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ARTÍCULOS

Mini-tunnel models influence the productivity of eucalyptus mini-stumps?

¿Los modelos de mini túnel influyen en la productividad de las minicepas de eucalipto?

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SUMMARY

Mini-cutting is the most common commercially used clonal propagation method in Brazil. However, several environmental interferences can impact this process, hindering the production of propagules. Mini-tunnel have been used to overcome these difficulties, allowing for an increase in the productivity of mini-stumps and the rooting of mini-cuttings. This study aimed to evaluate the effect of mini-tunnel models on the mini-stump productivity of two hybrid eucalyptus clones over two seasons. The study was conducted in the seedling nursery of a forestry company located in Alto Vale do Jequitinhonha, Minas Gerais, Brazil. Three models of the mini-tunnel were used: mini-tunnel 35 cm, 55 cm, 55 cm tubular, and without cover (control) over two seasons (cold and hot). The total and effective productivity of mini-cuttings were evaluated and the levels of chlorophyll A and B, total chlorophyll, height, leaf area, and dry matter of the mini-cuttings were determined. Together, season and use of the mini-tunnel influenced the productivity of the mini-cuttings of both clones. Use of the mini-tunnel resulted in high productivity, especially in the cold period. Mini-cuttings produced during the hot season showed greater growth, dry matter, and leaf area than those collected during the cold season. The use of mini-tunnels is an effective method to increase the productivity of clonal eucalyptus mini-gardens, as shown here by its overall positive influence on mini-cuttings of both genotypes.

Keywords: mini-cutting, mini garden, clonal propagation.

RESUMEN

El miniestaca es el método de propagación clonal más utilizado comercialmente en Brasil. Sin embargo, este proceso sufre varias interferencias ambientales que pueden dificultar la producción de propágulos. Los mini túneles se han utilizado para superar estas dificultades, permitiendo también un aumento de la productividad de las minicepas y el enraizamiento de las miniestacas. Este estudio tuvo como objetivo evaluar el efecto de los modelos de mini túneles sobre la productividad de minicepas de dos clones híbridos de eucalipto, en dos temporadas. El estudio se realizó en el vivero de plántulas de una empresa forestal ubicada en Alto Vale do Jequitinhonha, Minas Gerais, Brasil. Se utilizaron tres modelos de mini túneles: mini túnel de 35 cm, 55 cm, tubular de 55 cm y sin cobertura (control), en dos temporadas (frío y calor). Se evaluó la productividad total y efectiva de miniestacas y se determinaron los niveles de clorofila A y B, clorofila total, altura, área foliar y masa seca de las miniestacas. La temporada y el uso del mini túnel, en conjunto, influyeron en la productividad de las miniestacas de ambos clones. El uso del mini túnel resultó en una alta productividad, especialmente en el período frío. Las miniestacas producidos en la estación cálida mostraron mayor crecimiento, materia seca y área foliar, que los recolectados en la estación fría. El uso del mini túnel es un método eficaz para aumentar la productividad de los minihuertos clonales de eucalipto, como lo demuestra aquí su influencia positiva general sobre las miniestacas de ambos genotipos.

Palabras clave: miniestaca, mini jardín, propagación clonal.

INTRODUCTION

Species of the genus *Eucalyptus* are the most common commercially used species in Brazil's tree plantations, occupying 6.97 million hectares in 2019 (Indústria Brasileira de Árvores (IBA) 2020). They have advantages over other species, such as rapid growth, increased amount of dry matter, good seed production, ease of vegetative propagation, and good adaptability to a variety of silvicultural and industrial applications in the country (Siviero *et al*. 2019).

In Brazil, mini-cutting is the most widely used clonal propagation technique on a commercial scale for species of the *Eucalyptus* genus. This technique involves the use of shoots as a source of propagules. Mini-cuttings are provided by mini-stumps that are grown in clonal mini-gardens. The main advantages of mini-cuttings include greater productivity in a short period; a reduction in the area used for production; optimization for rooting; and improved seedling quality, especially in clones with rooting difficulties (Xavier 2013).

Climatic seasonality, observed in some regions of Brazil, leads to variation in environmental conditions, which in turn affects the induction of the rhizogenic processes of vegetative propagules. Therefore, in order to be successful in the cloning process, the season in which plant material is collected must be taken into consideration (Lima *et al*. 2022).

The search for technologies that increase the productive capacity of the nursery can improve the production of mini-stumps and the rooting of mini-cuttings as well as reduce the impact of environmental factors such as seasonality. Using plastic greenhouses for propagation changes the environment for plant development, including changes in the energy balance, temperature, humidity, and a reduction in transpiration (Khoshnevisan *et al*. 2013). Use of the mini-tunnel, which involves covering clonal mini-gardens with a plastic tunnel, has been shown to increase the production of cuttings.

