodora* seedlings produced in 30% colored, red, Blue and non-meshed meshes inside the greenhouse (No Mesh) at high and medium density. Frederico Westphalen, Rio Grande do Sul, Brazil, 2020.