

## Rooting of mini-cuttings of *Sequoia sempervirens* using different clones and cultural environments

Enraizamiento de miniestaquilla de *Sequoia sempervirens* utilizando diferentes clones y ambientes culturales

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### SUMMARY

*Sequoia sempervirens* is a fast-growing, long-lived tree with pest-resistant wood. The objective of this study was to quantify the adventitious rooting ability of sequoia clones in two environments as well as determining rooting behavior during the evaluation period. Mini-cuttings of five clones grown in a clonal mini-garden were planted for rooting in two different environments: a mini-tunnel and an intermittently fogging greenhouse. Weekly assessments (from 30 to 93 days) of the variables were performed: survival, rooted mini-cuttings and number of roots emitted. Rooting speed varied in relation to the clones, occurring mainly between 50 and 60 days after planting, with the appearance of new roots between 70 and 80 days. The cultivation environment promoted distinct responses in the rooting process, being earlier in the greenhouse environment, although with a higher index in the mini-tunnel at the end of the evaluation. The process of differentiation of mini-cuttings occurs initially with the formation of calluses, around 15 days, followed by rooting (between 30 and 60 days) and the subsequent appearance of new roots. The rooting of sequoia mini-cuttings can be done in a mini-tunnel, with good indexes for all clones. It is recommended to keep mini-cuttings in this environment for 90 days for good root formation.

**Key words:** vegetative propagation, greenhouse, mini-tunnel, rooting speed, logistic model.

### RESUMEN

*Sequoia sempervirens* es un árbol longevo de rápido crecimiento con madera resistente a plagas. El objetivo de este estudio fue cuantificar la capacidad de enraizamiento adventicio de clones de *S. sempervirens* en dos ambientes, así como determinar el comportamiento de enraizamiento durante el período de evaluación. Se plantaron miniestaquillas de cinco clones cultivados en minihuertos clonales para enraizar en dos ambientes diferentes: mini túnel e invernadero de nebulización intermitente. Se realizaron evaluaciones semanales (de 30 a 93 días) de las variables: supervivencia (%), mini estaquillas enraizadas (%) y raíces emitidas. La velocidad de enraizamiento varió en relación con los clones, ocurriendo principalmente entre 50 y 60 días después de plantados, con la aparición de nuevas raíces entre 70 y 80 días. El ambiente de cultivo promovió respuestas distintas en el proceso de enraizamiento, siendo más temprano en el invernadero de nebulización intermitente, pero con un índice más alto en el mini túnel al final de la evaluación. El proceso de diferenciación de mini estaquillas ocurrió inicialmente con la formación de callos, alrededor de 15 días, seguido de enraizamiento (entre 30 y 60 días) y la posterior aparición de nuevas raíces. El enraizamiento de las mini estaquillas se puede hacer en un mini túnel, con buenos índices para todos los clones, y se recomienda mantener las mini estaquillas en este ambiente durante 90 días para una buena formación de raíces.

**Palabras clave:** propagación vegetativa, invernadero, mini túnel, velocidad de enraizamiento, modelo logístico.

### INTRODUCTION

*Sequoia sempervirens* ((D. Don) Endl.) (sequoia) is a species of natural occurrence restricted to the coast of California and southwestern of Oregon, generally in an irregular coastal zone of 725 km long and 8-56 km wide (Ahuja

2009). Although the sequoia develops in humid places and at altitudes ranging from 30 to 760 m, its dispersion can extend to 915 m above sea level. It is a fast-growing tree, reaching a growth rate of 1-1.5 meters per year, with the largest trees reaching heights superior to 110 m (Olson *et al.* 1990). Sequoia wood is reddish, usually called redwood. It presents

low density (0.40 to 0.45 g cm<sup>-3</sup>), being easy to work and resistant to pests that attack wood. Its phenolic products confer exceptional chemical stability and can be used for raw wood, unfolding and paper production (Gale 1962).

*Sequoia* shows reproduction mainly by seeds and, often, by basal shoots (Ahuja 2009). Seed reproduction is a barrier to large-scale breeding, as seed germination rate is very low (mean 10 %) (Boe 1974). For asexual reproduction, classic literature has reported that the species has the potential for propagation by cuttings (Metcalf 1924), nevertheless no large-scale attempt for this type of reproduction had been reported until the 1960's (Roy 1966). The first studies on vegetative propagation by cuttings began around 1970. Already in the first studies, the necessity to use basal shoots obtained from cut of the trunk or the stimulation by hot water in the roots was evidenced.

The use of vegetative propagation can overcome the problems reported with the use of seeds, besides making it possible to increase production in a shorter period (Stuepp *et al.* 2013), to select genotypes of interest and to produce uniform populations with a larger phase control of development (Hartmann *et al.* 2011). Among the vegetative propagation methods, the mini-cutting technique shows effective and relatively fast results for obtaining clones (McMahon *et al.* 2014). This technique has some advantages, such as the reduction of the size of the propagules, increasing in the productivity of shoots per area and better rooting, often without the need to use growth regulators (Stuepp *et al.* 2015).

Several factors influence the performance of mini-cuttings, especially the environmental conditions to which the propagules are exposed during the adventitious rooting stage, directly interfering with the survival and percentage of rooting (Brondani *et al.* 2018). The temperature and relative humidity of the air in the rooting environment are emphasized among the factors and have direct implications in the physiological processes. To maintain adequate temperature and humidity for rooting, appropriate structures are required in the nursery phase. The advancement of technology has allowed the development of infrastructure used in the propagation of plants, such as nebulization chambers and greenhouses, often equipped with automated systems. However, intermittent misting systems are costly installations, hence to use this technology is unfeasible for smaller producers.

For small-scale and simple production of the plants, the construction of small greenhouses using low-cost materials (Vieira Neto *et al.* 2010) may be feasible. However, some studies have been developed with this proposal, with good results (Almeida *et al.* 2008, Brondani *et al.* 2018). Another factor that represents an important point for research, having direct effects on breeding gains, is the clonal effect on rooting. The ability to form adventitious roots in vegetative propagules is very restricted and the selection of specific genotypes may allow propagation of the species with future gains.

