

MINI-CUTTINGS ROOTING OF *Sequoia sempervirens* AT DIFFERENT IBA CONCENTRATIONS AND CLONES

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Resumo

Enraizamento de miniestacas de Sequoia sempervirens em diferentes clones e concentrações de AIB. Sequoia é uma espécie que proporciona grandes volumes de madeira, é resistente à decomposição e tem a produção de mudas por miniestaca como uma alternativa viável. O objetivo do trabalho foi avaliar o enraizamento de miniestacas de diferentes clones de *Sequoia sempervirens* e posterior emprego de distintas concentrações de AIB em clones com baixo enraizamento adventício. Foram utilizadas miniestacas de diferentes clones, oriundas de minijardim clonal. No experimento com regulador de crescimento utilizaram-se diferentes concentrações de AIB (de 0 mg L⁻¹ a 3000 mg L⁻¹). O material foi colocado para enraizar em estufim e avaliado aos 90 dias. Todos os experimentos foram instalados em delineamento inteiramente casualizado, com posterior avaliação da sobrevivência (%), miniestacas com calos (%), miniestacas enraizadas (%) e número de raízes (somente no experimento com diferentes concentrações de AIB). No primeiro experimento a sobrevivência das miniestacas foi alta (próximo a 95%) e o enraizamento apresentou grande variação entre os clones (22,8 a 100%). No segundo experimento, utilizando-se clones com enraizamento, anteriormente avaliado abaixo de 50%, submetidos à aplicação de AIB, observou-se alta sobrevivência (88,1 a 99,4%). Os clones apresentaram respostas variadas entre si, mas com melhora no enraizamento utilizando-se regulador de crescimento.

Palavras-chave: propagação vegetativa, regulador de crescimento, AIB, raízes.

Abstract

Sequoia is a species that provides large volumes of wood, is resistant to decomposition and has seedling production by mini-cutting as a viable alternative. The aim of this work was to evaluate the rooting of mini-cuttings of different clones of *Sequoia sempervirens* and subsequent use of different concentrations of IBA in clones with low adventitious rooting. Mini-cuttings of different clones were used, from clonal mini-garden. In the experiment with the growth regulator, different concentrations of IBA (from 0 mg L⁻¹ to 3000 mg L⁻¹) were used. The material was placed to be rooted in a mini-tunnels and evaluate at 90 days. All experiments were installed in a completely randomized design, with a subsequent evaluation of survival (%), mini-cuttings with calluses (%), mini-cuttings rooted (%) and number of roots (only in the experiment with different concentrations of IBA). In the first experiment, the survival of mini-cuttings was high (close to 95%) and rooting showed great variation among the clones (22.8 to 100%). In the second experiment, using clones that previously presented rooting less than 50%, submitted to IBA application, high survival (88.1 to 99.4%) was observed. The clones presented different responses among themselves, but with improve in the rooting using growth regulator.

Keywords: vegetative propagation, growth regulator, IBA, root.

INTRODUCTION

Sequoia sempervirens (D. Don) Endl.), is a conifer native to the west coast of the state of California and the southwest coast of the state of Oregon in the United States, corresponds to a well-known species, mainly because it is one of the highest, reaching more than 100 meters in ideal conditions of growth. It is a species used when there is a need for wood resistant to decomposition (OLSON *et al.*, 1990). The tree is highly valuable, not only for ornamental purposes but also for industrial purposes, due to its rather vigorous growth. The species is rarely attacked by disease or insects and is resistant to strong winds and other adverse weather conditions. Seed germination is highly variable, presenting many empty seeds and with malformed or infected embryos with several

parasites. Like most forest species, seed propagation is predominant for the species, although vegetative propagation through root shoots or lateral sprouts also occurs (KORBAN; SUL, 2007).

Vegetative propagation was used to produce sequoia seedlings (LUNA, 2008) by cuttings, but few large-scale that work in this sense have been reported in the literature (OLSON *et al.*, 1990). For species that present seasonality in the production and storage difficulty of the seeds, mini-cutting is an alternative. By means of this technique, it is possible to form a clonal bank in a greenhouse, with a supply of diversified material for the production of seedlings aiming at the conservation of the species, besides allowing the selection of matrices with superior phenotypic characteristics for commercial production, as wood quality for the production of wood (OLIVEIRA *et al.*, 2016).

