BOSQUE 44(3): 595-603, 2023 DOI: 10.4067/S0717-92002023000300595

Productivity of mini-cuttings of a hybrid clone of *Eucalyptus urophylla* x *Eucalyptus pellita* as a function of exposure time of mini-stumps to mini-tunnel

Productividad de miniestacas de un clon híbrido de *Eucalyptus urophylla* x *Eucalyptus pellita* en función del tiempo de exposición de minicepas a mini túnel

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SUMMARY

With the implementation of the mini-cutting technique in *Eucalyptus*, several alternatives have emerged to improve structures for plant propagation, growth, and development. A technology recently applied in the propagation of eucalyptus in clonal mini-gardens is the mini-tunnel, which has shown promise in increasing mini-stump productivity and the rooting of mini-cuttings. The aim of this study was to evaluate the effect of exposure times for mini-stumps to mini-tunnel on the productivity of mini-cuttings from a hybrid clone of *Eucalyptus urophylla* x *Eucalyptus pellita*. Four exposure times were tested: 0 days (control), 15, 30, and 45 days. The total productivity and effective productivity of mini-cuttings per mini-stump were evaluated, as well as the levels of chlorophyll *a*, *b*, and total chlorophyll, height, leaf area, and dry mass of the mini-cuttings. The results showed that a 45-day exposure period of mini-stumps to the mini-tunnel resulted in increased mini-cutting productivity, greater height, higher chlorophyll content, smaller leaf area, and reduced dry biomass of the mini-cuttings. These findings offer advantages for managing clonal mini-gardens.

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

RESUMEN

Con la implementación de la técnica de miniestaca en *Eucalyptus* han surgido varias alternativas para mejorar las estructuras de propagación, crecimiento, y desarrollo de las plantas. Una tecnología que se ha utilizado recientemente en la propagación de eucaliptos en mini jardines clonales es el mini túnel, que se ha mostrado prometedor, permitiendo aumentar la productividad de las minicepas y también el enraizamiento de las miniestacas. En ese sentido, este estudio tuvo como objetivo evaluar el efecto de los tiempos de exposición de minicepas a mini túnel sobre la productividad de miniestacas de un clon híbrido de *Eucalyptus urophylla* x *Eucalyptus pellita*. Se probaron cuatro tiempos de exposición: 0 (control), 15, 30, y 45 días. Se evaluó la productividad total y la productividad efectiva de miniestacas por minicepa y los contenidos de clorofila *a*, *b*, y total, altura, área foliar y masa seca de las miniestacas. El tiempo de exposición de 45 días de las minicepas al mini túnel resultó en mayor productividad de miniestacas, aumento de altura y contenido de clorofila, además de menor área foliar y biomasa seca de las miniestacas. Estos hallazgos ofrecen ventajas para el manejo de mini jardines clonales.

Palabras clave: miniestaca, mini jardín clonal, hibridación.

INTRODUCTION

Species and hybrids of the *Eucalyptus* genus play a fundamental role in the forestry sector as they offer an effective alternative to reduce the consumption of and pressure on natural forests (Lima *et al*. 2022). However, it is important to emphasize that the effectiveness of this approach is significantly influenced by the specific context of cultivation and the management practices employed. Brazil, a leader in forest productivity, is one of the primary producers of cellulose, paper, and wood panels globally, largely attributable to the favorable edaphoclimatic conditions and the technologies developed and implemented in the country (Indústria Brasileira de Árvores (IBA) 2020).

In recent decades, there has been a continuous increase in interest in clonal forestry, due to its potential to address issues related to certain diseases, heterogeneity, and the productivity of forest plantations (Xavier *et al*. 2013). The combination of hybridization with the cloning of superior genotypes presents a promising alternative with significant implications for forest improvement programs. It contributes positively to three critical components of the competitive process: productivity, quality, and production costs (Assis *et al*. 2004). Consequently, the benefits of using superior genotypes of *Eucalyptus* spp. should be maximized by employing economically viable techniques for their propagation (Freitas *et al*. 2017).

Among the clonal propagation methods employed on a commercial scale, mini-cutting stands out. This technique involves the use of mini-cuttings obtained from shoots that develop on mini-stumps cultivated within clonal mini-gardens, usually located inside greenhouses (Canguçu *et al*. 2022). In comparison to other vegetative propagation techniques, the utilization of mini-cuttings offers higher productivity within a shorter timeframe, reduces the required cultivation area, increases uniformity and the number of roots per cutting, and improves the quality of the root system (Xavier *et al*. 2013).