During the rhizogenic process the formation of groups of meristematic cells occurs, also happens at this stage the differentiation of these groups of cells in primitive roots and the development and emergence of the new roots, including the rupture of other tissues of the stem and the formation of vascular connections with the tissues conducting the cuttings (Hartmann *et al.* 2011). Only from the formation of root primordia is it possible to observe the appearance of roots. The determination of these phases is important for the creation of a pattern of rooting time, period in which each clone needs to remain in the rooting environment. This type of study is important, especially for sequoia, since there are no reports of studies in this sense.

This article is part of a project that aims at producing and cultivating the species in Brazil, through research involving selection of superior materials, vegetative propagation and clonal field tests. Our hypothesis for this study is that there is a difference in rooting of mini-cuttings among clones of *Sequoia sempervirens*, and that this may serve to select superior genetic material for this trait. In addition, these clones may respond differently depending on the rooting environments they are conditioned. This response can guide producers in the process of plants production. Likewise, we wanted to show by means of a mathematical model how the process of formation and development of roots occurs along the time of propagation, aiming at determining the time of maintenance of mini-cuttings in rooting environment.

In this way, the objective of this work is to identify the better environment for rooting of mini-cuttings of different clones of *Sequoia sempervirens*, besides determining the dynamics of rooting through mathematical modeling behavior in the root formation during the evaluation period.

## METHODS

Propagule sources for the test were selected from sequoia matrices (located in Sao Francisco de Paula, Rio Grande do Sul state, Brazil (29°27'44"S and 50°24'11"W) of approximately 40 years of age. The area is part of a sequoia plantation carried out between 1974 and 1975 (no correct definition date). Planted seedlings were originated from seeds from California (USA). The vegetative material was propagated by the conventional cutting technique, 120 days after total girdling or partial girdling of the parent plant. The material was collected from the parent trees and transported in Styrofoam® boxes with ice in the background (transport lasted approximately 4 hours) to the Forest Nursery located at University of the State of Santa Catarina- UDESC (Lages, Santa Catarina state, Brazil - 27°47'33" S and 50°18'04" W). After that, cuttings with a size of 10 cm containing a pair of acicular leaves were reduced to 50 % of the leaf area and treated with 6000 mg L<sup>-1</sup> of indole-3-butyric acid (IBA). Afterwards, they were inserted in tubes (180 cm<sup>-3</sup>) containing vermiculite of medium granulometry and commercial substrate (peat, ex-

panded vermiculite, pine bark and charcoal) and arranged in a mini-tunnel for rooting.

After cutting, five-month-old rooted cuttings were acclimatized in a shade house (covered with shading screen 30-35 % and average 23 °C) for 30 days and transferred to the greenhouse (covered with low-density polyethylene and average 28 °C) to complete the acclimation for 30 days more. Subsequently, plants with approximately 20 cm of height were transferred to pots with 5L capacity, filled with commercial substrate (50 %) (the same used for cutting) and vermiculite of medium granulometry (50 %) was added. Fifteen days after the installation, the time necessary to adapt the plants to the system, the apex of the plant main bud was cut at a height of 10 cm ( $\pm$  2 cm), forming the mini-stumps for the establishment of the clonal mini-garden. During four months, monthly pruning of the mini-stumps was carried out.

The nutrient solution used in the clonal mini-garden was commercial fertilizer composed by: 10 % N (water soluble), 42 % P<sub>2</sub>O<sub>5</sub> (water soluble), 10 % K<sub>2</sub>O (water soluble), 0.6 % Mg, 0.1 % Fe and 0.02 B. Fertigation was done twice a week, with each mini-stump receiving 50 mL of solution composed by diluting 1.5 grams of the fertilizer per liter of water. After the period of formation of the mini-stumps, the sprouts were collected to perform the experiments.

Five clones with higher availability of propagating material from the clonal mini-garden, named A113, A116, A140, A227 and A228 were used, constituting the factor "A" of the experiment. The shoots obtained from these clones were prepared mini-cuttings with 8 to 10 cm in length with a pair of acicular leaves reduced to 50 % of the leaf area.

The mini-cuttings were placed in 180 cm<sup>3</sup> polypropylene tubes, containing vermiculite of medium granulometry and commercial substrate (1:1, v/v), where the insertion of approximately 2 cm of the base of the mini-cutting in the substrate was carried out, without the use of growth regulator. According to the manufacturer's description the commercial substrate used consists of peat, expanded vermiculite, pine bark and charcoal. The chemical and physical characteristics according to substrate analysis were: pH = 6.6; electrical conductivity = 0.13 mS cm<sup>-1</sup>; wet density = 450.8 kg m<sup>-3</sup>; dry density = 302.7 kg m<sup>-3</sup>; current humidity = 32.8 (%); total porosity = 72.2 (%); aeration space = 17.0 (%); readily available water = 12.2 (%); buffer water = 2.4 (%); remaining water = 40.5 (%); CRA10 - water holding capacity at 10 cm = 55.1; CRA50 = 42.9 and CRA100 = 40.5.

After planting, the tubes containing the mini-cuttings were conditioned in two different rooting environments (factor B): a mini-tunnel, located in the UDESC Forest Nursery, and a greenhouse intermittently fogging (GIF) of a forest company located in Otacilio Costa (Brazil). The mini-tunnel is a simple structure with plastic cover, showing 8.0 x 1.20 x 0.9 m (length x width x height), located under the shade house. The GIF is automated, with temperature control (general temperature between 20-30 °C)

and relative humidity (RH > 80 %); both variables were controlled automatically by thermostat and humidistat. The preparation and planting of the material in the containers were carried out in the UDESC Forest Nursery. The pots were transported soon after the procedure to the nursery of Otacilio Costa and kept in an air-conditioned environment during the trip, not exceeding one hour of travel between the two nurseries.