The mini-cutting provides increases in rooting, in addition to favoring the development of a better quality root system for vigor, uniformity and volume, which positively reflects the survival and performance of the clone in the field (OLIVEIRA *et al.*, 2012). However, it is important to know the factors that affect root formation, directly related to the success of cuttings production.

Some of the main factors involved in the rooting of mini-cutting are the hormonal balance, genetic constitution of the plant (potential and genetic variability within the species), endogenous level of inhibitors, nutritional conditions, propagation/youthfulness effect of propagule, season is collected, abiotic factors (temperature, light, humidity), substrate quality and the use of growth regulators. Regarding growth regulators, auxins were usually, considered as the main inducers of adventitious rooting, especially in difficult rooting species (DIAS *et al.*, 2012). Among the most used, indole butyric acid (IBA) is used because of its efficiency, stability and lower toxicity over a wide concentration range. The sequoia cuttings respond favorably to the hormonal treatment (NAVROSKI *et al.*, 2015). As the maturation effect of the donor plant of the species increases, the auxin concentrations increase (up to a certain limit) so that there is a maximization in the success in rooting (LUNA, 2008).

Considering that the clonal effect and the application of exogenous auxin present high importance in the clonal propagation, it was expected to find substantial differences between the tested clones, mainly due to the high number of genetic materials. For low-rooting clones without the use of growth regulator, it was expected that their use may increase root formation. In this context, we aim to evaluate the rooting of mini-cuttings of different clones *Sequoia sempervirens* and the subsequent use of different concentrations of IBA in clones with low adventitious rooting.

MATERIALS AND METHODS

As propagule sources for mini-cuttings were select from sequoia matrices (located in Sao Francisco de Paula, Rio Grande do Sul state, Brazil) of approximately 40 years of age, propagated by the conventional cutting process, 120 days after partial or complete annealing of the matrix tree. The material was collected from the parent trees and transported to the Forest Nursery located in Lages, Santa Catarina state, Brazil. After that, cuttings with a size of 10.0 ± 2 cm of length and 0.7 ± 2 cm of diameter containing a pair of acicular leaves reduced to 50% of the leaf area and treated with 6.000 mg L^{-1} of indole-3-butyric acid (IBA). Then, they were inserted in tubes (180 cm^3) containing vermiculite of medium granulometry and commercial substrate (1:1 v/v) and arranged in a mini-tunnel for rooting. The mini-tunnel is a structure located under the shadehouse and with the following dimensions: $8.0 \times 1.20 \times 0.9$ m (length x width x height).

After cutting, five-month-old rooted cuttings were acclimatizing in a shade house for 30 days, then transferred to greenhouse to complete the acclimation for another 30 days until it the seedlings reaches 20 cm in height. Subsequently, were transferred to pots with 5L vases filled with peat and pine bark-based commercial substrate (50%) and medium-sized vermiculite (50%). Fifteen days after the installation, the time necessary to adapt the seedlings to the system, the apex of the cuttings the main bud was cut at a height of 10 cm (± 2 cm), forming the mini-stumps for the establishment of the clonal mini-garden. Monthly, for four months, pruning of the mini-stumps was carried out.

The nutrient solution used in the clonal mini-garden was the base of the commercial fertilizer composed of 10% N (water soluble); 42% P_2O_5 (water soluble); 10% K_2O (water soluble); 0.6% Mg; 0.1% Fe and 0.02 Br. 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 collect to perform the experiments.

Mini-cutting of different clones and application of IBA

The first experiment consisted of the rooting of mini-cuttings of different clones kept in a clonal mini-garden system. Sixteen treatments (clones) were used: A100, A113, A115, A116, A117, A126, A127, A130, A131,

A133, A135, A136, A138, A140, A227 e A228). A completely randomized design was used, with 10 replicates of 10 stakes each. No growth regulator was used for this experiment.

With the clones that low rooting (<50%), a second experiment was realized, using different concentrations of IBA. The first factor was composed of 4 clones (A100, A117, A126 and A138) and the second factor was IBA concentrations (0, 1000, 2000 and 3000 mg L⁻¹). Eight replications of 10 mini-cuttings each used; the design was completely randomized in a two-factorial scheme.

