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## VEGETATIVE RESCUE AND ROOTING OF CUTTINGS OF DIFFERENT STOCK PLANTS OF *Sequoia sempervirens*

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**ABSTRACT:** *Sequoia* is a fast-growing, long-living species, producing durable timber. The aim of this study was to test different methods for the vegetative rescue of *Sequoia sempervirens* trees over 40 years old, made at different periods of the year, and, later, testing the rooting of individualized cuttings in planned arrays. Twenty-four individual sequoias were rescued, applying girdling and semi-girdling at three different heights (-10, 0, and 30 cm). The first collection was made 90 days after application of the treatments, being repeated at 150, 240, and 360 days. The percentage of budding trees and the number of shoots per array were registered. In all collections, shoots produced cuttings, which were placed for rooting in mini-tunnels. Cutting survival (%), rooting (%), and number of roots were registered, per array, and per collection. The species vegetative rescue proved to be efficient for the production of shoots for stem cuttings, especially with girdling at 30 cm and semi-girdling at -10 cm. However, it is difficult to define the best method, mainly because of the genetic effect among stock plants. The rooting of cuttings, of recovered material, presented good results (average >65%), also with great differences among stock plants. The potential for rooting of cuttings varied according to different planting periods, with high rooting rates in all seasons, especially in summer. *Sequoia sempervirens* shows the potential for vegetative rescue and cloning by rooting of cuttings, and this may lead to new studies, with a view towards fixing clones.

## RESGATE VEGETATIVO E ENRAIZAMENTO DE ESTACAS DE DIFERENTES MATRIZES DE *Sequoia sempervirens*

**RESUMO:** *Sequoia* é uma espécie de rápido crescimento, longeva e de madeira durável. O objetivo do estudo foi testar diferentes métodos de resgate vegetativo em árvores com mais de 40 anos de idade de *Sequoia sempervirens*, realizados em distintas épocas do ano e posterior enraizamento de estacas individualizadas por planta matriz. Resgataram-se 24 indivíduos de sequoia, aplicando-se anelamento e semianelamento em três diferentes alturas (-10, 0 e 30 cm). A primeira coleta foi efetuada 90 dias após aplicação dos tratamentos, sendo repetida aos 150, 240 e 360 dias. Contabilizou-se a porcentagem de árvores com brotações e o número de brotações por matriz. Em todas as coletas as brotações originaram estacas que foram colocadas para enraizar em estufim. No enraizamento das estacas analisou-se a sobrevivência (%), enraizamento (%) e número de raízes por matriz por coleta. O resgate vegetativo da espécie mostrou-se eficiente na produção de brotações para a estaquia, destacando-se o anelamento a 30 cm e o semianelamento a -10 cm. Entretanto, há dificuldade em definir qual o melhor método, principalmente em função do efeito genético entre matrizes. O enraizamento de estacas do material resgatado apresentou bons resultados (média >65%), também com grande diferença entre as matrizes. Diferentes épocas de estaquia alteraram o potencial de enraizamento, contudo, com altos índices de enraizamento em todas as estações do ano, principalmente no verão. *Sequoia sempervirens* apresenta potencial de resgate vegetativo e clonagem por estaquia, sendo possível conduzir novos trabalhos com vistas a fixação de clones.

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## INTRODUCTION

The demand for the cultivation of new species is increasing, and timber from the noblest species is increasing its economic value. Among the most valuable species is the *Sequoia sempervirens* ((D. Don) Endl.), popularly known as the sequoia.

The species occurs naturally in Western North America, especially in California (USA). Its distribution range is narrow, occurring in the coastal zone (latitude 42°20'N and 35°83'N), which corresponds to a large belt with the occurrence of heavy fog, where it is dominant or codominant (MEASON et al., 2016). Average annual temperatures in that region range between 10 and 16 °C, and the difference between the minimum and maximum annual average does not surpass 16.7 °C. The species tolerates extreme temperatures, both positive and negative, and can survive under -10 °C and above 38 °C (OLSON et al., 1990).

Timber from *Sequoia sempervirens* is highly durable, with low contraction rates when dry, and is structurally stable (COWN, 2008). These properties and its appearance make it desirable for timber production. In the United States, timber from the species is widely used for decks, outdoor furniture, boards, and other products, in which durability, appearance, and stability are important (COWN and MCKINLEY 2009).

Like most conifers, sequoias propagate mostly by sexual reproduction. However, germination (in average 10%) and seedlings survival rates are very low (BOE, 1974). In addition, younger plants present lower seed viability, while higher values were obtained with 250-year-old trees (OLSON et al., 1990).

Thus, vegetative propagation techniques can be used for propagation of superior genotypes, more productive and tolerant to certain situations. Stem cuttings present satisfactory and relatively fast results in producing genetically superior clones (HUNT et al., 2011; MAJADA et al., 2011). However, the rooting potential of cuttings, and the quality of the seedlings are affected by cyclophysis (degree of maturation effects of the donor plant), and topophysis (effects related to the cutting position on the donor plant architecture) (LUNA, 2008).