The use of mini-tunnels is a recent forestry practice, so there are few studies that have investigated its effectiveness (Batista *et al*. 2015, Pereira *et al*. 2019, Nascimento *et al*. 2020, Vallejos-Torres *et al*. 2021, Lima *et al*. 2022). Thus, further research is needed to understand the physiological changes that occur in mini-stumps as a result of changes in the environment and to relate them to the production and quality of the mini-cuttings and seedlings generated. This study aimed to evaluate the effects of mini-tunnel models on the productivity of two hybrid *Eucalyptus* mini-cuttings in a clonal mini garden over two seasons.

METHODS

Study location and station. The study was conducted from July 2019 to February 2020 in the seedling nursery of a forestry company located in Alto Vale do Jequitinhonha, Minas Gerais, Brazil. According to Köppen's classification, the region is considered to be Cwa climate, characterized by cold, dry winters and hot, humid summers. The average temperature in the region is 21.2 ºC and the annual average relative humidity varies between 60 and 70 %. The average annual rainfall is 1,132 mm (Martins *et al*. 2018).

Genetic material and formation of the clonal mini garden. Two superior hybrid genotypes were used: *Eucalyptus grandis* W. Hill ex Maiden × *Eucalyptus urophylla* S. T. Blake (clone 1) and (*Eucalyptus camaldulensis* Dehnh × *Eucalyptus grandis)* × *Eucalyptus urophylla* (clone 2). Both were obtained through controlled pollination. The seedlings, which originated from sprouts in the clonal garden and were rooted in a greenhouse, were planted in 2013 in fiber cement gutters containing gravel (granulometry between 2 and 5 mm) and crushed stone, with 10 × 10 cm spacing, under a semi-hydroponic irrigation system.

After 21 days, to adapt the seedlings to the semi-hydroponic system, apical sprouting was pruned to form mini-stumps. The clonal mini garden was created through the development of basal buds, induced by the breakdown of apical dominance. Following the company's operating procedure, mini-cuttings were collected periodically for the commercial production of seedlings, until the experiments were initiated.

Management, nutrition of mini-stumps, and experimental design. Before the initiation of the experiment, the mini clonal garden was kept open, and weekly cleaning was carried out to remove dead leaves, shoots, and mini-stumps. The mini-stumps received a nutrient solution via drip irrigation, distributed seven times per day at a total flow rate of 9.3 L m^{-2}.

The nutrient solution was composed of powdered monoammonium phosphate (1.55 g L^{-1}), magnesium sulfate (0.5 g L^{-1}), powdered potassium chloride (0.6 g L^{-1}), chloride calcium (0.5 g L^{-1}), zinc sulfate (4 mg L^{-1}), iron chelate (24 mg L^{-1}), boric acid (11 mg L^{-1}), manganese sulfate (14 mg L^{-1}), and copper sulfate (2 mg L^{-1}). The electrical conductivity of the nutrient solution was maintained between 1.5 and 1.8 mS m^{-2} and the pH was adjusted to 5.7 (± 0.1), corrected with 1 M hydrochloric acid and 1 M sodium hydroxide.

Four gutters were used in the experiment, and three mini-tunnel models were established. The first had dimensions of 0.80 × 16.30 × 0.35 m (mini-tunnel 35 cm) and the second had dimensions of 0.80 × 16.30 × 0.55 m (mini-tunnel 55 cm). Both of the structures were made of steel cables. The third mini-tunnel had dimensions of 0.80 × 16.30 × 0.55 m (55 cm tubular mini-tunnel) and was made with iron in a tubular format. The control treatment was uncovered (figure 1). The mini-tunnels were covered with a plastic film of low-density polyethylene, with a thickness of 150 µm.

After assembling the mini-tunnels, the mini-cuttings were cut weekly for 15 days (two harvests), except for the last 7 days before the experimental evaluations. The 7 days were defined according to the frequency of the company's operational collection and were in compliance with the recommendations of Xavier *et al*. (2013). Two simultaneous experiments were carried out, one for each respective clone, over two seasons representing the cold period (July to August) and the hot period (November to December).

A randomized block design was used in a subdivided plot scheme, with the four plots being the mini-tunnel models and the subplots being the two seasons of the year, with three blocks (beginning, middle, and end of the gutter) containing six mini-stumps each (repetition).