Mini-cutting and growing conditions

From each clone, mini-cuttings were prepared whit 8 to 10 centimeters in length, the basal portion being bevel cut and the upper portion being incised transversely, a pair of a pair of acicular sheets being cut in half. In the first experiment, there was no use of growth regulator. In the second experiment, the cuttings were immersed in hydroalcoholic solution, with the base of the cuttings remaining in contact with the solution for a period of 15 seconds. After preparation, the mini-cuttings were arrange to be rooted in 180 cm³ polypropylene tubes containing vermiculite of medium granulometry and commercial substrate (1: 1 v/v) with the addition of 6 g L⁻¹ of Osmocote®, where it was made the insertion of approximately 2 cm of the base of the mini-cutting in the substrate.

The commercial substrate for plants, according to the manufacturer, is composed of peat, expanded vermiculite, pine bark and charcoal. The chemical and physical characteristics, after analysis of the substrate, are as follows: pH = 6.6; electrical conductivity = 0.13 mScm⁻¹; wet density = 450.8 kg m⁻³; dry density = 302.7 kg m⁻³; 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. The physical and chemical characterization of the substrate was Laboratory of Substrates of the Department of Horticulture and Forestry of the Federal University of Rio Grande do Sul.

The mini-cuttings were a condition in mini-tunnel (structure presenting 8.0 m long x 1.20 m wide x 0.9 m high). The temperature inside the mini-tunnel generally ranges between 20-32 °C and relative humidity of the air remains above 80%, and irrigation was performed by micro-sprinkler for 5 minutes, 5 times a day. The mini-cuttings remained in this condition for 90 days, at which time the evaluation of the experiment was performed.

Evaluated variables and statistical analysis

In both experiments, the percentage of survival, percentage of mini-cuttings that had calluses and percentage of rooted mini-cuttings were evaluated. In the experiment with different concentrations of growth regulator in low rooting clones, the number of roots was also evaluate, a parameter that was not analyzed in the previous experiment, since the seedlings from this experiment were destined to the field planting. The count of the number of roots implies in the disintegration of the substrate, causing serious damages to the roots of the cuttings. To evaluate the number of roots, the complete removal of the substrate, subsequent root washing and counting of all roots were performed visually.

Surviving mini-cuttings were those that had live wood, old leaves or young shoots, rooted or not. For calluses, the mini-cuttings were considered alive, with undifferentiated cell mass at the base, rooted or not. The percentage of rooting was considered on the total, not only on the surviving. It was considered as rooted mini-cuttings with induction of root origin of at least 2 mm in length.

After checking the normality of the data by means of the Kolmogorov-Smirnov and homogeneity by the Bartlett test, the analysis of variance was performed. When necessary, the data were transform by the function $(x + 0.5)^{0.5}$ and the means were compared by the Scott-Knott test, at 5% probability.

RESULTS

In the first experiment, evaluating the rooting of mini-cuttings of different clones, there was a significant difference between the three variables analyzed (Table 1). Mean survival between clones was high, almost 95%. Only four clones showed mini-cutting survival less than 90%. Regarding callus formation, with the exception of clones A116 and A140, all others presented more than 80%.

The rooting, the main variable observed when working with the mini-cutting technique, showed great variation among the clones. Some clones (A113, A115, A127 and A136) showed 100% or very close rooting. Half of the evaluated clones (eight) showed rooting indices between 66.7% and 87.8%, and only four of them (A100, A117, A126 and A138) presented rooting less than 50%.

For the four clones that obtained rooting less than 50% in the first experiment, after being submitted to IBA application, there was a significant effect of the clone factor for survival (%) and callus formation (Table 2). For clone root number and IBA concentrations, there was no significant effect on the three variables analyzed.

The survival of mini-cuttings varied between clones, ranging from 88.1% (A138) to 99.4% (A126). Callus formation was also high for most clones, remaining close to 90%, except for A117, which presented lower calogenic formation. The number of roots presented an average of 3.5, showing no significant difference between clones and concentrations of IBA used.

In relation to the rooting percentage, there was an interaction between the clones and the IBA concentrations. For all clones, the quadratic function presented a better adjustment (Figure 1). For clones A126, A138 and A100, rooting without growth regulator was less than 25%, with significant elevation when 1,000 mg L⁻¹ of IBA was used. Clone A117 was the only one to present rooting near 40% without the use of IBA and to maintain rooting near this value with the use of growth regulator.

