Climatic oscillations in the production of *Eucalyptus benthamii* x *E. dunnii* shoots in mini-clonal hedge

Oscilaciones del clima en la producción de brotes en mini-jardín clonal de *Eucalyptus benthamii* x *E. dunnii*

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SUMMARY

Detailed knowledge on the factors influencing the production of minicuttings, especially focusing on management and environmental optimization is important. Therefore, this study aimed at evaluating the influence of temperature, relative humidity and PAR (photosynthetically active radiation) and their oscillations in mini-clonal hedges of three *E. benthamii* x *E. dunnii* hybrid clones regarding survival, vigor and productive capacity of ministumps. A mini-clonal hedge with a semi-hydroponic system was formed, with five replicates of five ministumps per clone, submitted to 41 successive collections of minicuttings during one year. A multiple linear regression model for minicutting production was adjusted according to maximum, average and minimum climatic variables, considering a period from 0 until 16 weeks before collection. Ministumps from three clones showed 100 % survival throughout the year. Clone 1 showed higher production (8,000 minicuttings m^{-2} month^{-1}) and clone 3 showed the lowest values for this variable (less than 5,000 minicuttings m^{-2} month^{-1}). The adjusted multiple linear regression model was formed by the maximum PAR, maximum humidity and temperature range of different weeks before collection. Maximum PAR (1,440-1,600 μmol m^{-2} s^{-1}) was the most influential variable in the adjusted model and positively related to minicutting productivity. Based on ideal climatic values found by the regression model, it is possible to suggest that climatic oscillations do not favor ministump development.

Key words: cloning, subtropical *Eucalyptus*, photosynthetically active radiation, minicutting.

RESUMEN

Considerando la necesidad de conocimiento detallado de factores influyentes en la producción de plantas clonales, centrándose en la gestión y optimización del medio ambiente, el objetivo fue evaluar la influencia de la temperatura, humedad relativa y PAR (radiación fotosintéticamente activa) y sus fluctuaciones en miniestacas de tres clones híbridos de *E. benthamii* x *E. dunnii*. Se evaluó supervivencia, vigor y capacidad productiva de miniestacas. Se formó un cultivo clonal semi-hidropónico, con cinco réplicas de cinco miniestacas por clon, sometidas a 41 cosechas sucesivas de propágulos en el curso de un año. Se ajustó un modelo de regresión lineal múltiple para la producción de miniestacas en función de variables climáticas (máxima, media y mínima), considerando un período de 0 a 16 semanas antes de la toma de muestras. Las minicepas de tres clones mostraron 100 % de supervivencia a lo largo del año. El clon 1 destacó con los mayores rendimientos (8.000 miniestacas m^{-2} mes^{-1}) y el clon 3 con los menores (> 5.000 miniestacas m^{-2} mes^{-1}). El modelo de regresión se formó con la PAR máxima, máxima de humedad y rango de temperatura de diferentes semanas anteriores a la cosecha. La PAR máxima (1.440-1.600 μmol m^{-2} s^{-1}) fue la variable que más influyó en el modelo ajustado, mostrando correlación positiva con la productividad de miniestacas. A partir de los valores climáticos ideales encontrados por el modelo de regresión se sugiere que las fluctuaciones del clima no son beneficiosas para un buen desarrollo de las minicepas.

Palabras chave: clonación, *Eucalyptus* subtropical, radiación fotosintéticamente activa, miniestacas.

INTRODUCTION

The genetic improvement through refined techniques provided clones selected for several regions and commercial purposes, enabling the implementation of reforestation projects in areas previously not indicated, given the lack of seminal genetic material adapted to such purpose (Xavier *et al.* 2009). The occurrence of severe frosts in the Southern region is the main factor limiting the development of most species of the *Eucalyptus* genus. The species

Eucalyptus benthamii Maiden *et* Cambage and *E. dunnii* Maiden were among the most planted species in areas where frosting occurs (Paludzyszyn Filho *et al*. 2006).

The selected clones are propagated mainly by the minicutting technique, which is considered as the most economically viable, since it enables multiplying the selected genotypes with desirable characteristics at a lower cost, in a shorter period and with higher rooting potential compared to conventional cutting (Assis and Máfia 2007).