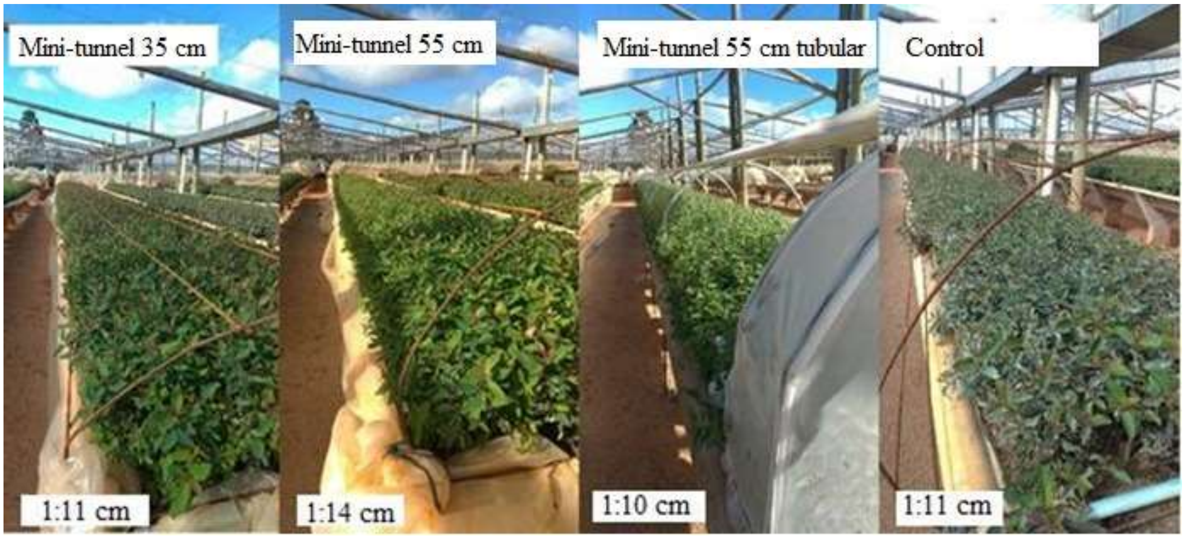


Figure 1. Mini-tunnel models used in the clonal-propagation of two hybrid *Eucalyptus* clones.

Modelos de minitúneles utilizados en la propagación clonal de dos clones híbridos de *Eucalyptus*.

Evaluations and data analysis. Before gathering the mini-cuttings, the levels of chlorophyll A and B and total chlorophyll were measured in a non-destructive way based on five leaves per repetition, using a ClorofiLOG portable chlorophyll meter (Falker®, model: CFL 1030).

The total productivity, effective productivity, height (cm), dry matter (g), and leaf area (cm²) of the mini-cuttings were determined. The number of mini-cuttings greater than 5 cm in height was used as the metric for total productivity, and the number of mini-cuttings greater than 9 cm was used as the metric for effective productivity (minimum height for staking according to the company's operational standard).

The height of the mini-cuttings was measured with a millimeter ruler. Dry matter was determined using an analytical balance (0.0001 g), after drying the material in an oven with forced air circulation at 65 ºC, until a constant weight was achieved. Five leaves of each repetition were collected, scanned, and digitized using ImageJ software (Rasband 2018) to determine the leaf area.

Initially, Shapiro-Wilk and Bartlett tests were performed on the residuals. When normality and homogeneity assumptions were not met, the data were transformed using the BoxCox method. Given the assumptions, the data were subjected to analysis of variance and compared using Tukey's test at the 5 % significance level. R software was used for statistical analyses (R Core Team 2021).

RESULTS

The highest levels of chlorophyll A and total chlorophyll for clone 1 were measured during the cold period, whereas the chlorophyll B content was higher in the warmer season. These values differed considerably, measured at the 5 % significance level. The control treatment showed the lowest values of chlorophyll A and B and total chlorophyll content and differed significantly from treatments in the 35 cm and 55 cm mini-tunnels, with the latter two being statistically equivalent (figure 2A). The levels of chlorophyll A and B and total chlorophyll of the mini-cuttings of clone 2 (figure 2B) were significantly influenced by the season (P = 0.00067), with the highest values found in the cold period. There was no difference in the impact of this variable among the evaluated mini-tunnel models for clone 2. Due to heat damage caused to the mini-stumps of the treatment with a 55 cm tubular mini-tunnel, this condition could not be repeated in the hot period for clone 1.

In the cold season, the total productivity of mini-cuttings from the mini-stumps of clone 1 (figure 3A) was significantly higher for treatments with mini-tunnels than for the control treatment (without mini-tunnels). However, in the hot season, the total productivity did not differ between treatments. A significant difference was also observed for the 35 cm mini-tunnel model, in which the total productivity was higher in the cold season. In the control treatment, a numerically higher average was observed during the hot season, however this value was not statistically significant. The effective productivity of the mini-cuttings (figure 3B) showed similar results to the total productivity. Significantly higher averages were observed in the cold season for treatments with mini-tunnels. In the hot season, there were no differences between treatments. Within the control treatment, there was higher effective productivity of mini-cuttings in the hot period than in the cold period.