The implementation of mini-cuttings has given rise to various innovations aimed at improving the facilities dedicated to plant propagation, growth, and development. One such technology that have gained prominence in the propagation of eucalyptus within mini clonal gardens is the mini-tunnel. This structure is essentially a small greenhouse designed in the shape of a tunnel and covered with plastic. It provides favorable conditions for the growth and production of mini-stumps, thereby increasing the overall productivity of the nursery (Pereira *et al*. 2019).

The environment created within the mini-tunnels, where the temperature and humidity are higher, induces a series of observable changes in leaf morphology. These alterations manifest as reduced leaf size, narrower leaf shape, and lighter pigmentation (Batista *et al*. 2015). Studies conducted with various eucalyptus species have demonstrated that the use of mini-tunnels leads to increased mini-stump productivity, enhanced rooting success, and a reduction in callus formation during the rooting process (Assis 2011, Batista *et al*. 2015, Lima *et al*. 2022, Canguçu *et al*. 2022).

In this regard, the present study is conducted to evaluate the effect of varying durations of mini-stump exposure to a mini-tunnel environment on the productivity of mini-cuttings derived from a hybrid clone of *Eucalyptus urophylla* S. T. Blake x *Eucalyptus pellita* F. Muell within a commercial nursery setting.

METHODS

The experiment was conducted during the period between October and December 2018 within the seedling nursery of Aperam BioEnergia, located in the municipality of Itamarandiba, Minas Gerais (17° 51’ 24’’ S and 42° 51’ 40’’ W), at an elevation of 910 m a.s.l. The region’s climate is classified as Cwa according to the Köppen classification, characterized by cold, dry winters and hot, humid summers (Alvares *et al*. 2013). Throughout the study, the total recorded rainfall was 83 mm, with an average temperature of 22 ºC (mean minimum temperature of 17 ºC and mean maximum temperature of 27 ºC).

The clonal mini-garden was composed of four fiber cement gutters, elevated 1 m above the ground, with dimensions measuring 0.8 x 16.3 m, a slope of 10 %, and a depth of 0.3 m. The gutters were equipped with a 5 cm layer of crushed stone to improve drainage, and 25 cm of gravel (granulometry between 2 and 5 mm) (figure 1A). In each gutter, mini-stumps of a hybrid clone of *Eucalyptus urophylla* x *Eucalyptus pellita* were cultivated for a duration of 30 months, with a spacing of 10 x 10 cm, utilizing a semi-hydroponic irrigation system. This specific hybrid genotype was obtained through controlled pollination and selected after reaching 96 months of age. The mother tree, which served as the origin for the clones, was initially planted in the city of Itamarandiba in 2001, with a spacing of 3 x 3 meters. By the time it reached 88 months of age, this mother tree had a basic density of 583 kg m^{-3} and an average annual increment of 35 m^3 per hectare per year.

The mini-stumps (figure 1C) were obtained using seedlings derived from shoots of the second generation from the staking process within the clonal mini-garden. These seedlings were initially rooted in a greenhouse and subsequently transplanted into the gutters in May 2016 (figure 1A). Following a 21-day adjustment period to the semi-hydroponic system, the apices of the seedlings were pruned to a height of 6 to 8 cm from the base (figure 1B).

The mini-tunnel employed to cover the gutters had a tubular structure measuring 0.80 x 16.30 x 0.50 m (width x length x height). It was constructed using galvanized steel and enclosed with low-density polyethylene plastic film with a thickness of 150 µm (figure 1D).

Before the installation of the experiment, the clonal mini-garden remained open, and a weekly cleaning removed leaves, shoots, and any deceased mini-stumps. The irrigation and mineral nutrition of the mini-stumps were managed through an automated drip fertigation system, with seven daily applications and a flow rate of 9.3 L m^{-2} day^{-1}. Table 1 provides details of the stock solutions used in the fertigation process.