The water management in the mini-tunnel included five irrigations daily (09:00 a.m.; 11:30 a.m.; 01:30 p.m.; 3:30 p.m.; 6:00 p.m) of 7 minutes each, using the micro sprinkler system. In the GIF, irrigation was performed by nebulization, with variable periods according to humidity and temperature. At both places, the data collection of moisture and temperature was performed by means using Politerm datalogger, model Pol-49, with the readings being performed every 10 minutes. The average weekly values of temperature and humidity in the two rooting environments can be observed in figure 1.

The experiment was conducted during the first week of April until the first week of July (2017), under a completely randomized design, in a 5 x 2 factorial scheme (five clones and two environments), using five replicates of 50 mini-cuttings per unit (five mini-cuttings per replicate at each evaluation). Weekly evaluations were carried out (10 weeks), starting from the 30<sup>th</sup> day after planting, until the 93<sup>rd</sup> day, when there was stabilization of the rooting. Mini-cuttings evaluated in one period were no longer used in the next evaluations to avoid interference with the withdrawal and replacement of the same.

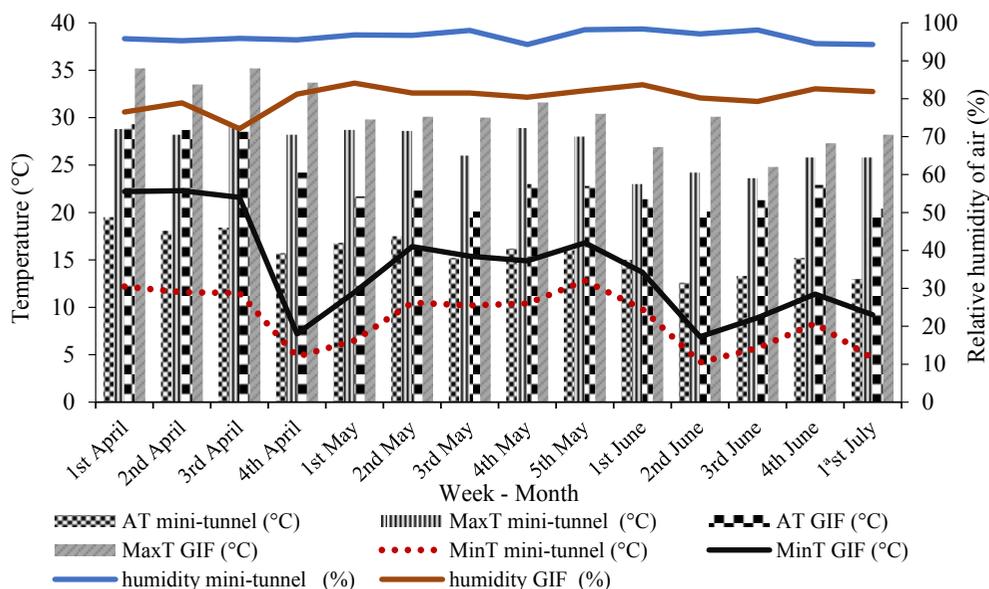
The survival of mini-cuttings (%), callus formation (%), rooting (%), a number of roots emitted by root mini-cutting and root origin were evaluated. Survivors were mini-cuttings that had live wood, old leaves or young shoots, rooted or not. The percentage of rooted mini-cuttings was considered on the total, not only on the surviving. Rooted mini-cuttings were those that had root primordia larger than 2 mm. To determine the origin of the roots, the site was classified into: roots with origin of callus (figure 2A), roots with origin in the woody tissue (it could be of the cortex, exchange, vascular ray or parenchyma of the phloem) (figure 2B) and in both sites (figure 2C).

After checking the normality of the data by the Kolmogorov-Smirnov test and the homogeneity by the Bartlett test, the statistical analysis was performed. When necessary, the data were transformed by the function  $(x + 0.5) / 0.5$ . The data of the final evaluation (93 days) were submitted to analysis of variance and when there was significant difference by the *F* test the means were compared by the Scott-Knott test (clone factor) or *t* test (local factor) at 5 % probability. The analyses were applied by the statistical program R software.

The data distributions of the two variables were then adjusted using an appropriate regression model for estimating the rooting of mini-cuttings according to the evaluation on the time. After testing by linear, exponential, poly-

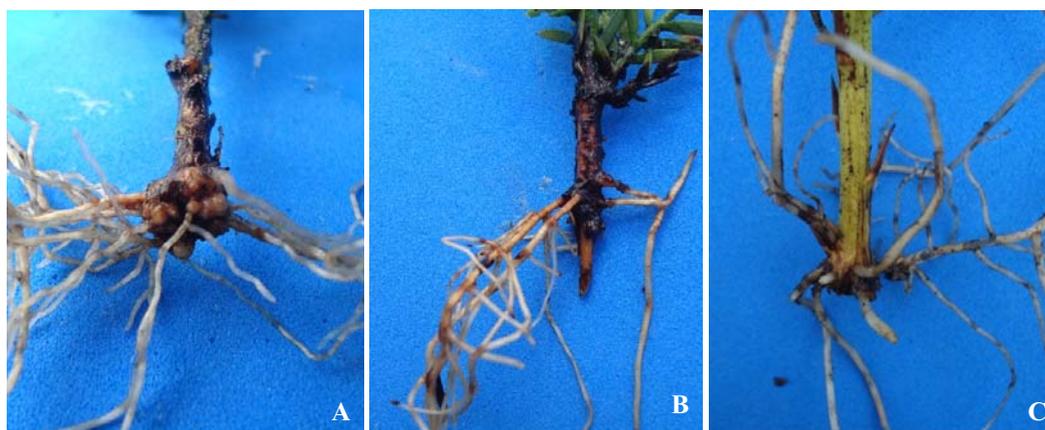
nomial and logistic models, the logistic model showed the best-fitting results. For the logistic regression, three models were tested: with four parameters, with three parameters, and an asymptotic. Their quality was further evaluated to select the best model, using the Akaike information criterion (AIC) (Akaike 1973), the Bayesian criterium (BIC)

and the standard error of the estimate ( $S_{yx}$ ). The logistic function is used to describe changes in the dependent variable (in the case of this study survival (%), callus formation (%), rooting (%) and number of roots) as a function of time. The logistic regression model of this work was then adjusted according to the function with four parameters [1]:



**Figure 1.** Weekly averages (first week of April to first week of July) of average temperature – AT (°C), maximum temperature - MaxT (°C), minimum temperature - MinT (°C) and average relative humidity - humidity (%) in the two environments (mini-tunnel and greenhouse intermittently fogging - GIF) in the rooting of mini-cuttings of *Sequoia sempervirens*.