However, it is important to understand the factors affecting the sprout production and root formation, and their implications for the successful production of clone seedlings. One of the main limitations in the propagation of *Eucalyptus* spp. in subtropical regions is the decline in production and rooting of seedlings during the winter period (Assis *et al*. 2004). Studies evaluating the influence of climatic factors at different stages of production of clonal seedlings of species and of *Eucalyptus* can be found in literature (Scarassati and Guerrini 2003, Máfia *et al*. 2006, Cunha *et al*. 2009, Brondani *et al*. 2010, Brondani *et al*. 2012, Trueman *et al*. 2013ab). However, the direct influence of climate, especially regarding climatic oscillations, has not yet been elucidated.

In some places in southern Brazil, for example, nurseries and plantations with subtropical species such as *E. benthamii* and *E. dunnii* occur where climate variables (especially light and temperature) oscillate excessively along the day, among days and among weeks, and may interfere with the propagation process (Hartmann *et al*. 2011). Thus, the climatic influence is concealed when using average climatic data for the period or seasons to correlate environmental and production factors.

Therefore, the need for studies further exploring the influence of climatic factors in the minicutting process, especially regarding climatic oscillations, is suggested. Consequently, the objective of this study was to evaluate the influence of the oscillations of climatic variables (temperature, humidity and light) in the production of shoots in miniclonal hedges of *Eucalyptus benthamii* x *E. dunnii* hybrids.

METHODS

The study was conducted between July 2007 and June 2008 in the Forest Species Propagation Laboratory (Laboratório de Propagação de Espécies Florestais) of Embrapa Forests (Embrapa Florestas), located in Colombo - PR (25°20' S and 49°14' W, 950 m). According to Köppen, the climate of the region is temperate, type Cfb. The temperature of the coldest month ranges from -3 °C to 18 °C. The region is always humid, with rains every month, the hottest month temperature below 22 °C and at least four months with temperature above 10 °C.

Clonal seedlings obtained through the minicutting process of three natural hybrids of *Eucalyptus benthamii* x *E. dunnii* (identified as clone 1, 2 and 3) with approximately one year were used to form the mini-clonal hedges.

The seedlings with approximately 120 days of age and 15 cm height were transferred to a semi-hydroponic channel system in sand and planted at a 10 x 15 cm spacing. Pruning of apical budding ± 7 cm of the stem was carried out eighteen days after being planted in the channel, keeping a pair of leaves to induce the development of side shoots and subsequent formation of ministumps, as described by Brondani *et al.* (2009).

The mini clonal hedge was maintained in non-acclimatized greenhouse conditions and covered with transparent polyethylene (150 microns), where the ministumps underwent successive shoot collections during the one year experimental period. Due to the period of the year, the time interval between each sampling procedure ranged from 7 to 15 days, in attempt to maintain the standardization regarding shoot selection, where shoots smaller than 5 cm and possessing less than three pairs of leaves were maintained in the ministump for subsequent ministump sampling (Brondani *et al.* 2009).

The ministumps received a nutrient solution through a drip irrigation system three times a day at a total daily flow of 5 L m^{-2}. The nutrient solution consisted of monoammonium phosphate (0.04 g L^{-1}), magnesium sulfate (0.40 g L^{-1}), potassium nitrate (0.44 g L^{-1}), ammonium sulfate (0.31 g L^{-1}), calcium chloride (0.79 g L^{-1}), boric acid (2.88 mg L^{-1}), manganese sulfate (3.70 mg L^{-1}), sodium molybdate (0.18 mg L^{-1}), zinc sulfate (0.74 mg L^{-1}) and hydro iron powder (81.80 mg L^{-1}) (Brondani *et al*. 2009a). The electrical conductivity was maintained at 1.6 mS m^{-2}, 25 °C, and the adjusted pH at 5.5 (± 0.1), corrected with commercial hydrochloric acid (muriatic acid) and sodium hydroxide (NaOH), both 1 Molar, during the renovation of the nutrient solution (every three weeks).

The survival of ministumps and the production of minicuttings during the experimental period was assessed through 41 successive shoot collections. The production of minicuttings was evaluated per ministump per sampling (MPS) and transformed into production of minicuttings per square meter per month (PMM) using the following equation [1]:

$$PMM = ((MPS/0.015)*(30/IS)) \qquad [1]$$

where, PMM: production of minicuttings per square meter per month; MPS: production of minicuttings per ministump per sampling; 0.015: area occupied by a ministump in m²; 30: number of days of a month; IS: interval between sampling (For each sampling the real interval between the previous sampling was used).