The experiment employed one gutter for each exposure duration of mini-stumps under the mini-tunnel (0 - control, 15, 30, and 45 days). The process of covering the gutters with plastic film was carried out in stages (figure 1E), beginning with the gutter designated for the 45-day duration. After 15 days, the film was placed on the gutter designated for the 30-day duration, and finally, after an additional 15 days, on the treatment specified for a 15-day duration. The control treatment remained uncovered with plastic film throughout the entire experimental period.

Following the establishment of the treatments, the mini-cuttings (figure 1F) were harvested on a weekly basis, except for the final seven days leading up to the experimental evaluations. The seven-day interval was determi-

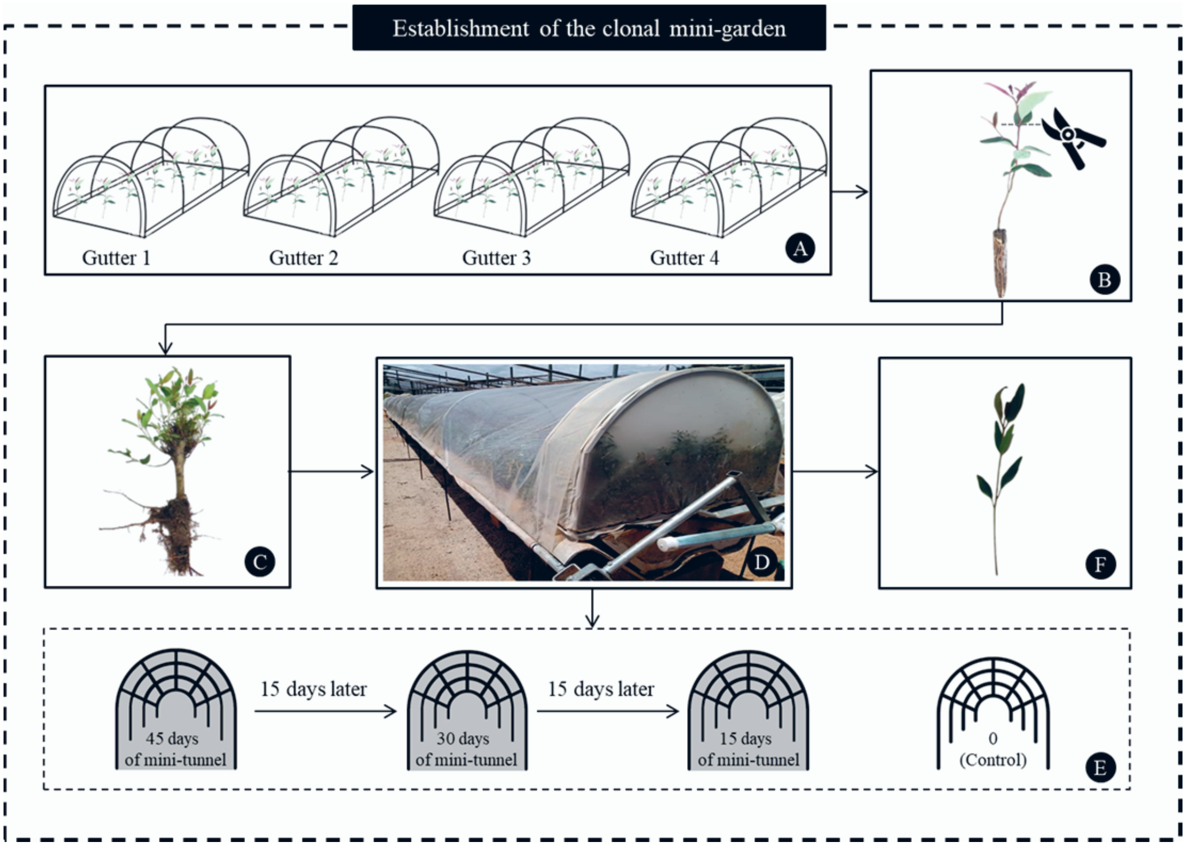

Figure 1. Flowchart of the establishment of the clonal mini-garden of *Eucalyptus urophylla* x *Eucalyptus pellita*. A) Seedlings transplanted to fiber cement gutters, B) Apical pruning, C) Mini-stump, D) Overview of the mini-tunnel over the gutter, E) Staggered application of plastic film, F) Mini-cutting.