For clone 2, the total productivity of mini-cuttings per mini-stump was higher in the cold season for the 55 cm mini-tunnel, 55 cm tubular mini-tunnel, and control treatments. Only the 35 cm mini-tunnel treatment showed higher averages in the hot season, differing from the others

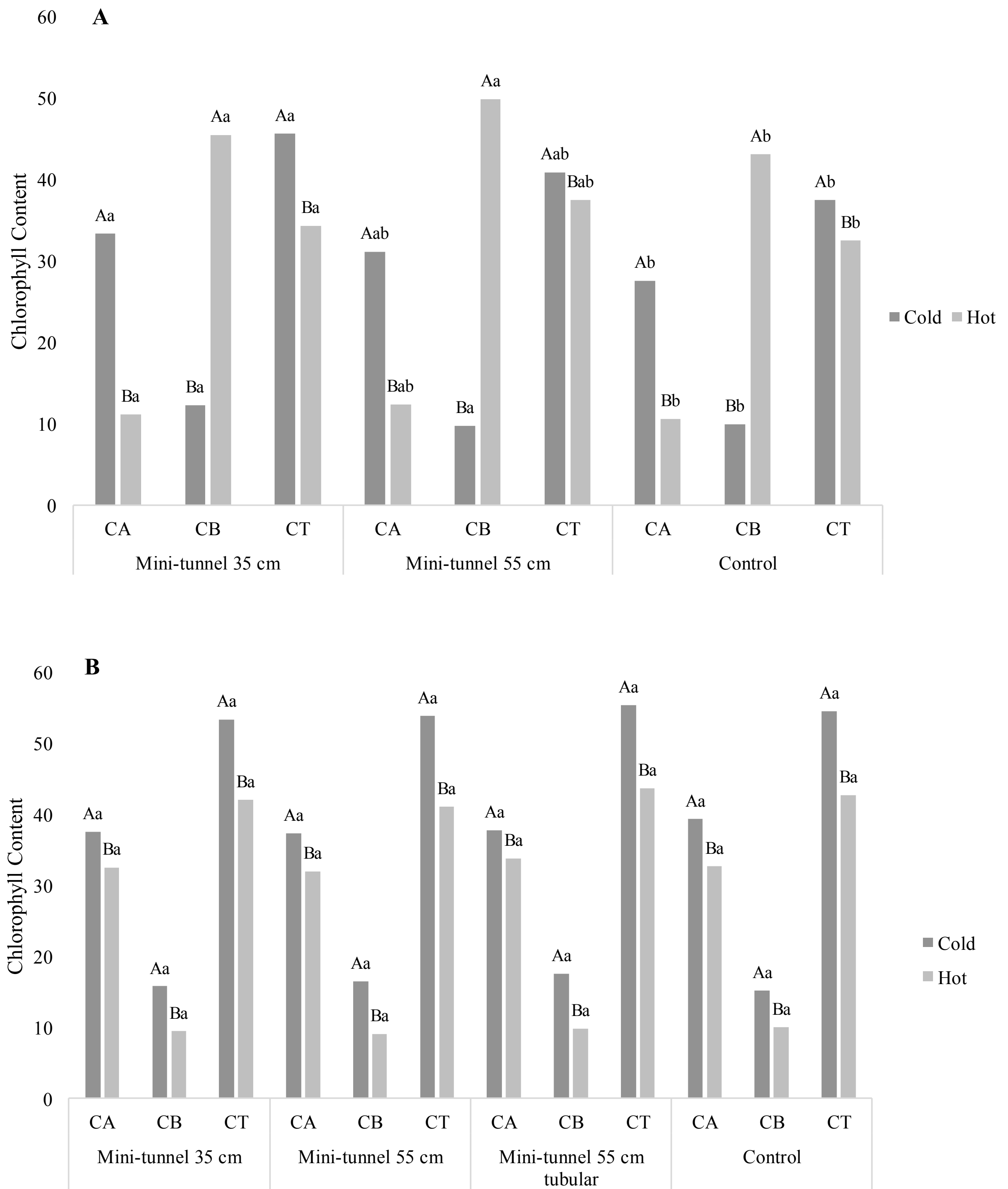
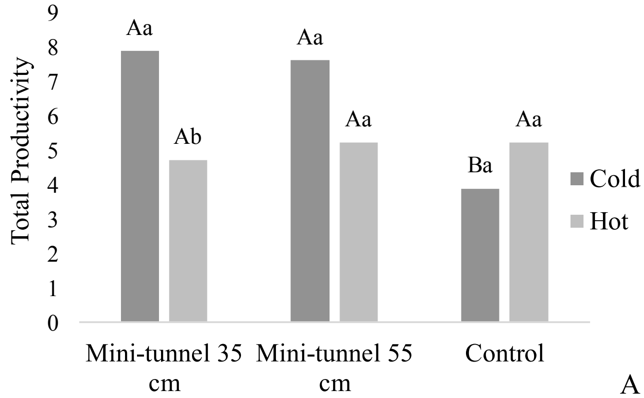


Figure 2. Chlorophyll content of clone 1 (A) and clone 2 (B) mini-cuttings as influenced by mini-tunnel models, over two seasons. Uppercase letters establish the comparison between the seasons in the same treatment (mini-tunnel models) and lowercase letters represent the comparison between the mini-tunnel models in the same treatment (seasons). Averages followed by the same letter do not differ from each other, by the Tukey test at 5 % significance.