Promedios semanales (desde la primera semana de abril hasta la primera semana de julio) de temperatura promedio - AT (°C), temperatura máxima - MaxT (°C), temperatura mínima - MinT (°C) y humedad relativa promedio - humedad (%) en los dos ambientes (mini túnel e invernadero de nebulización intermitente - GIF) durante el enraizamiento de mini estaquilla de *Sequoia sempervirens*.



**Figure 2.** Roots origin in *Sequoia sempervirens* mini-cuttings; A) roots with origin of callus; B) roots with origin in the woody tissue and, C) roots emerging from calluses and woody tissue.

Origen de las raíces en mini estaquilla de *Sequoia sempervirens*; A) Raíces con origen de callo; B) raíces con origen en el tejido leñoso, C) raíces que emergen de callos y tejido leñoso.

$$Vi = \varphi_1 + \frac{\varphi_2 - \varphi_1}{1 + \exp[(\varphi_3 - Ti)/\varphi_4]} + \varepsilon_i \quad [1]$$

Where:  $V_i$  = Variable observed in the  $i^{\text{th}}$  mini-cutting;  $T_i$  = time of the  $i^{\text{th}}$  mini-cutting regarding the days after planting;  $\varphi_i$  = regression coefficients and  $\varepsilon_i$  = random error.

The parameter  $\varphi_1$  is the upper horizontal. The parameter  $\varphi_2$  is the lower horizontal asymptote where the variable goes to positive infinity. The parameter  $\varphi_3$  is the middle response parameter, which represents the variable value at the inflection point, when the response is  $(\varphi_1 - \varphi_2)/2$ , *i.e.* halfway between the upper and lower asymptote. The parameter  $\varphi_4$  is the scale parameter.

With the equation obtained for each clone and site, the maximum rooting potential expressed by the coefficient  $\varphi_2$  was determined. The functions were adjusted for each rooting site, for each clone and for the variation of the clones at each site. Weekly, starting from the 15 day, the development of calluses and roots were recorded through photographs, seeking to create a pattern of root development.

## RESULTS

For survival (%) and callus formation (%) in the sequoia mini-cuttings in the different evaluation periods, due to the clones and rooting sites, there was no significance and adjustment of the original logistic function, or any of the equations tested. Mean survival was 98 % and callus mean was 35.5 %, considering weekly evaluations of 30 to 93 days in both sites and for the five clones tested. About rooting, based on the shape of the curve and the estimates of the parameters  $\varphi$ , it was possible to verify that there are differences in the rooting percentage in the evaluated rooting environment (mini-tunnel and GIF) and in the five clones tested (figure 3).

In clones A113, A116 and A140, the rooting started earlier in the GIF than in the mini-tunnel. At 30 days, there was no rooting of mini-cuttings in the mini-tunnel, while in GIF, rooting of clones A113 and A140 were observed. In clone A113 rooting progressively increased in GIF until approximately 80 days after planting, when there was stabilization of the curve. In the mini-cuttings kept in the mini-tunnel, rooting only occurred after the 50<sup>th</sup> day. However, rooting reached stabilization ( $\varphi_2$ ) at 60 days. The clone A113 presented final rooting near 80 % in both rooting environment ( $\varphi_2$  mini-tunnel = 82.27 % and  $\varphi_2$  GIF = 87.46 %).

Rooting in GIF also started previously (evaluation at 37 days) for clone A116, reaching stabilization near 60 days. In this environment, the maximum final rooting was less than 90 %, being smaller in relation to the mini-cuttings kept in the mini-tunnel, which presented 100 % 80 days after planting. The clone A140 exhibited a similar

rooting percentage in both environments, being higher in the mini-tunnel at the end of the evaluation. It is important to emphasize the precocity in the rooting of this clone in relation to the others, because before the 60 days more than 80 % of the mini-cuttings already had roots.

The most important difference in rooting between the two environments was observed for clone A227, which had a final rooting slightly higher than 50 % in GIF ( $\varphi_2$  = 52.543) and 100 % in the mini-tunnel. Probably this result is due to the interaction of genetic characteristics of the clone versus environmental effect. Unlike clone A227, clone A228 showed similarity in rooting percentage. Only after 70 days were better results observed for the mini-tunnel, in which there was also higher final rooting for this clone.