Diagrama de flujo para el establecimiento del mini jardín clonal de *Eucalyptus urophylla* x *Eucalyptus pellita*. A) Plántulas trasplantadas a canaletas de fibrocimento, B) Poda apical, C) Minicepa, D) Vista general del mini túnel sobre la canaleta, E) Aplicación escalonada de película plástica, F) Miniestaca.

Table 1. Fertilizers and quantity of solutions used in the fertigation of mini-stumps of *Eucalyptus urophylla* x *Eucalyptus pellita*, in the clonal mini-garden.

Fertilizantes y cantidad de soluciones utilizadas en la fertirrigación de minicepas de *Eucalyptus urophylla* x *Eucalyptus pellita*, en el mini jardín clonal.

Fertilizers	Quantity of stock solution
Monoammonium phosphate	1.55 g L^{-1}
Magnesium sulfate	0.5 g L^{-1}
Potassium chloride	0.6 g L^{-1}
Calcium chloride	0.5 g L^{-1}
Zinc sulfate	4 mg L^{-1}
Hydroiron	24 mg L^{-1}
Boric acid	11 mg L^{-1}
Manganese sulfate	14 mg L^{-1}
Copper sulfate	2 mg L^{-1}

Source: Aperam BioEnergia.

ned in accordance with the company's operational collection frequency and the recommendation of Xavier *et al*. (2013).

A randomized block design was employed, consisting of four treatments (mini-tunnel exposure times) and three blocks (beginning, middle, and end of the gutter), with six mini-stumps per plot.

Before collecting the mini-cuttings, chlorophyll levels were assessed non-destructively by measuring chlorophyll *a*, *b*, and total in five leaves per plot, using the portable chlorophyll meter ClorofiLOG (Falker®, model: CFL 1030).

The following variables were determined from the collected mini-cuttings: total productivity and effective productivity of mini-cuttings per mini-stump, as well as the height, leaf area, and dry mass of the mini-cuttings. Total productivity considered the number of mini-cuttings taller than 5 cm, while effective productivity included the number of mini-cuttings taller than 9 cm (the minimum height required for staking according to the company's operational standard). Mini-cutting height was measured using a ruler, and dry mass was determined by drying the material in an oven with forced air circulation at 65 ºC, until a constant weight was reached, followed by weighing on an analytical scale. Leaf area was determined by collecting five leaves from each plot, scanning them, and digitizing them using the Digital Determinator of Areas (DDA) software (Ferreira *et al*. 2008).

The collected data underwent the Shapiro-Wilk test to assess the normality of the residuals and the Bartlett test to assess the homogeneity of variances. Given that the assumptions were satisfied, we proceeded with the analysis of variance and conducted mean comparison using Tukey's test at a 5 % significance level, when significant differences were observed between treatments. All analyses were performed using the R software (R Core Team 2018).

RESULTS

There was no statistically significant effect of the mini-tunnel exposure times on the total productivity of mini-cuttings per mini-stump ($P > 0.05$). The values observed for the control treatment and 15 and 30 day exposure times to the mini-tunnel were 3.4, 3.2, and 2.9 mini-cuttings / per mini-stump, respectively. However, in the 45-day exposure time, there was a 73 % increase in the variable compared to the control treatment, resulting in 5.9 mini-cuttings / per mini-stump (figure 2A). Regarding effective productivity, there was a significant effect of the mini-tunnel exposure times ($P < 0.05$). The 45-day period (4.1 mini-cuttings / mini-stump) showed a statistically significant difference from the 30-day period (1.6 mini-cuttings / mini-stump), representing a 156% increase (figure 2B). The visual comparison between the mini-stumps subjected to a 45-day exposure period to the mini-tunnel and those from the control treatment is illustrated in figure 3.

Regarding mini-cutting height, the highest average was found in the treatment with 45 days of mini-tunnel exposure (10.5 cm), while the lowest was in the control treatment (8.9 cm). There was no statistical difference between them (figure 4). Figure 5 illustrates the visual appearance of the mini-cuttings after 45 days of mini-tunnel exposure and the mini-cuttings from mini-stumps that were not covered by the mini-tunnel.

The mini-cuttings produced during 45 days of mini-tunnel had a lower average leaf area (3.6 cm²) when compared to the other treatments, showing a 22 % reduction from the control treatment (4.4 cm²) (figure 6). The visual aspect of the leaf area of the mini-cuttings after the 45-day period under the mini-tunnel is illustrated in figure 7, allowing a visual comparison with the mini-cuttings from the control treatment.