Contenido de clorofila de las miniestacas del clon 1 (A) y del clon 2 (B) en función de los modelos de mini túnel durante dos temporadas. Las letras mayúsculas establecen la comparación entre las temporadas en un mismo tratamiento (modelos de mini túnel) y las minúsculas representan la comparación entre los modelos de mini túnel en el mismo tratamiento (temporadas). Medias seguidas de la misma letra no difieren entre sí, utilizando la prueba de Tukey al 5 % de significación.



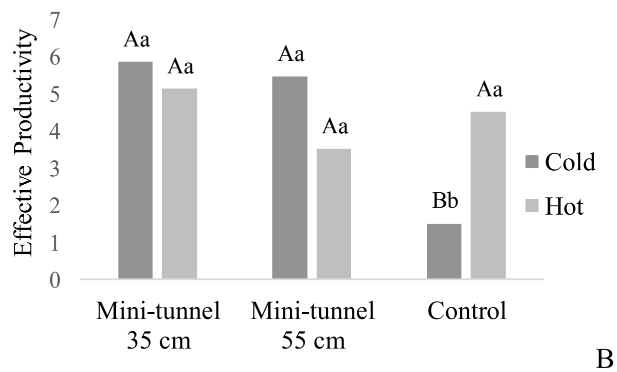


Figure 3. Total productivity (A) and effective productivity (B) of mini-cuttings by mini-stumps of clone 1 as influenced by mini-tunnel models, over two seasons. Lowercase letters establish the comparison between the seasons in the same treatment (mini-tunnel models) and uppercase letters represent the comparison between the mini-tunnel models in the same treatment (seasons). Averages followed by the same letter do not differ from each other, by the Tukey test at 5 % significance.

Productividad total (A) y productividad efectiva (B) de miniestacas por minicepas del clon 1 en función de los modelos de mini túnel, durante dos temporadas. Las letras minúsculas establecen la comparación entre las temporadas en un mismo tratamiento (modelos de mini túnel) y las mayúsculas representan la comparación entre los modelos de mini túnel en el mismo tratamiento (temporadas). Medias seguidas de la misma letra no difieren entre sí, utilizando la prueba de Tukey al 5 % de significación.

at the 5 % significance level. The control treatment was significantly influenced by the season, with the averages observed as lower in the hot season than in the cold season (figure 4A). Season had a significant influence on the effective productivity of the mini-cuttings from the mini-stumps, with the cold period showing a lower number for this parameter than the hot period. Statistically, this variable showed no difference among the tested mini-tunnel models (figure 4B).

The average heights of the mini-cuttings of clone 1 were significantly higher in treatments with the 35 cm and 55 cm mini-tunnels than in the control treatment without a mini-tunnel (figure 5A). The height of the clone 2 mini-cuttings was not influenced by the tested mini-tunnel models (figure 5B). The mean heights were shorter during the cold period than during the hot period for both clones.

In the cold period, the dry matter of the mini-cuttings of clone 1 differed significantly among treatments, with the control condition (without mini-tunnel) showing higher values than the other conditions (figure 6A). Under all treatments, the average values of dry matter were significantly higher in the hot season than in the cold season. Similar to clone 1, clone 2 showed higher dry matter averages in the hot period, especially with the control treatment (figure 6B).

The mean values for leaf area of the mini-cuttings of both clones differed significantly between seasons (figure 7). The average leaf area was lower in the cold period (7.00 cm^2 - clone 1 and 7.56 cm^2 - clone 2) than in the warm period (10.70 cm^2 - clone 1 and 8.62 - clone 2). This variable did not differ significantly among the mini-tunnel models, at the 5 % significance level.

DISCUSSION

Chlorophylls are greenish pigments present in chloroplasts. They specialize in light absorption, which, in turn, is used in photosynthesis. Chlorophylls A and B are abundant in green plants, with type A being the most common (Taiz *et al*. 2017). Chlorophyll content was higher in treatments using mini-tunnels, for clone 1 in the hot period and for both clones in the cold period. Plants grown in low-light environments, such as mini-tunnels, often have higher levels of chlorophyll in their leaves, which may be related to a compensatory mechanism used by the species for survival in low levels of solar radiation (Lima *et al*. 2010). The effects of mini-tunnel use were studied on *Corymbia* and *Eucalyptus* species throughout the seasons and found that the environmental changes in mini-tunnels minimize stress on the stock plant and promote higher photosynthetic rates due to the higher concentrations of chlorophyll A and B (Lima *et al.* 2022).