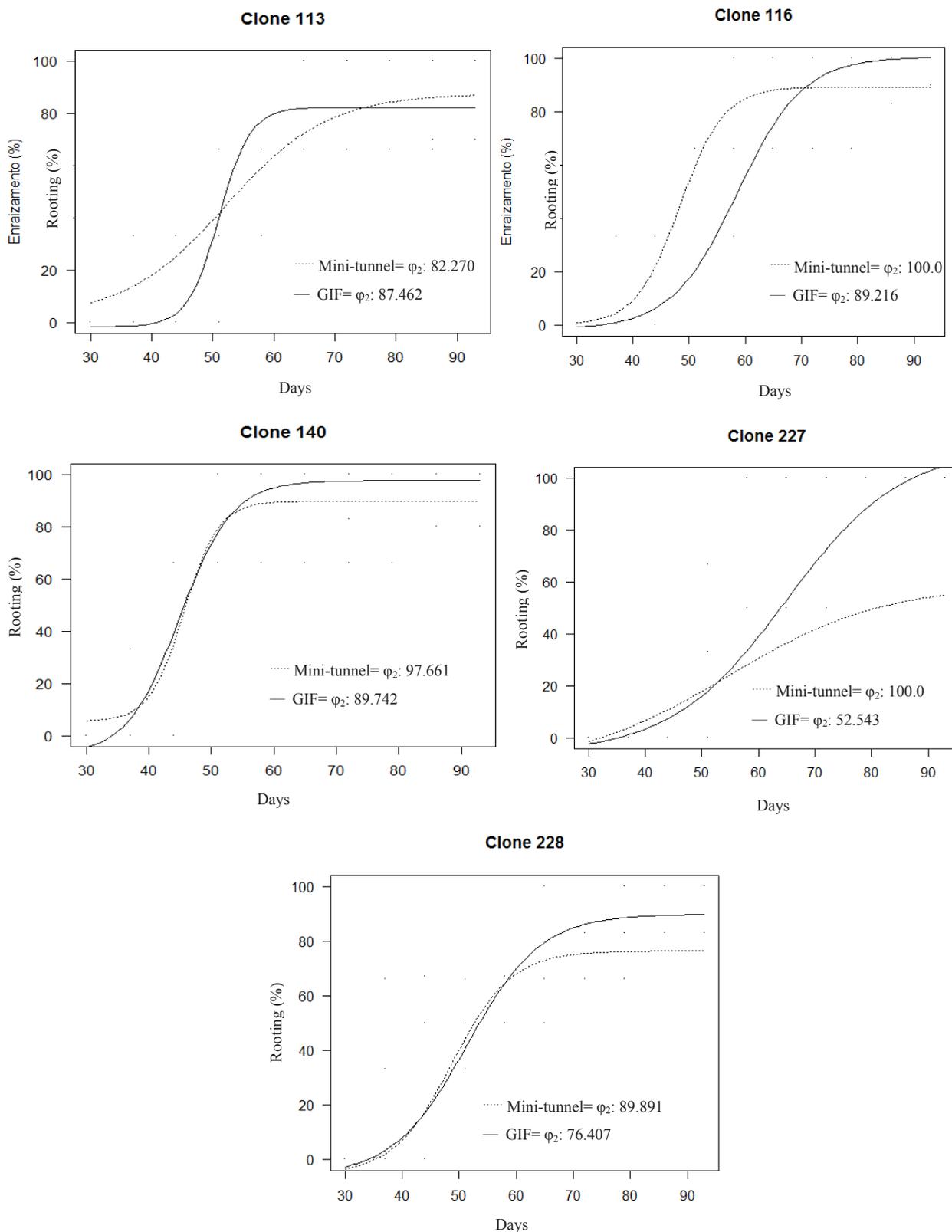
In relation to the number of roots per mini-cutting there was variation in each clone (figure 4). For clones A116 and A140 the GIF presented the best results, exceeding 10 roots per mini-cutting. For clones A227 and A228, the mini-tunnel environment provided improvement in rooting, ranging from 6 to 10 roots in the last evaluation (93 days). Clone A113 presented similar behavior at 93 days of evaluation in both environments.

In the analysis of variance, using data only from the last evaluation (93 days), there was no difference for survival, with the overall mean of 98 %. The callus formation ( $P = 0.045$ ), rooting ( $P = 0.0049$ ) and number of roots ( $P = 0.0023$ ) showed interaction between the environment (mini-tunnel and GIF) and clones.

In relation to callus formation, mini-cuttings in the mini-tunnel showed higher callogenic formation (table 1). Clone A228 presented the highest average and clone A140 the lowest percentage. Rooting showed no difference among the clones in the mini-tunnel, with results higher than 85 %. In the GIF, the clone A227 showed inferior rooting (also in relation to the mini-tunnel), the only one with a value lower than 85 %. Regarding the number of roots, there was also no difference among the clones, with each mini-cutting showing approximately five to nine roots. In the GIF there was a considerable difference among the clones, ranging from 4.1 (A227) to 14.7 (A140). Between the environments, mini-cuttings maintained in GIF presented higher values for clones A116 and A140.

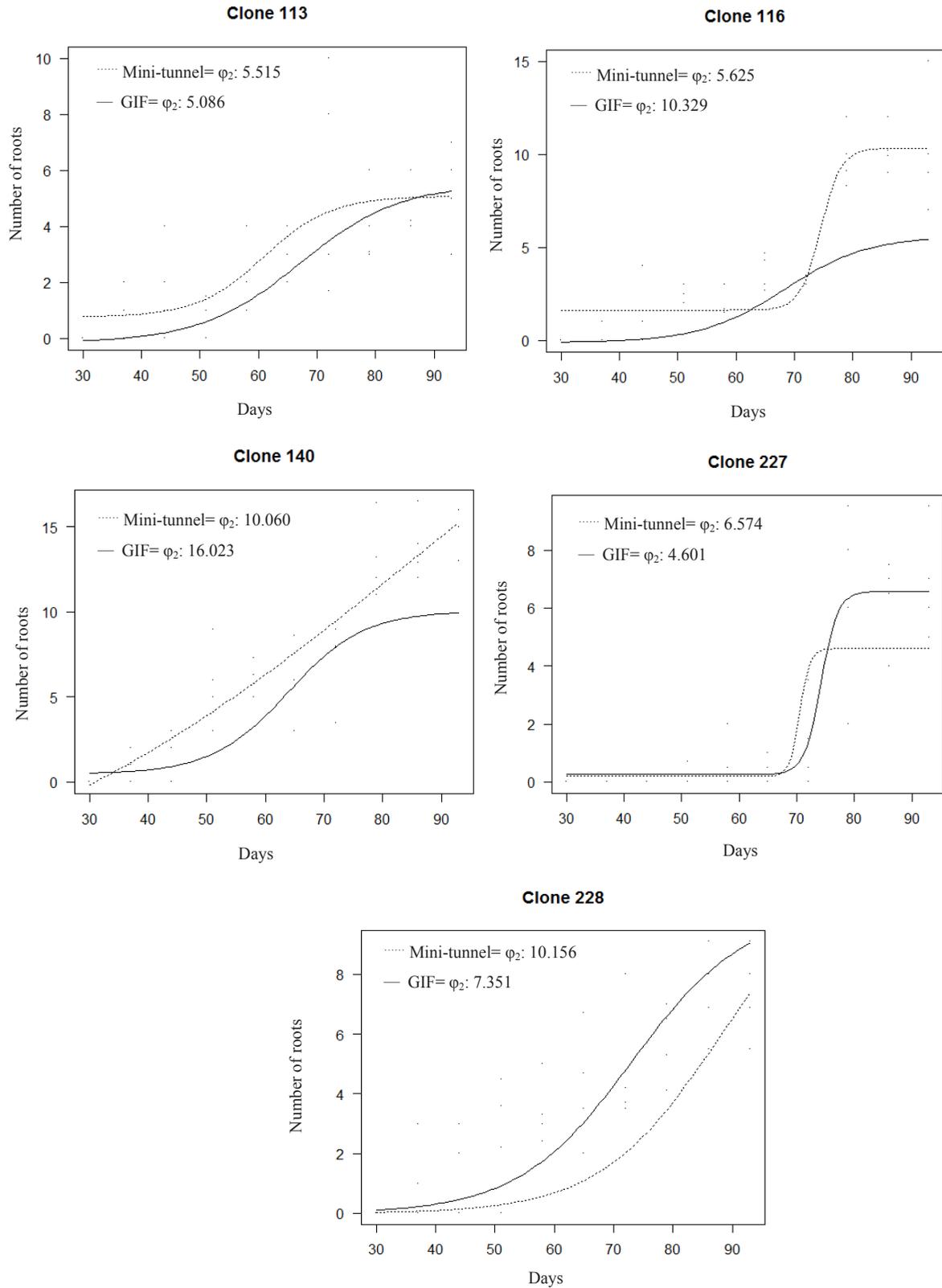
The root origin shows high variation among the evaluated clones. Most clones present callus as root origin, except for clones A116 and A228 in which woody tissue or both sites (callus and woody tissue) predominate as root origin (table 2).