For dry mass, there was a decrease in the variable with increasing mini-tunnel time, where the control treatment (0.17 g) was statistically superior to the 45-day treatment (0.11 g) (figure 8).

There was a linear increase in the levels of chlorophyll *a* and total chlorophyll with the increase in the time of the mini-stumps under the mini-tunnel. Regarding chlorophyll *b*, there was a reduction up to 30 days, and from that point on, an increase in the value of the variable was observed (figure 9).

DISCUSSION

The productivity of the mini-stumps stands out as one of the main factors for the success of mini-cutting, being directly influenced by proper management and mineral nutrition (Pimentel *et al*. 2019). In the present study, the solution of macro and micronutrients positively favored the nutritional status of the mini-stumps, and the presence of the mini-tunnel for a longer time proved to be important in the production process of mini-cuttings of *Eucalyptus urophylla* x *E. pellita*. Productivity values of *Eucalyptus* mini-stumps implanted in clonal mini-garden have been reported by several authors: 1.0 mini-cutting / mini-stump

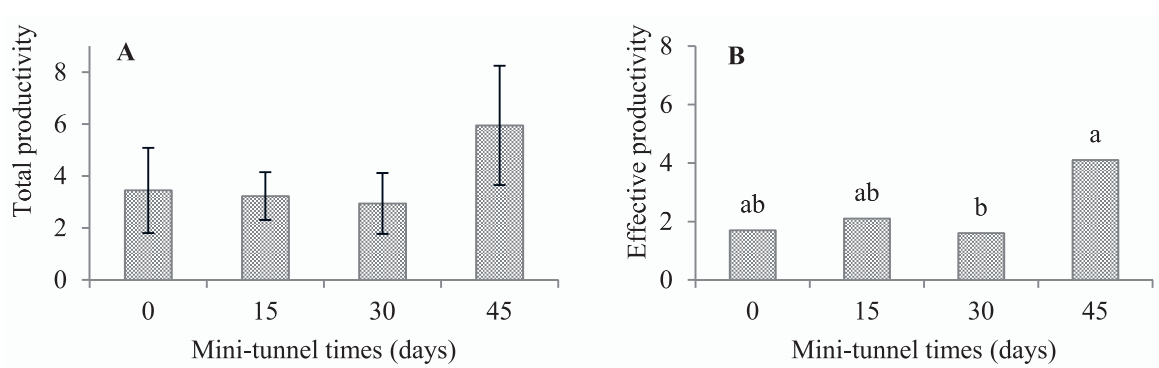

Figure 2. A) Total productivity and B) effective productivity of mini-cuttings per mini-stump of *E. urophylla* x *E. pellita* in response to four mini-tunnel exposure times. Bars indicate standard deviation. Means followed by the same letter do not differ from each other according to the Tukey test at 5 % significance.

A) Productividad total y B) Productividad efectiva de miniestacas por minicepa de *E. urophylla* x *E. pellita* en respuesta a cuatro tiempos de mini túnel. Las barras indican la desviación estándar. Medias seguidas de la misma letra no difieren entre sí por la prueba de Tukey al 5 % de significación.

in clones of *E. globulus* (Alfenas *et al*. 2009); 2.9 mini-cuttings / mini-stump in hybrids of *Eucalyptus urophylla* x *E. grandis* (Cunha *et al*. 2009); 3.2 mini-cuttings / mini-stump in hybrids of *Eucalyptus urophylla* x *E. grandis* (Souza *et al*. 2014); 1.9 mini-cuttings / mini-stump in hybrids of *Eucalyptus globulus* (Freitas *et al*. 2017).

The decrease in productivity values at 15 and 30 days of mini-tunnel exposure, compared to the control treatment, can be attributed to the need for initial adaptation of the mini-stumps to the new environmental conditions (Peguero-Pina *et al*. 2020). In the environment protected by the mini-tunnel, the increase in temperature and relative humidity of the air can make the mini-stumps more sensitive during the adaptation process, leading to more gradual growth, and consequently, a reduction in productivity. On the other hand, the significant increase in productivity after

Figure 3. Visual comparison between a mini-stump from the control treatment (A) and a mini-stump exposed to 45 days of mini-tunnel (B).