The dates of the 41 samples were transformed into weeks of the year in which samples were made. Therefore, the experiment began the 26th week of 2007 (July) and ended the 25th week of 2008 (June).

The temperature and relative air humidity of the mini clonal hedges were monitored daily and every hour,

throughout the experiment, using a thermostat and humidistat (FieldChart NOVUS 1.70® system), respectively. Photosynthetically active radiation data (PAR) (μmol m^{-2} s^{-1}) were obtained from the global solar radiation measure (w s^{-1}) obtained at the SIMEPAR Weather Station (Paraná Meteorological System; Sistema Meteorológico do Paraná), approximately 5 kilometers to the site where the experiment was conducted. The conversion of solar radiation into PAR was carried out following Valrlet-Grancher *et al.* (1993) and Pereira *et al.* (2002), where 1 W of global solar radiation corresponds to 2.02 μmol m^{-2} s^{-1} of PAR.

A reduction of 20 % in the input of solar radiation in the environment due to the plastic used to cover the greenhouse was considered (Martins *et al.* 1999). This reduction was also confirmed measuring the solar radiation (Luxímetro analógico Extech LT300) in five different sites and three reading periods (8 hours, 13 hours, 17 hours), and out of the greenhouse in an open area without shading.

The experiment was conducted in a randomized and factorial (3X41) design, where the factors were three clones and 41 successive samples of shoots along the year. A total of five repetitions and five ministumps per repetitions were used, amounting to 25 ministumps per clone. The data underwent a Shapiro-Wilk normality test ($P < 0.05$). Afterward, an Analysis of Variance was conducted (ANOVA) ($P < 0.01$ and $P < 0.05$) among the characteristics measured.

The climatic data (temperature, humidity and PAR) of 0 (sampling week) to 16 weeks before carrying out sampling procedures in the mini clonal hedge were considered to build the Multiple Regression model. The climatic data were characterized weekly by the maximum, average and minimum values, and temperature range as an additional variable for temperature. Subsequently, variables that explained over 5 % of the total variation and that were significant at a 5 % probability level were included in the model.

RESULTS

The values most commonly observed for clone 1 were higher than those observed for the other clones, reaching the highest production values (approximately 8,000 mini-cuttings m^{-2} month^{-1}), as observed in the probability density. Clone 3 had the lowest performance among the three clones evaluated, with a production below 5,000 mini-cuttings m^{-2} month^{-1} in most samples. Clone 2 presented average performance (figure 1 and figure 2A).

A significant effect among clones, samples and in the interaction of these two factors on the production of mini-cuttings was observed per square meter per month (PMM) ($P < 0.0001$). The ministumps of the three evaluated clones exhibited 100 % survival after the 41 sampling were made in the one year period.

The ministumps of the three clones had an overall similar result, with low production in the initial samples, which increased until the maximum production, decreasing again afterwards. In addition, the maximum production for the three clones was reached in the first week of 2008 (figure 2A). The weekly climatic values obtained in the mini clonal hedges in the experimental period are represented in figures 2B, 2C and 2D.