The process of differentiation of mini-cuttings aiming at root development starts, in most experiments, with callus formation. At about 15 days (the 3<sup>rd</sup> week after planting), the visual appearance of this type of structure at the base of the mini-cutting begins, usually with small structures and with clear appearance (figure 5A). Rooting for most of the tested clones starts from the 4<sup>th</sup> and 5<sup>th</sup> week (figure 5B), which originates from the callus or woody tissue, presenting initially few roots with important thickness



**Figure 3.** Rooting of *Sequoia sempervirens* clones as a function of time in greenhouse intermittently fogging (GIF) and mini-tunnel.  $\varphi_2$ : coefficient of the maximum potential of rooting. Weekly, starting from the 15 day, the development of calluses and roots were recorded through photographs, seeking to create a pattern of root development.

Enraizamiento de clones de *Sequoia sempervirens* en función del tiempo de residencia en el invernadero de nebulización intermitente (GIF) y mini tunel.  $\varphi_2$ : coeficiente del potencial máximo de enraizamiento. A partir del día 15, el desarrollo de callos y raíces fue registrado semanalmente mediante fotografías para crear un patrón de desarrollo radical.



**Figure 4.** Number of roots in mini-cuttings of *Sequoia sempervirens* clones as a function of time in greenhouse intermittently fogging (GIF) and mini-tunnel.  $\phi_2$ : coefficient of the maximum potential of rooting.

Número de raíces en mini estaquillas de clones de *Sequoia sempervirens* en función del tiempo de residencia en el invernadero de nebulización intermitente (GIF) y mini tunel.  $\phi_2$ : coeficiente del potencial máximo de enraizamiento.

(figure 5C and 5D). From the 6<sup>th</sup> to 7<sup>th</sup> week (between 40 - 50 days) a progressive increase in rooting (figure 5E) and the emergence of more fine roots occur. After the 8<sup>th</sup> week (and observed until the 13<sup>th</sup>), there was a high increase in

the number of roots, and the mini-cutting was observed to present growth of the aerial part, developing an apical dominance (figure 5F, 5G and 5H).

## DISCUSSION

Survival was high in the sequoia mini-cuttings, showing good control of the environment conditions for maintenance of the material during the rooting process, regardless of the site (mini-tunnel or GIF). In addition, it can be observed that the species has a potential for propagation by the mini-cutting technique, and is similar to classical species in the propagation by cloning in Brazil, especially the genus *Eucalyptus* (Brondani *et al.* 2010, Borges *et al.* 2011).

Regarding rooting, the difference between the mini-cuttings maintenance sites shows the importance of the environment conditions to maximize the rooting. In the GIF, rhizogenic process started earlier for most of the clones, and it was possible to observe mini-cuttings rooted as early as 30 days. This rapid rooting is surprising when it comes to a species of conifer that generally has low indexes and slower processes, especially in important genera such as *Picea*, *Tsuga*, *Abies* and *Pinus* (Hartmann *et al.* 2011). This result, besides the environment conditions, which will be treated next, is attributed to the process of reinvigoration in vegetative rescue, when full or partial girdling was used, and to rejuvenation with the use of successive pruning in the clonal mini-garden (Wendling *et al.* 2014).

The highest temperature obtained in the GIF (figure 1) was responsible for the early rooting contrasted with the mini-tunnel in most of the clones. The mean temperature obtained in the GIF during the first 30 days was around 25 to 28 °C. On the other hand, the average was less than 20 °C. With this, the species shows a certain preference for higher temperatures in the initial phase of cell differentiation and root development. Lower temperatures may inhibit the activity of endogenous auxins (Corrêa and Fett-Neto 2004). Studies have shown that there is a reduction of

**Table 1.** Callus formation (%), rooting (%) and number of roots in mini-cuttings of *Sequoia sempervirens* in relation to the use of different clones and environments, 93 days after planting.

Formación de callos (%), enraizamiento (%) y número de raíces en mini estaquillas de *Sequoia sempervirens* en relación con diferentes clones y ambientes, 93 días después de plantados.