Comparación visual entre una minicepa del tratamiento control (A) y una minicepa expuesta a 45 días de mini túnel (B).

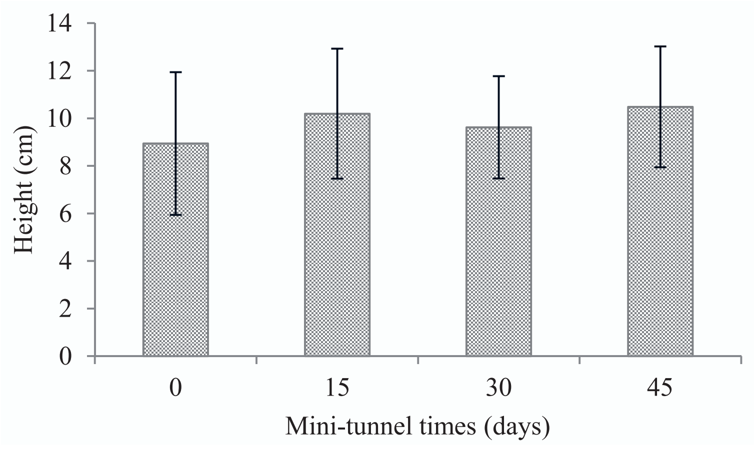

Figure 4. Average height of mini-cuttings of *E. urophylla* x *E. pellita* in response to four mini-tunnel exposure times. Bars indicate standard deviation.

Altura media de miniestacas de *E. urophylla* x *E. pellita* en respuesta a cuatro tiempos de mini túnel. Las barras indican la desviación estándar.

45 days of exposure to the mini-tunnel environment can be explained by several factors: complete adaptation of the mini-stumps to the new environment, which favors the cell division process; development of a more robust root system during this period, allowing for greater absorption of water and nutrients; increase in chlorophyll content, indicating an improvement in the plant's ability to carry out photosynthesis and, consequently, generate more energy for the growth and development of mini-cuttings, even if they have reduced leaf area (Hartmann *et al*. 2011, Taiz *et al*. 2017).

The results regarding mini-cutting height are consistent with the observations made by Batista *et al*. (2015) and Oliveira (2016) who, while working with *Eucalyptus* mini-cuttings, noted greater heights in plants grown under the mini-tunnel. According to these authors, the elongation of the shoots can be attributed to the reduced light exposure in the plots covered with the mini-tunnel, which is coated with polyethylene plastic film.

The smaller leaf area observed in the mini-cuttings produced during the longest exposure period in the mini-tunnel suggests a morphological adaptation in the plants

Figure 5. Visual aspect of the height of mini-cuttings. A) Mini-cuttings after 45 days of mini-tunnel, B) Mini-cuttings from mini-stumps that were not covered with mini-tunnel.

Aspecto visual de la altura de las miniestacas. A) Miniestacas después de 45 días de mini túnel, B) Miniestacas de minicepas que no fueron cubiertos con mini túnel.

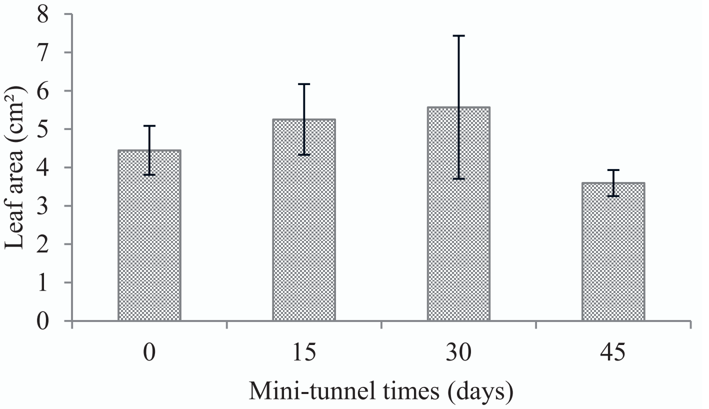

Figure 6. Leaf area of mini-cuttings of *E. urophylla* x *E. pellita* in response to four mini-tunnel times. Bars indicate standard deviation.

Área foliar de miniestacas de *E. urophylla* x *E. pellita* en respuesta a cuatro tiempos de mini túnel. Las barras indican la desviación estándar.