The productivity of the mini-stumps stands out as a key deciding factor for the success of mini-cuttings. The productivity of the mini-stumps is influenced by variables such as the type of clonal mini-garden, the nutritional management used, and seasonality (Pimentel *et al*. 2019). The use of the mini-tunnel in the clonal mini-garden had different effects on the productivity of mini-cuttings from mini-stumps depending on the season of propagule collection (cold or hot). In gutters covered with mini-tunnels, the higher productivity of mini-cuttings observed primarily in the cold season may be related to the temperature changes that occur near the canopy of the mini-stumps. The cell division process and consequently the emission of sprouts and the formation of plant roots are favored by higher temperatures to a certain extent (Lima *et al*. 2022). A study by Oliveira (2016) showed that the temperature in mini-gardens covered with mini-tunnels is 10.6 % higher in the cold period and 13.0 % higher in the hot period, relative to the temperature of gutters that do not use mini-tunnels. A study by Batista *et al*. (2015) showed that mini-tunnels increase mini-cutting production by 53 %. However, contrary to the present study, Batista *et al.* (2015) observed that mini-tunnel use in the cold season did not improve productivity of the mini-stumps of the clones studied, suggesting the influence of seasonality and the genotype being propagated.

Another factor that influences the productivity of mini-stumps and the quality of mini-cuttings is the presence of pathogens, such as the powdery mildew fungus (*Oidium* sp.), which preferentially affects buds and shoots. During our experiments, powdery mildew was observed in the channels of both genotypes, but its presence was more accentuated in those without coverage. A plant containing this pathogen may have tapering limbs with one half generally narrower than the other half, and leaves with a whitish, powdery cover and wrinkled appearance. The attack on sprouts results in overgrowth, death, and the consequent loss of propagule quality. This disease spreads through the air, by contact between healthy and sick plants, and via rain splashes. It occurs mainly in the dry season in the genus *Eucalyptus*, which in the studied area coincides with the cold period (Bovolini *et al*. 2018).

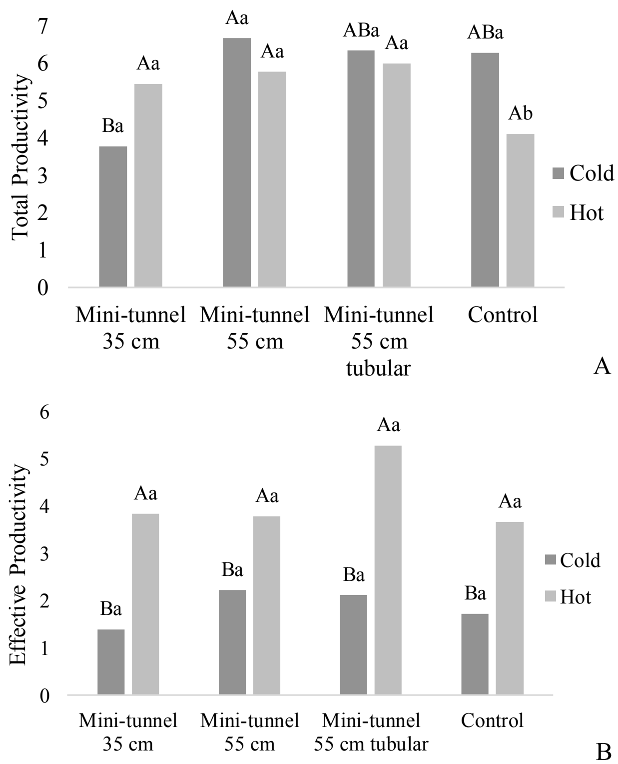


Figure 4. Total productivity (A) and effective productivity (B) of mini-cuttings from mini-stump of clone 2 as influenced by mini-tunnel models, over two seasons. Lowercase letters establish the comparison between the seasons in the same treatment (mini-tunnel models) and uppercase letters represent the comparison between the mini-tunnel models in the same treatment (seasons). Averages followed by the same letter do not differ from each other, by the Tukey test at 5 % significance.

Productividad total (A) y productividad efectiva (B) de miniestacas por minicepas del clon 2 en función de los modelos de mini túnel, durante dos temporadas. Las letras minúsculas establecen la comparación entre las temporadas en un mismo tratamiento (modelos de mini túnel) y las mayúsculas representan la comparación entre los modelos de mini túnel en el mismo tratamiento (temporadas). Medias seguidas de la misma letra no difieren entre sí, utilizando la prueba de Tukey al 5 % de significación.