Thus, the following descriptive regression model was adjusted [2], with a coefficient of determination (R²) of 69.2 % (figure 3):
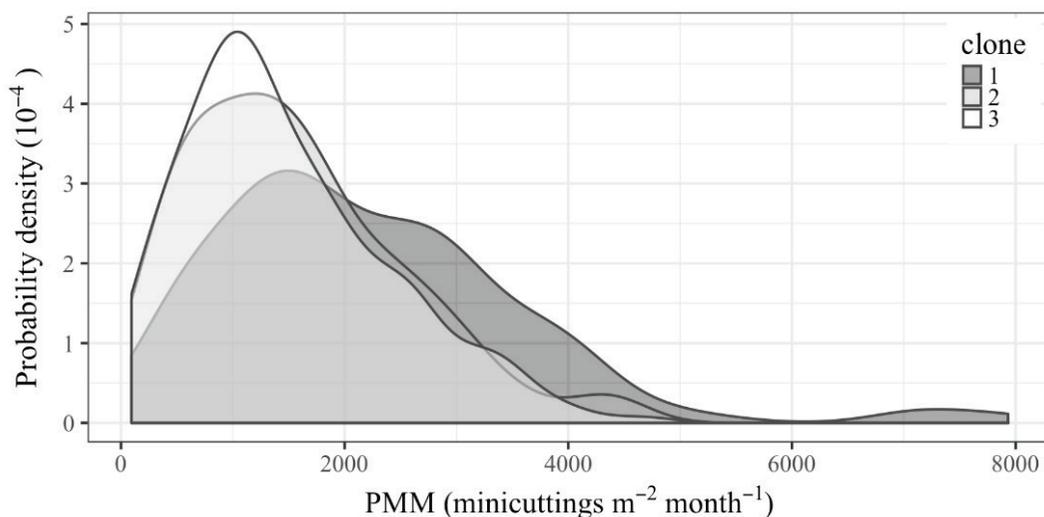
Figure 1. Probability Density for the production of minicuttings per square meter per month *Eucalyptus benthamii* x *Eucalyptus dunnii* in mini clonal hedge.

Densidad de probabilidad para la producción de brotes por metro cuadrado al mes en mini-jardín clonal de *Eucalyptus benthamii* x *Eucalyptus dunnii*.
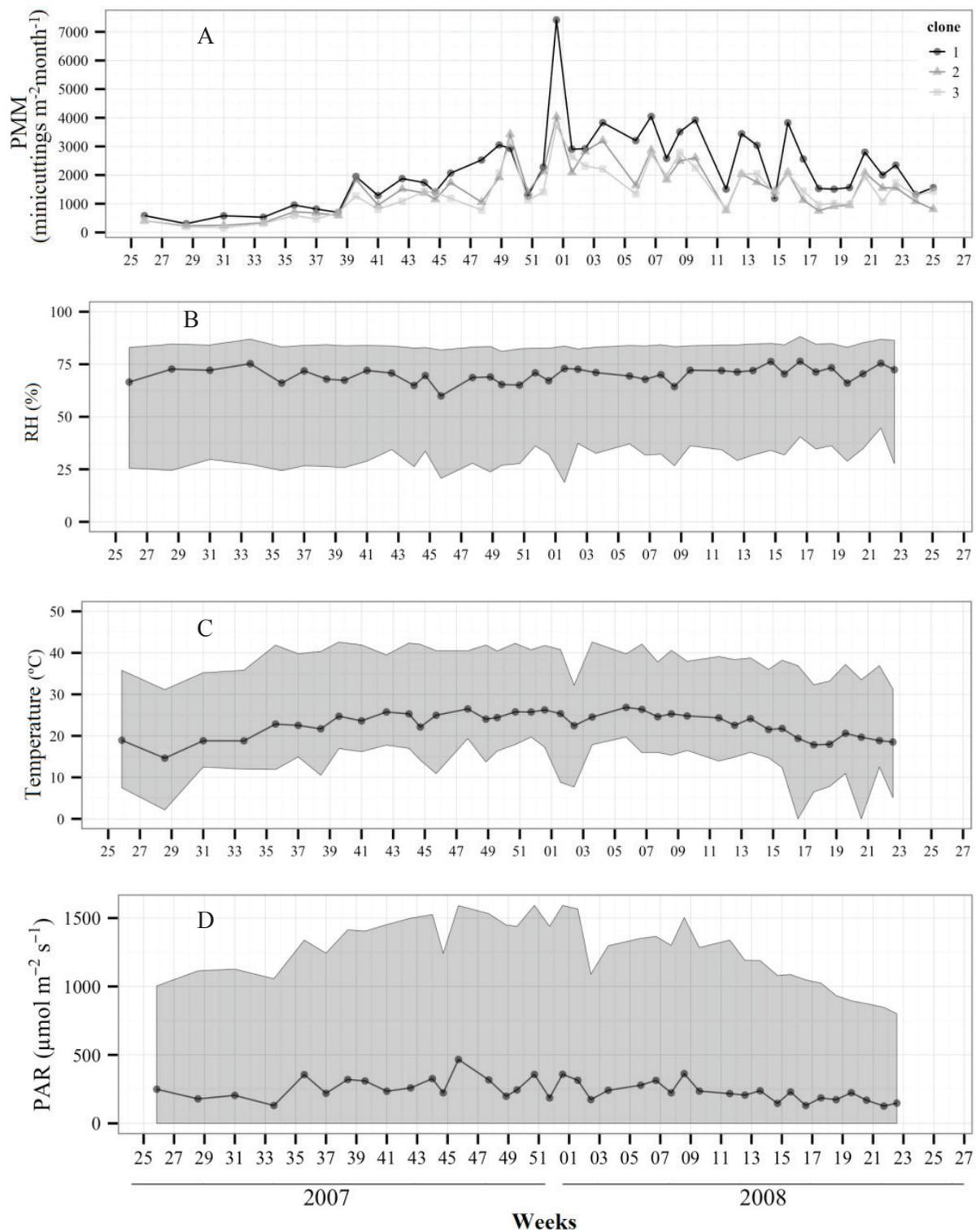
Figure 2. A) Production of minicuttings per square meter per month *Eucalyptus benthamii* x *E. dunnii*; (B) Relative humidity (RH); (C) Air temperature and (D) photosynthetically active radiation (PAR) maximum, medium and minimum (range in gray) depending on different weeks in mini-clonal hedge.