Tabela 1. Sobrevivência (%), formação de calos (%) e enraizamento (%) de miniestacas de *Sequoia sempervirens* obtidas de diferentes clones.

Table 1. Survival (%), callus formation (%) and rooting in the mini-cuttings of *Sequoia sempervirens* obtained from different clones.

Clone	Variable		
	Survival (%)	Callus (%)	Rooting (%)
A136	100.0 a*	95.2 a	98.1 a
A133	100.0 a	90.3 a	84.1 b
A131	100.0 a	100.0 a	66.7 b
A138	100.0 a	88.3 a	22.8 d
A100	100.0 a	87.9 a	36.9 c
A127	98.7 a	82.0 b	98.7 a
A115	98.4 a	100.0 a	95.4 a
A113	98.1 a	95.0 a	100.0 a
A227	95.6 a	91.0 a	87.8 b
A130	97.2 a	86.7 a	77.5 b
A116	96.4 a	75.0 b	84.2 b
A135	95.2 a	80.4 b	79.5 b
A117	88.7 b	98.0 a	48.7 c
A140	86.5 b	78.7 b	86.0 b
A228	86.4 b	85.1 b	86.4 b
A126	82.1 b	83.5 b	39.8 c
<i>P</i> -valor	<0.001	0.035	<0.001
CV%	19.2	24.4	22.1
Overall average	94.4	88.6	73.3

* Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test, 5% error probability.

Tabela 2. Sobrevivência (%), formação de calos (%) e número de raízes em miniestacas de *Sequoia sempervirens* em função dos diferentes clones e concentrações de AIB.

Table 2. Survival (%), callus formation (%) and number of roots in *Sequoia sempervirens* mini-cuttings according to the different clones and IBA concentrations.

		Survival (%)	Callus (%)	Number of roots
		CLONE	A100	92.1 b*
	A117	92.4 b	76.5 b	3.7
	A126	99.4 a	92.3 a	3.2
	A138	88.1 b	92.5 a	3.4
	0	94.2 ^{ns}	92.5 ^{ns}	2.9 ^{ns}
IBA (mg L ⁻¹)	1,000	92.7	84.0	3.3
	2,000	94.3	85.0	3.7
	3,000	91.7	85.0	3.8
	Média	93.0	87.2	3.5
	CV (%)	14.0	15.3	37.2

* Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test, 5% error probability. ^{ns} - Not significant at 5% probability of error.

For clones A126, A117 and A100, the concentration indicated by the fit of the equations is close to 1,500 mg L⁻¹ of IBA. Only for clone A138 that the maximum response can be obtain with concentration, close to 2,000 mg L⁻¹. For all clones, the use of the highest concentration of IBA (2000 mg L⁻¹) presented similar results.