Clone	Mini-tunnel	GIF
	Greenhouse intermittently fogging	
Callus formation (%)*		
A113	62.5 Ab <sup>1</sup>	49.5 Ab
A116	87.5 Aa	31.0 Bb
A140	50.0 Ab	18.7 Bc
A227	50.0 Ab	72.5 Aa
A228	87.5 Aa	89.0 Aa
Rooting (%)*		
A113	87.5 Aa	85.0 Aa
A116	100.0 Aa	95.0 Aa
A140	100.0 Aa	95.0 Aa
A227	100.0 Aa	58.0 Bb
A228	93.7 Aa	95.7 Aa
Number of roots*		
A113	6.0 Aa	5.2 Ac
A116	5.3 Ba	10.5 Ab
A140	8.9 Ba	14.7 Aa
A227	6.8 Aa	4.1 Ac
A228	8.7 Aa	7.4 Ac

<sup>1</sup> Means followed by upper in the line and lower case letters in columns do not differ by the Scott-Knott test (clone factor) and *t* test (local environment), at 5 % probability. \* = *P* < 0.001.

**Table 2.** Origin of the roots (callus, woody tissue or callus and woody tissue) in different clones of *Sequoia sempervirens* during the rooting process.

Origen de las raíces (%) (callo, tejido leñoso o callo y tejido leñoso) en los diferentes clones de *Sequoia sempervirens* durante el proceso de enraizamiento.

Clone	Origin of the roots (%)		
	Callus*	Woody tissue*	Callus and woody tissue*
A113	54.5 b <sup>1</sup>	38.6 a	6.9 a
A116	29.5 a	34.1 a	36.4 b
A140	50.0 b	31.2 a	18.8 a
A227	48.7 b	25.6 a	25.7 b
A228	33.3 a	31.3 a	35.4 b

<sup>1</sup>Averages followed by equal letters in the columns do not differ by Scott-Knott's test, at 5 % probability. \* = *P* < 0.001.



**Figure 5.** Evolution in the mini-cutting rooting process of *Sequoia sempervirens* after planting: A) 3<sup>rd</sup> week; B) 4<sup>th</sup> and 5<sup>th</sup> week; C) 6<sup>th</sup> week; D) 7<sup>th</sup> week; E) 8<sup>th</sup> week; F) 9<sup>th</sup> week; G) 10<sup>th</sup> week and H) 11<sup>th</sup> week.

Evolución en el proceso de enraizamiento de mini estaquillas de *Sequoia sempervirens* después de: A) 3<sup>a</sup> semana; B) 4<sup>a</sup> y 5<sup>a</sup> semana; C) 6<sup>a</sup> semana; D) 7<sup>a</sup> semana; E) 8<sup>a</sup> semana; F) novena semana; G) 10<sup>a</sup> semana y H) 11<sup>a</sup> semana.

basal IAA (indole acetic acid) transport when the temperature is reduced from 25 °C to 4 °C (Garrido *et al.* 2002), which may influence the decrease of the root induction in the mini-cuttings during the colder seasons or slow process of differentiation. In this study, minimum temperatures were recorded in the mini-tunnel, near 5 °C (4<sup>th</sup> week of April), which probably reduced the root starting.

Influence of temperature during the organogenesis of adventitious roots was observed on cuttings of *Pinus elliotii* var. *elliotii* Engelm. x *Pinus caribaea* var. *hondurensis* (Sénécl) Barr. *et* Golf., where low temperatures influenced the survival of the cuttings, although but reducing the percentage of rooting (Rasmussen *et al.* 2009). In contrast, high temperatures can induce water deficit and trigger numerous cellular processes that affect cycle and cell division (Costa *et al.* 2013). High temperatures alter the metabolism of the propagules and may lead to the denaturation of enzymes with reduced absorption of nutrients that ultimately reduce the rhizogenic capacity of the tissues and promote the callus induction at the base of the propagules (Trueman *et al.* 2013). The lowest rooting

at the end of the evaluations for most clones in GIF may be related to the higher maximum temperature in this environment (figure 1), which was over 35 °C in some moments. Sensitivity to deleterious effects in high temperature conditions may also be related to the genetic variation existing in the species (Ahuja 2009), adaptive characteristics of each genotype (Hartmann *et al.* 2011) or even by epigenetic factors (Allavena and Rossetti 1986).

In general, the GIF showed average temperature (figure 1) closer to those indicated for the rooting of subtropical or tropical species (20-25 °C), mainly *Eucalyptus* spp. (Brondani *et al.* 2018). However, the fact that sequoia originates from colder areas with a high thermal variation (Olson *et al.* 1990), lower temperatures or with larger thermal variation between day and night can represent a benefit to the rooting of the species, such as seen for some species of conifers (Hartmann *et al.* 2011).

Each species requires a distinct temperature range, in which optimal adventitious rooting can be achieved. The average, maximum and minimum temperatures of the day, at determined time intervals, are parameters that must be

considered in the propagation environment, being adapted for each species, hybrid or clone. Different species have different preferences in the rooting process. Corrêa and Fett-Neto (2004) observed in *Eucalyptus saligna* Smith the range of 25 to 30 °C, while for *Eucalyptus globulus* Labill. the ideal average temperature is around 20 °C.

With the exception of clone A113, the others had a higher rooting at the end of the experiment (93 days) for the mini-cuttings kept in the mini-tunnel. This fact (most rooting in the mini-tunnel) can be explained by the maintenance of higher humidity in the mini-tunnel environment, as well as the damage to the process due to the high temperatures, as previously mentioned. Although the GIF has moisture content close to or higher than 80 %, there seems to be larger adaptation of the species to higher levels of this factor. Humidity is one of the external factors that most contribute to the occurrence of rooting in the cuttings, and its maintenance is recommended over 80 %, thus preserving the turgescence of the propagules. According to Hartmann *et al.* (2011), plastic coverings in the rooting environment are recommended to maintain high humidity by forming a film of water vapor on the leaves. In case of wilting or dehydration of mini-cuttings, even for a short period, the chance of rooting is reduced drastically, and may lead only to the formation of calluses.

The use of mini-tunnels in vegetative propagation has increased in recent years, mainly in the mini-cutting of species of the genus *Eucalyptus*, promoting acceleration and elevation of rooting indices. Despite this, the most common use of these structures is in the coverage in the clonal mini-garden (Batista *et al.* 2015). The use of simple mini-tunnels, such as those used in this study, for the maintenance of cuttings/mini-cuttings during the rooting process can be an inexpensive and easily installed alternative. Due to the results obtained in the rooting of sequoia and the data of humidity and temperature observed, it is possible to recommend the use of this structure for the most varied species, and the type and interval of the irrigation should be adjusted.