(A) Producción de brotes por metro cuadrado al mes de *Eucalyptus benthamii* x *E. dunnii*; (B) humedad relativa (RH); (C) temperatura del aire y (D) radiación fotosintéticamente activa (PAR) máxima, media y mínima (achurado gris) en función de diferentes semanas en mini-jardín clonal.

$$PMM = 37,220.0665 + 0.9649 \, PAR_{max8} - 131.1838 \, \Delta t_{13} - 250.7426 \, U_{max2} - 365.8483 \, U_{max10} + 208.0783 \, U_{max15} \quad [2]$$

Where: PAR_{max8} = average of the maximum PAR the 8th week before sampling; Δt_{13} = mean temperature range the 13th week before sampling; U_{max2} = average of the highest relative air humidity the 2nd week before sampling; U_{max10} = average maximum relative air humidity the 10th week before sampling; U_{max15} = average relative air humidity the 15th week before sampling.

The model fit was suitable, even for the extreme values observed ($< 1,000$ and $> 5,000$ minicuttings m⁻² month⁻¹ (figure 3).

The maximum PAR recorded the 8th week before sampling had a significant positive correlation, explaining 30.1 % of the variation observed in the minicutting production values. The maximum PAR recorded the 8th week before sampling was higher than 1,500 µmol m⁻² s⁻¹ (figure 2D) for the highest productivity of the first week of 2008, for clone 1 (over 7,000 minicuttings m⁻² month⁻¹) (figure 2A). The production of minicuttings ranged from 900 to 1,500 minicuttings m⁻² month⁻¹ for the three clones in the last sample (25th week of 2008), with a maximum PAR being recorded 8 weeks before (only 1000 µmol m⁻² s⁻¹; figure 2D).

The variable temperature was part of the model response, having a negative correlation with the temperature range of the 13th week before sampling (12.3 % explained observed variation). The highest temperature range recorded in this study was observed between weeks 16 and 20, with a minimum of 0 °C and maximum ≥ 35 °C (figure 2C), which can exert negative effects in subsequent sampling procedures.

The relative humidity influenced productivity in different weeks, where the maximum air humidity of week 2 and 10 were negatively correlated with production, representing 12.0 % and 9.2 % of the variation of the adjusted model, respectively. However, the maximum relative air humidity of week 15 was positively correlated with production, accounting for 5.6 % of the variation. The highest values of maximum humidity in the experimental period ranged from 84 % to 88 % (figure 2B).

DISCUSSION

The ministumps showed high efficiency in producing minicuttings (100 % survival in the experimental period). High ministump survival has been reported for the *Eucalyptus* genus (Wendling *et al*. 2000, Titon *et al*. 2003, Wendling *et al*. 2003, Brondani *et al.* 2012). It is noteworthy that for all those studies, except Brondani *et al.* (2012) (evaluating 27 samples), the highest survival (above 90 %) was recorded for only four of the 14 shoot samples, without assessing the long-term behavior of the ministumps.