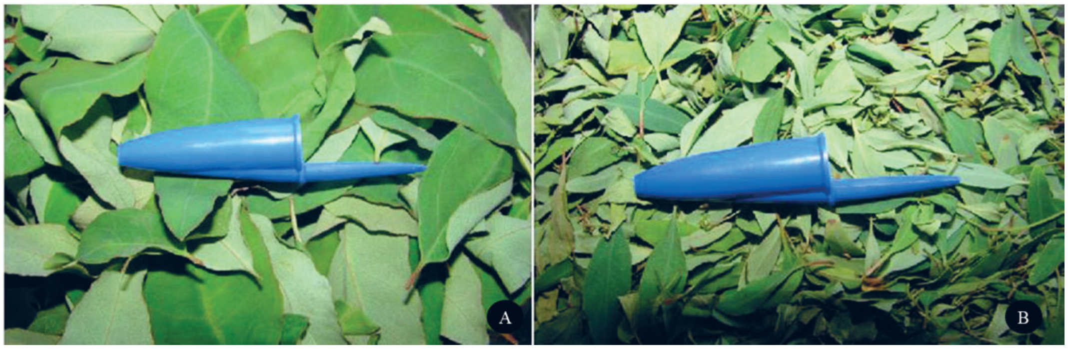

Figure 7. Visual comparison of the leaf area between the mini-cuttings of the control treatment (A) and those exposed to the mini-tunnel for 45 days (B).

Comparación visual del área foliar entre las miniestacas del tratamiento control (A) y las expuestas al mini túnel durante 45 días (B).

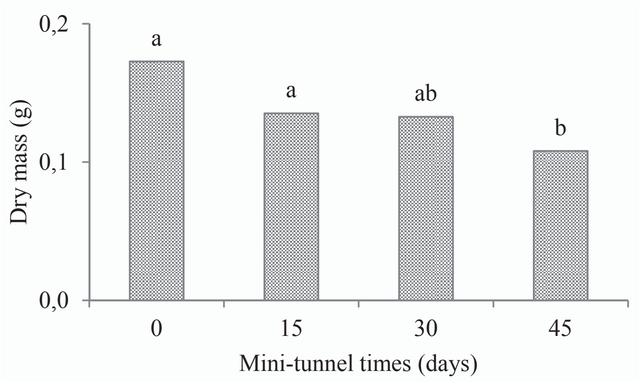

Figure 8. Dry mass of mini-cuttings of *E. urophylla* x *E. pellita* in response to four mini-tunnel exposure times. Means followed by the same letter do not differ from each other according to the Tukey test at 5 % significance.

Masa seca de miniestacas de *E. urophylla* x *E. pellita* en respuesta a cuatro tiempos de mini túnel. Medias seguidas de la misma letra no difieren entre sí por la prueba de Tukey al 5 % de significación.

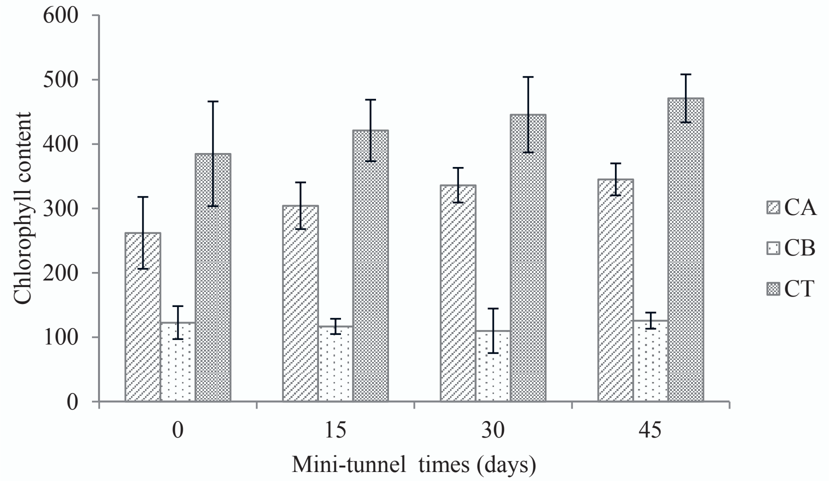

Figure 9. Chlorophyll *a* (CA), *b* (CB), and total (CT) contents of *E. urophylla* x *E. pellita* mini-cuttings in response to four mini-tunnel exposure times. Bars indicate standard deviation.