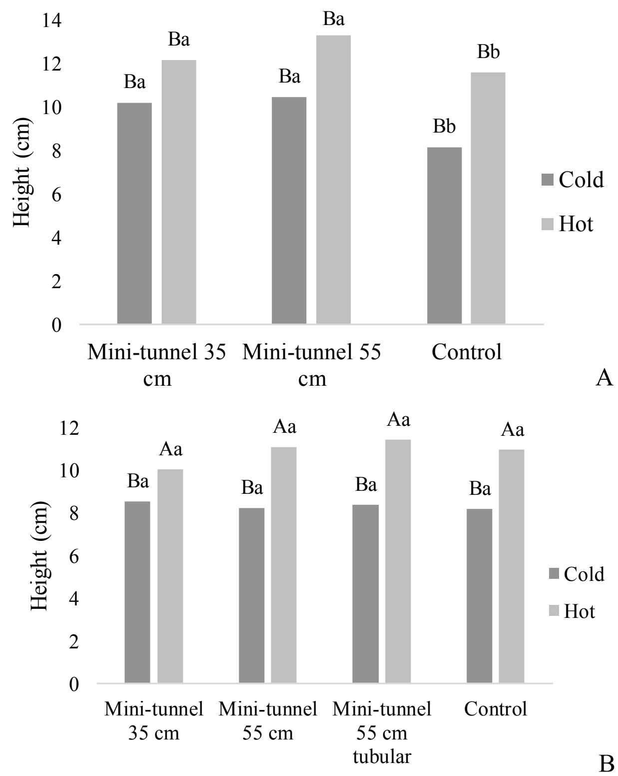


Figure 5. Average height of the mini-cuttings of clone 1 (A) and clone 2 (B) as influenced by mini-tunnel models, over two seasons. Uppercase letters establish the comparison between the seasons in the same treatment (mini-tunnel models) and lowercase letters represent the comparison between the mini-tunnel models in the same treatment (seasons). Averages followed by the same letter do not differ from each other, by the Tukey test at 5 % significance.

Altura media de las miniestacas del clon 1 (A) y clon 2 (B) en función de modelos de mini túnel, durante dos temporadas. Las letras mayúsculas establecen la comparación entre las temporadas en un mismo tratamiento (modelos de mini túnel) y las minúsculas representan la comparación entre los modelos de mini túnel en el mismo tratamiento (temporadas). Medias seguidas de la misma letra no difieren entre sí, utilizando la prueba de Tukey al 5 % de significación.

Studies on the cultivation of *Eucalyptus* mini-stumps in environments protected by mini-tunnels have shown a significant reduction in the occurrence of powdery mildew (Martins and Silveira 2014, Ruiz 2019), due to the reduced chance of contamination by wind and rain. This may explain the greater effective productivity of mini-cuttings and the higher averages of height, leaf area, and consequently, dry matter of the mini-cuttings for both clones in the hot period.

Etiolation is the process of abnormal elongation in plants, a compensatory mechanism used to search for light when there is a lower incidence of solar radiation (Guimarães *et al*. 2018). Clone 1 was influenced by the type of mini-tunnel used, whereas clone 2 was only influenced by the season. The highest average heights observed in the production of *Eucalyptus* mini-cuttings using mini-tunnels were in accordance with those reported by Batista *et al*. (2015) and Oliveira (2016). This was the case for

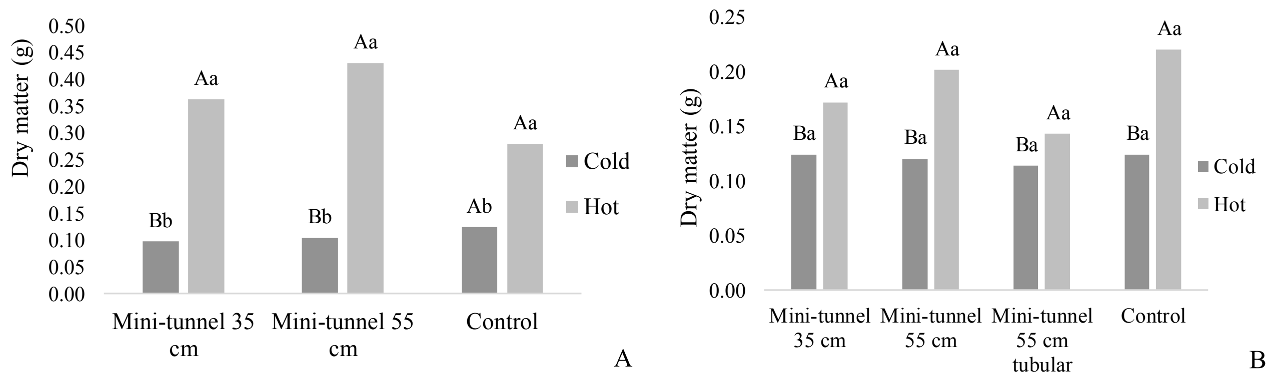


Figure 6. Dry matter of clone 1 (A) and clone 2 (B) mini-cuttings as influenced by mini-tunnel models, over two seasons. Lowercase letters establish the comparison between the seasons in the same treatment (mini-tunnel models) and uppercase letters represent the comparison between the mini-tunnel models in the same treatment (seasons). Averages followed by the same letter do not differ from each other, by the Tukey test at 5 % significance.

Materia seca de las miniestacas del clon 1 (A) y clon 2 (B) en función de modelos de mini túnel, durante dos temporadas. Las letras minúsculas establecen la comparación entre las temporadas en un mismo tratamiento (modelos de mini túnel) y las mayúsculas representan la comparación entre los modelos de mini túnel en el mismo tratamiento (temporadas). Medias seguidas de la misma letra no difieren entre sí, utilizando la prueba de Tukey al 5 % de significación.