In this study, ministump production dropped after reaching a maximum (figure 2A), even without reaching mortality, which may be associated not only with climate change, but also with the occurrence of a physiological exhaustion. A contrasting behavior has been reported for other clones of the same hybrid *E. benthamii* x *E. dunnii*, where the ministumps showed only a temporary exhaustion (Brondani *et al.* 2012), as evidenced by the increased production in the last samples. However, physiological exhaustion and low vigor of the strains over time can be rela-
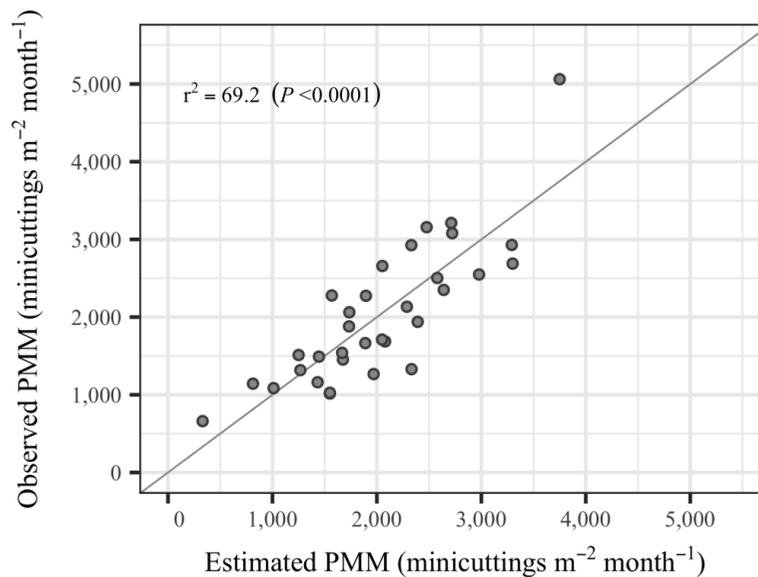
Figure 3. Estimated and observed minicutting production according to the multiple linear regression model obtained for *Eucalyptus benthamii* x *Eucalyptus dunnii* in mini-clonal hedge.

Producción de brotes estimados e observados según el modelo de regresión lineal múltiple obtenido para *Eucalyptus benthamii* x *Eucalyptus dunnii* en mini-jardín clonal.

ted to the competition of the root system for environmental and nutritional factors as well as to the decreased amount of stored reserves (Reis and Reis 1997).

The behavioral difference among clones may be associated with the genotype, one of the factors that affect vegetative propagation, so that behavioral differences between species and clones of the same species exist (Xavier *et al.* 2009, Hartmann *et al.* 2011). Brondani *et al.* (2012) evaluated other clones of the same hybrid (*Eucalyptus benthamii* x *E. dunnii*) and observed different performances among the clones in the different shoots collected throughout the year.

The production of minicuttings of *Eucalyptus* spp. may range from 315 to 3,400 minicuttings m^{-2} month^{-1} depending on the species, on the clone and on the mini-clonal hedge adopted (Alfenas *et al.* 2009). The results of this study show that the three clones had similar productivity according to literature for that genus, even with performances differing among each other. Brondani *et al.* (2012) recorded values ranging from 1,500 to 1,800 minicuttings m^{-2} month^{-2} for the clones of the same hybrid (H12, H19 e H20).

Low production in the first samples may be associated with the need for an initial adaptation of the ministumps to the environment, in addition to the pruning of the ministump to break apical dominance, which makes dormant buds reactive after some samples, resulting in an increased growth stimulation in the following weeks (Wendling *et al.* 2003). The oscillation among samples for the three clones is another factor to be highlighted. The clones exhibited weeks of high production followed by a low production of minicuttings. This result supports findings of Titon *et al.* (2003) regarding minicuttings of *Eucalyptus grandis*, where the authors attributed the results to the temporary exhaustion of the ministumps. Brondani *et al.* (2012) also recorded a cyclic production of ministumps of the hybrid *E. benthamii* x *E. dunnii* according to the different seasons. For Wendling *et al.* (2000), the production oscillations are associated with temperature changes: higher temperatures are related to the higher productivity of the ministumps due to the higher vegetative growth found under these conditions, unlike what occurs under lower temperatures.