Contenido de clorofila *a* (CA), *b* (CB) y total (CT) de miniestacas de *E. urophylla* x *E. pellita* en respuesta a cuatro tiempos de mini túnel. Las barras indican la desviación estándar.

(Batista *et al*. 2015). Leaf area is a critical parameter for biomass production and accumulation, and changes in leaf shape represent one of the plant's responses to extreme environmental conditions, such as prolonged exposure to high temperatures (Taiz *et al*. 2017). Consequently, a reduction in leaf area may represent a mechanism to enhance vegetative propagation efficiency with the same amount of available photoassimilates, while maintaining an optimal water balance to facilitate cell division (Mendonça *et al*. 2010, Batista *et al*. 2015). Moreover, since reducing leaf area by 50 % is a common practice in commercial nurseries (Xavier *et al*. 2013), utilizing mini-cuttings produced under the mini-tunnel conditions may lead to operational benefits and reduce the risk of repetitive stress injuries among workers, as these mini-cuttings inherently have reduced leaf area (Santana *et al*. 2013, Batista *et al*. 2015).

The decrease in dry biomass observed in the mini-cuttings with longer exposure to the mini-tunnel conditions suggests that the allocation of photoassimilates in the mini-stumps favored the production of a greater number of mini-cuttings, even with reduced biomass. This allocation strategy may be an adaptation to cope with extreme environmental conditions (Oliveira 2016). Dry mass reflects the plant's capacity to increase its dry weight through photosynthesis. In other words, dry mass production depends on the efficiency with which intercepted photosynthetically active solar radiation is converted into increasing carbohydrate levels (Ataíde *et al*. 2010, Sanquetta *et al*. 2014).

Chlorophylls are the primary pigments present in chloroplasts, responsible for capturing solar radiation, which is then converted into chemical energy during the photosynthesis process (Taiz *et al*. 2017). Often, plants grown in environments with limited light availability, such as those under a mini-tunnel, exhibit higher levels of chlorophyll in their leaves. This phenomenon may be related to a compensatory mechanism employed by the species to cope with the reduced amount of available radiation (Lima *et al*. 2010). Despite the reduction in leaf area observed with prolonged exposure to the mini-tunnel, the total chlorophyll content increased over a 45-day period, surpassing that of the control treatment. These findings are consistent with a study by Silva *et al*. (2004), which reported elevated chlorophyll levels in *Eucalyptus grandis* seedlings subjected to water stress.

The results of this study suggest that the use of a mini-tunnel is an advantageous technology for the clonal propagation of eucalyptus, and has the potential to increase the productive capacity of clonal mini-gardens, in line with studies conducted by Lima *et al*. (2022) and Canguçu *et al*. (2022). As hybrids between *Eucalyptus urophylla* and *Eucalyptus pellita* have gained attention in the energy sector, particularly due to their properties like basic density and growth rate (Rocha *et al*. 2022), the propagation of these hybrid individuals can be further enhanced by the use of the mini-tunnel. It is important to highlight that the application of this technology is relatively recent in the field of forestry, and there is a noticeable gap in current information, both concerning *Eucalyptus* and other forest species.

CONCLUSIONS

The 45-days exposure of the mini-stumps to the mini-tunnel environment led to increased productivity of mini-cuttings, greater height, and higher chlorophyll content. Furthermore, mini-cuttings obtained from mini-stumps exposed to mini-tunnel conditions for 45 days exhibited reduced leaf area and lower dry biomass, which can be advantageous for the management of clonal mini-gardens.

AUTHOR CONTRIBUTIONS

FMR: Conceptualization, Formal analysis, Investigation, Methodology, Writing - first draft, Writing - review and editing; LFM: Formal Analysis, Investigation, Writing - review and editing; MT: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing - review and editing; SJOF: Research, Writing - review and editing; ELMM: Writing - review and editing; NSM: Writing - review and editing.

ACKNOWLEDGMENT

To the Federal University of Vales do Jequitinhonha and Mucuri and the Postgraduate Program in Forest Science, for structure and logistics. To Aperam Bioenergia, for the technical support. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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Recibido: 11/06/23
Aceptado: 02/09/23