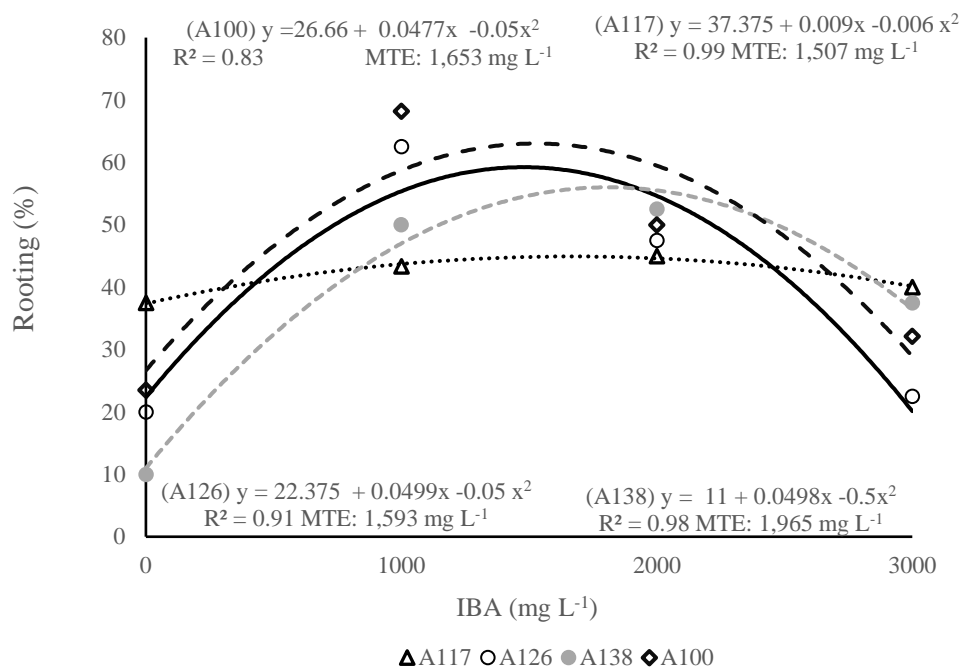


Figura 1. Enraizamento de miniestaquia de *Sequoia sempervirens* (Clones: A100, A117, A126 e A138) em diferentes concentrações de AIB.

Figure 1. Mini-cutting rooting of *Sequoia sempervirens* (Clones: A100, A117, A126 and A138) at different IBA concentrations.

DISCUSSION

The good survival results of the mini-cuttings for the 16 clones demonstrate an adequate control of temperature and, especially, humidity provided in the rooting environment (mini-tunnel) during the conduction of the experiment, and it is indispensable that it presents adequate conditions for the maintenance of mini-cuttings (STUEPP *et al.*, 2013). In addition, the very ability of the mini-cuttings of the species to survive, resembling other vegetative propagation species such as *Eucalyptus* (BRONDANI *et al.*, 2010).

Callus formation in sequoia mini-cuttings is common (NAVROSKI *et al.*, 2015). Some clones present all of these with the presence of calogenic structures, some of which are less well formed, such as clone A116 (75%). The roots of the sequoia mini-cuttings, as well as other genus of conifers (*Cryptomeria*, *Larix*, *Podocarpus*, *Picea* e *Taxodium*) usually have roots arising from external calogenic tissues (HARTMANN *et al.*, 2011). According to the same authors, in some species the formation of callus is a precursor of the formation of adventitious roots. However, calogenesis has been reported as an obstacle to the rooting of propagules of various forest species (SINGH; ANSARI, 2014).

In relation to rooting, a high variation was found in the results, with good indexes for most clones, with only four of them with averages below 50%. The low rate of rooted mini-cuttings may be related to the recalcitrance of these four clones. Thus, indirect selection may be unfavorable for growth or adaptive characteristics due to the propagation of specific genotypes (more likely to be mini-cutting), resulting in loss of clones in the selection process (OLIVEIRA *et al.*, 2015).

For the great majority of the clones, the rooting indexes were superior to 80% and can be considered as very promising for the species, mainly because it is a material only reinvigorated by the technique of girdling in the field trees and one pruning in the clonal mini-garden. Work developed with *Sequoia sempervirens* by Luna (2008) showed inferior rooting to the present study, with values between 30 to 35%. Similar rooting (80%) was achieved only in the work of Navroski *et al.* (2015). All the works referenced made use of cuttings for the rooting, and no reports of works were done using clonal mini-garden, with later use of mini-cutting in the production of cuttings of the species.

This great difference in rooting between clones reveals possible genotypic effects for this trait. Dantas *et al.* (2016) comments that the genetic component is one of the factors that most influence the rooting, having a greater or lesser natural ability to form roots between the different clones.

In a study with *Eucalyptus cloeziana*, propagated by mini-cutting, there was the indication of an expressive genetic control in rooting (OLIVEIRA *et al.*, 2015). The same authors inferred that there were experimental rigor and good reliability in the prediction of genotypic values, with the possibility of identifying superior clones for adventitious rooting. Still according to these authors, the presence of considerable variation in rooting between clones indicates the possibility of practicing selection among sequoia clones propagated through mini-cutting, and it is possible to obtain significant genetic gains.

The lack of work related to the selection of superior genotypes for rooting in a forest improvement program is probably a consequence of the elimination of productive genotypes to other characteristics of interest. The selection of rooting of cuttings/mini-cuttings occurs naturally in nurseries, since recalcitrant genotypes/clones for rooting was discarded (OLIVEIRA *et al.*, 2015), especially in species already cultivated. However, for species still in initial breeding trials, such as sequoia, the disposal of these less rooting materials should not be done, since clones with difficulty in being propagated by vegetative means may have good silviculture characteristics in the field, making them interesting long-term.