The positive effect of the PAR in minicutting production is due to the fact that the PAR is the main responsible for activating the photosynthesis process, converting light energy into chemical energy, which will be stored and remobilized to cellular metabolic activities (Galvani 2009). However, this effect of PAR on the growth of plants depends on its intensity, quality and duration, since the intensity and duration of light should be enough for an accumulation of carbohydrates superior to those spent in respiration (Hartmann *et al.* 2011). The authors emphasize that a higher synthesis of cytokinins occurs in high light conditions, substances related to shoot growth.

The low radiation in the mini clonal hedges in certain months of the year and the reduction of light passing caused by the ceiling dusting of such environments, which in most cases have fixed cover (Alfenas *et al.* 2009), may limit the entry of light needed for proper ministump development. To overcome this problem, the maximum radiation in a mini-clonal hedge, usually achieved between noon and 2 p.m., may be optimized through some structural changes in a greenhouse, such as the use of a retractable roof, which can be opened on sunny days seeking a larger solar radiation and the installation of a light supplementation in the mini-clonal hedge, especially on days of lower photoperiod, in which maximum radiation is reduced.

Some authors have reported a positive effect of light on mini clonal hedges of *Eucalyptus* spp. (Scarassati and Guerrini 2003, Cunha *et al.* 2009, Brondani *et al.* 2012), however, without determining the luminous intensity range that led to the highest shoot development. The average PAR suitable to maximize the production of *Eucalyptus grandis* x *E. urophylla* microcuttings in a greenhouse environment (considering 8 samples) was 1,458 μmol m^{-2} s^{-1} (Scarassati y Guerrini 2003).

The adjusted model indicated that increased temperature range did not favor minicutting production. The biochemical reactions of the enzymes will be accelerated if they are not held within a suitable temperature range for enzyme functioning. Values above or below a certain temperature range can cause denaturation of enzymes, causing loss of their biological function and, on the other hand, in growth rate (Taiz and Zeiger 2004). It is highlighted that short term shifts in the ideal temperature lead to a decreased photosynthetic activity, which is resumed as soon as the optimum temperature is restored (Battaglia *et al.* 1996).

Literature discusses the influence of relative humidity especially in the rooting phase (mist above 80 % in greenhouse environment) (Alfenas *et al.* 2009, Xavier *et al.* 2009, Hartmann *et al.* 2011) and not in the production of minicuttings in the mini-clonal hedge. Excessive humidity should be avoided for the mini-clonal hedge environment for hindering gas exchange and favoring the incidence of diseases and nutrient leaching, making the shoots physiologically less able to take root (Alfenas *et al.* 2009). The negative influence of the maximum relative humidity of few weeks indicates that the mini-clonal hedge environment should be dry for productivity to increase. However, the maximum relative humidity of 15 weeks before sampling was positively correlated with production. Thus, environmental management must be well planned since the ideal maximum relative humidity values differ among the different shoots development phases.

It is suggested thereby, that the maximum humidity values remain constant, because while humidity is benefiting the sprouting stage in development, it will also affect other stages. Note that humidity is negatively related to air temperature, where increasing one leads to the reduction of the other. This pattern suggests the need of controlling both variables simultaneously to reach a better efficiency in maintaining the propagation environment, with climatic conditions that favor an increase in minicutting productivity.

According to the maximum values found in this study (figure 2B), maximum relative humidity values above 84 % are not suitable for a good production of shoots. In addition, humidity values above 80 % influence negatively the productivity of ministumps of *E. grandis*, *E. urophylla* and hybrids of these two species (Cunha *et al.* 2009). However, the microstumps of the hybrid E. *grandis* x *E. urophylla* had higher production with higher maximum (92 %), average (80 %) and minimum (51 %) humidity, highlighting that greenhouse environment, naturally with high relative humidity, was established as a mini-clonal hedge (Scarassati and Guerrini 2003).

CONCLUSIONS

The production of shoots from the three clones of *E. benthamii* x *E. dunnii* was affected by the oscillations of the climatic variables recorded in different weeks before sampling. Maximum PAR explained most of the variations observed, followed by maximum humidity and temperature range. From the ideal climatic values found by the regression model, it is suggested that high climatic oscillations are not beneficial for an adequate development of ministumps and that superior climate control aiming at optimal ranges can optimize the production of shoots.

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