Corroborating with the results of the present study, where the mini-tunnel resulted in high rooting rates, Brondani *et al.* (2018) working with *Corymbia citriodora* (Hook.) K.D. Hill *et al.* L.A.S. Johnson, *E. urophylla* S.T. Blake and *E. benthamii* Maiden *et al.* Cambage, also obtained better rooting of micro-cuttings maintained in a mini-tunnel kept in the shade house. Studying the effect of mini-tunnels in the propagation of cuttings of *Ficus carica* L., Pipattanawong *et al.* (2008) found an increase in temperature and humidity with the use of the structure, a fact that promoted the early formation of calluses, shoots and roots. In *Hamelia patens* Jacquin (Nour and Elgimabi 2009) and *Citrus limettioides* Tan. (Hussain *et al.* 2016) better rooting results of cuttings were also found when they were kept in mini-tunnels with plastic cover. All the studies related the improvement in the rooting to higher humidity obtained with the use of the plastic structures, such as mini-tunnels.

The rooting of mini-cuttings and the number of roots

were not significant for all clones. From the root initiation until the end of the evaluations (93 days) it was possible to observe differences, more pronounced in the clone A227 by the difference between the sites, and clone A140 by the precocity in the root initiation and stabilization. It can be observed that the genotype capacity and speed of rooting of mini-cuttings is similar to that found by Mankessi *et al.* (2009) and Oliveira *et al.* (2012) in species of the genus *Eucalyptus*.

The rooting velocity of mini-cuttings indicates differences among the clones regarding the rhizogenic process, reflecting distinction in the ideal time of permanence of the mini-cuttings in the rooting house. Results showing genotypic differences were also obtained in work with different progenies of *Anadenanthera macrocarpa* (Benth.) Brenan (Dias *et al.* 2012) and *Eucalyptus grandis* Hill (ex Maiden) clones (Melo *et al.* 2011).

It is known that the process of rooting in woody species is genotype dependent. Different species, hybrids and clones of the same parent may require different cultivation conditions and, consequently, varied indices and rooting processes (Mokotédi *et al.* 2000). The formation of adventitious roots in mini-cuttings can be directly and indirectly controlled by some genes (Silva *et al.* 2012), as *SCARECROW* and *SCARECROW-LIKE* (Sanchez *et al.* 2007), *PtRR13*, *PtAIL1*, *PtAGL16* and *PtMYB36* (Rigal *et al.* 2012) resulting in a low auxin level or lack of co-factors of rooting. The genetic determination in rooting is known for several conifers, such as *Pinus taeda* L., *Larix* sp, *Tsuga heterophylla* Raf. and *Pinus elliottii* Englem × *P. caribaea* var. *hondurensis* (Shepherd *et al.* 2005). This knowledge of genetic effect is directly related to the success or failure of plants production through mini-cuttings, since there may be premature discarding of some clones because they present a slower root emission.

An important fact in the vegetative propagation of sequoia is the formation of calluses in mini-cuttings. In this study, the presence of callus was higher in the mini-tunnel than in GIF. This fact can be attributed to larger temperature oscillation in mini-tunnel environment. However, callus formation is common in the vegetative propagation of conifers (Hartmann *et al.* 2011) mainly in sequoia (Navroski *et al.* 2015). In addition, there seems to be a genetic effect interacting with environmental effect on rooting, evidenced by the low appearance of callus in clone A140, mainly in the GIF environment. Other clones (mainly A228) present almost obligatory formation, for later rooting process.

It is important to consider that callus and root formation are independent processes, and the simultaneous occurrence is derived from the dependence of similar internal and environmental conditions (Goulart *et al.* 2014). However, in some plants callus formation may be a precursor to adventitious root formation as is commonly the case in sequoia.

The development of rooting, obtained through visual weekly frequency observations, shows that the process of cell differentiation is rapid for the species. After 15 days of planting it is possible to observe the appearance of callo-

genic structures at the base of mini-cuttings. As described by Salvador (2011), with the preparation of a mini-cutting, from the longitudinal or bevel cut made at its base, a lesion occurs both in the tissues of the xylem and in the phloem, resulting in a trauma followed by a cicatrization process, forming then a suberin cover that reduces dehydration in the injured area. In this region a mass of disorganized parenchyma cells is formed, with little differentiation and in different stages of lignification, called callus.

In the process of rooting mini-cuttings, generally the higher and faster the degree of cell differentiation, the better the possibility of adventitious rooting of the species (Dias *et al.* 2012). This fact was observed in sequoia, since, from the 4<sup>th</sup> week, an increase in the growth of the callogenetic structures was observed, involving practically the whole radius of the base of the mini-cutting (figure 5B), however without rooting.

Sequoia rooting usually starts with the appearance of a root, followed by the emergence of others after a few days. In the beginning, few thick roots emerge, often arising from callogenetic structures, although, they may also arise from woody tissues. The process evolves with the appearance of new roots and, mainly, secondary roots. This type of root, thinner, is desirable because it exhibits a higher absorption of water and nutrients (Freitas *et al.* 2009) and improves the structuring of the substrate.

Finally, as observed by the mini-cutting rooting percentage, it is possible to keep some clones for less time in the mini-tunnel or GIF. However, as visualized in the rooting process, it is recommended to maintain them for up to 90 days, to improve the quality of rooting and, especially, for the formation of thin roots.

## CONCLUSIONS

The clones tested show different rooting speeds, with high average occurring mainly between 50 and 60 days after mini-cutting. The rooting environment presents a difference in the rooting process, being earlier in the GIF, nevertheless with a higher percentage in the mini-tunnel at the end of the evaluation. The number of roots was influenced by the clones and the two evaluated environments, presenting a higher formation of new roots between 70 and 80 days after mini-cutting.

The use of a mini-tunnel is indicated for the rooting of mini-cuttings of *Sequoia sempervirens* for its low cost and good rooting results. It is recommended to maintain them in this environment for a period of 90 days for proper root formation.

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