Concerning the application of IBA to clones with roots of less than 50%, observed in the first experiment, a difference was again observed between the clones for survival and callus formation in the mini-cuttings. In relation to rooting, the effect of the exogenous application of auxin on the four clones was observed. Without the use of IBA, three of the four clones showed low root formation (<25%). Already with the use of 1,000 mg L⁻¹ of IBA there was increased in rooting, reaching almost 70% for clone A100.

Adventitious root formation is a demanding process of high energy, involving cell division, in which the predetermined cells move from their morphogenetic pathway to act as mother cells for the beginnings of the root; therefore, need hormonal stimuli for root initiation (GEHLOT *et al.*, 2014). This efficiency for root induction depends on the presence of endogenous auxin levels, which may vary according to age, propagule position and genotype. When the responsible factor is the genotype or clone, one can make use of exogenous growth regulators, especially in cases of superior clones (WENDLING *et al.*, 2014).

The rooting of cuttings/mini-cuttings was especially controlled by endogenous levels of plant regulators, mainly auxins (LI *et al.*, 2009). As noted in some clones in the present experiment, exogenous auxin applications may increase rooting percentage due to its ability to act on plant tissues located near the excision region (ZHU *et al.*, 2010), and may be related to concentrations and time of immersion (WENDLING *et al.*, 2010). Therefore, the application of IBA acts as a metabolizing agent and signal for the induction of rooting. During external contact with the cell, IBA induces changes in the metabolism of enzymes, carbohydrates, RNA, DNA and proteins. These changes in the rooting region may inhibit or promote adventitious root regeneration, especially during cell division and differentiation (KOMATSU *et al.*, 2011).

Generally, the concentrations considered optimal for root formation are very particular to each situation (BRONDANI *et al.*, 2010). This fact was observed in the present study, where the clones presented different behaviors regarding the need to use IBA (Figure 1). Almeida *et al.* (2007), testing concentrations of 0 to 6000 mg L⁻¹ of IBA observed that some clones of *Eucalyptus cloeziana*, a species considered difficult to vegetative propagation, presented higher rhizogenic potential of mini-cuttings only when treated with the highest concentrations of the plant regulator. The application of plant regulators is subject to the physiological and genetic conditions of the matrix plant and may not result in a significant increase in rooting, as was observed for clone A117 in this study.

The application of auxin induces the rooting in species of conifers as *Pinus elliottii* var. *elliottii* x *P. caribaea* var. *hondurensis* (HUNT *et al.*, 2011), *Pseudotsuga menziesii* (COPES; MANDEL, 2000) and *Pinus radiata* (RICCI *et al.*, 2008), among others. In general, auxin may be more beneficial in species or clones difficult to root or with less predisposition to rooting (HUNT *et al.*, 2011), as observed in the present study. However, in many cases, auxin does not provide an improvement in rooting (DIAS *et al.*, 2012), showing that some species have a natural ability to rooting mini-cuttings, not justifying the application of indolebutyric acid for the induction of adventitious roots.

Above 2,000 mg L⁻¹, the application of IBA caused a reduction in the rooting of the mini-cuttings. This is because the exogenous auxin has exceeded its maximum utilization point, from which point it becomes inhibitory (POP *et al.*, 2011). In this sense, Hartmann *et al.* (2011) states that when applying plant regulators, there is a stimulating effect on the induction of roots, but this stimulus reached a maximum point, from which it becomes inhibitory. A similar result, with a reduction in rooting from an ideal concentration, has also been report in *Sequoia sempervirens* (NAVROSKI *et al.*, 2015) and other species such as *Taxus wallichiana* (KAUL, 2008) and *Handroanthus heptaphyllus* (OLIVEIRA *et al.*, 2015).

CONCLUSIONS

- There is wide variation in mini-cutting rooting among the sequoia clones evaluated.
- The survival and callus formation exhibit less variation, presenting an average of close to 90%.
- Clones that presented rooting less than 50% in the first experiment and were submitted to application with IBA reported varied responses, but with improved in the rooting.

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