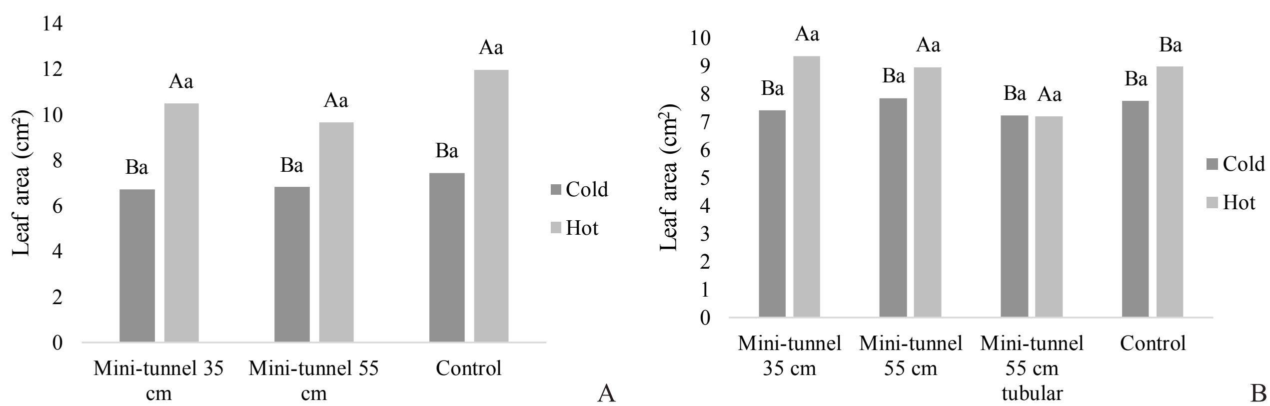


Figure 7. Leaf area of mini-cuttings of Clone 1 (A) and Clone 2 (B) as influenced by mini-tunnel models, over two seasons. Uppercase letters establish the comparison between the seasons in the same treatment (mini-tunnel models) and lowercase letters represent the comparison between the mini-tunnel models in the same treatment (seasons). Averages followed by the same letter do not differ from each other, by the Tukey test at 5 % significance.

Área foliar de las miniestacas del Clon 1 (A) y Clon 2 (B) influenciados por modelos de mini túnel, durante dos temporadas. Las letras mayúsculas establecen la comparación entre las temporadas en un mismo tratamiento (modelos de mini túnel) y las minúsculas representan la comparación entre los modelos de mini túnel en el mismo tratamiento (temporadas). Medias seguidas de la misma letra no difieren entre sí, utilizando la prueba de Tukey al 5 % de significación.

treatments in which a mini-tunnel was covered with a polyethylene plastic film. Environments with conditions of low irradiance force plants to use different strategies to absorb sufficient light energy for use as chemical energy (Moraes *et al*. 2013).

The amount of dry matter is a good indicator of the efficiency and growth potential of the plant throughout its life cycle. For both clones, the average dry matter values were higher in the hot season than in the cold season. According to Ataíde *et al*. (2010), plant development depends on the adequate conversion of intercepted solar energy into increasing amounts of carbohydrates, with growth in dry mass resulting from the accumulation of these substances in vegetables.

Leaf area is an important parameter in determining the photosynthetic capacity, the soil-water-plant relationship, and the production and accumulation of biomass in different cultures (Bastos *et al*. 2012). Sanquetta *et al*. (2014) reported that leaf area is one of the most important biophysical characteristics of plants, because it determines the amount of light energy available to perform photosynthesis and is therefore related to plant growth. The results obtained in this study show that the two genotypes were significantly influenced only by the season of the year. This contrasts with the results reported by Batista *et al*. (2015), who found that mini-cuttings produced under a mini-tunnel showed significantly smaller leaf areas than those produced under the control treatment for all clones tested, indicating that a morphological change was triggered in the plant. Changing leaf shape is one of the response mechanisms available to plants in extreme environmental conditions, such as prolonged exposure to high temperatures or low relative humidity. Very low relative humidity may cause a wide vapor pressure gradient, leading to a water deficit in the plant, even when there is the adequate water content in the soil (Taiz *et al*. 2017).

The average, maximum, and minimum temperatures of the day at certain time intervals are important parameters that must be considered for propagation and need to be adapted for each species, hybrid, or clone (Pereira *et al*. 2019). The presence of the mini-tunnel and the season both influenced the productivity of the studied clones, but varied according to the genotype. Therefore, further research is needed regarding the benefits of using a mini-tunnel and its interaction with environmental conditions for different cultivated genotypes in order to determine the best form of application.

CONCLUSIONS

Together, the season and the use of a mini-tunnel influenced the productivity of mini-cuttings for the two genotypes in this study. Mini-cuttings produced during the hot season showed greater growth, dry matter, and leaf area than those collected during the cold season. The mini-tunnel promoted the productivity of mini-cuttings for both clones, primarily during the cold period. Thus, the use of mini-tunnels is advantageous, as it increases the productivity of clonal *Eucalyptus* mini-gardens, as shown here by its overall positive influence on mini-cuttings of both genotypes tested.

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