To facilitate the collection of viable shoots for rooting, and achieve greater material juvenility, it is possible to use vegetative rescue (MEIER et al., 2012; O'HARA and BERRILL, 2009). Two commonly used procedures for the rescue of adult plants are felling and girdling to induce epicormic budding (STUEPP et al., 2014). These buds have morphological and physiological characteristics of juvenile plants, of fundamental

importance for the recovery of the rizogenetic capacity (MELO et al., 2012; WENDLING et al., 2013).

Propagation, via stem cuttings, of superior sequoia genotypes, can assist in the deployment of this species in Brazil, mainly in the Southern region. This area presents climatic similarities with the species original areas, and traditionally faces adaptation problems of local noble species, due to the cold climate. Commercial or experimental plantations, conducted in locations with similar climate, show enormous potential for the species. In Chile, sequoias produced up to 30 m<sup>3</sup>/ha/year, in a 30 to 40-year rotation (VILLANUEVA, 1995), and in New Zealand, they exhibited an increment of up to 50 m<sup>3</sup>/ha/year, in a 40-year rotation in optimum sites (PALMER et al., 2012).

In this work we seek to deepen studies initiated by Navroski et al. (2005), with the vegetative propagation of sequoia in Brazil, as well as other studies carried out in Chile (RAMOS-VILCHES, 2004), New Zealand (MEASON et al., 2016) and the United States (LUNA, 2008; O'HARA and BERRILL, 2009). Thus, the study aimed at testing different methods of vegetative rescue of *Sequoia sempervirens*, performed at different times of the year, and later, rooting of individualized cuttings, through planned arrays (genetic control).

## MATERIALS AND METHODS

### Rescue of vegetative material

The area where vegetative rescue was effected is located in the São Francisco de Paula National Forest (FLONA), located in the municipality of São Francisco de Paula, in the State of Rio Grande do Sul (RS), Brazil; between coordinates 29°24' and 29°27'S, and 50°22' and 50°25'W, with a maximum elevation of 923 m. Average annual rainfall estimate in the area is 2.252 mm. It rains regularly every month of the year, with more intense rainfall occurring during spring and summer. According to the Köppen climate classification, climate in the region is type Cfb, mesothermal, super humid, with bland summer and cold winter. Frost formation is frequent with eventual snowfall. The entire region is subject to constant and intense fogs, and prevailing winds are E/SE/NE (BACKES, 1999). The average temperature, obtained from the meteorological station at FLONA, for the coldest month (June/2015), was 11.5 °C, and for the hottest month (January/2015) it was 20.7 °C (trial period - August 2014 to July 2015).

The Brazilian System of Soil Classification (SBCS) classifies soils found in FLONA as Aluminic Humic Cambic, Ferric Argiluvic Chernosolic, and Eutrophic Lithic Neosol (STRECK et al., 2002). According to soil analysis (0-40 cm), rescue location features the following chemical attribution: pH: 4.6; SMP index: 4.5; Ca: 2.44; Mg: 0.62; Al: 7.2; H+Al: 25.9; effective CTC: 10.5;

O.M.: 3.6%; O.C.: 2.1%; Clay: 26%; P (Mehlich): 3.2; K: 124; K: 0.32; Cu (Mehlich): 1.0; Zn (Mehlich): 1.9; Fe (Mehlich): 30.3; and Mn: 25.2.

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). Of the original plantation (approximately 50 plants, observed through spacing), 24 trees survived, which were used for the vegetative rescue experiment, plus three trees (A100, A227 and A228) located at FLONA, which were also used in the experiment of rooting in the different seasons.

Total height (h) of all trees and the diameter at breast height (DBH) were measured using a Vertex IV hypsometer and a tape measure, respectively. Individuals used for genetic rescue had an average DBH of 52.3 cm (11.6 - 95.6 cm), and an average height of 26.4 m (11.7 - 37.3 m).

The rescue experiment was laid out in a 6 (factor A) x 4 (factor D) factorial scheme. Factor A constituted by the rescue procedure with the following treatments: T1 (-10 cm, girdling); T2 (-10 cm, semi-girdling); T3 (0 cm, girdling); T4 (0 cm, semi-girdling); T5 (30 cm, girdling) and T6 (30 cm, semi-girdling) and factor D constituted by the four collections performed at 90, 150, 240, and 360 days, after girdling and semi-girdling. Each treatment of factor A constituted of four repetitions for each tree, each. D factor (time of collection) was evaluated in the same tree, in each collection. Trees were selected for treatment systematically, to minimize differences in soil and selection of stock plant, for certain treatments.

Girdling and semi-girdling were performed by cutting two transverse lines, on the trunk of each selected tree, with a chainsaw, cutting only the thickness of the bark. Later, with the aid of a carpenter chisel and machete, a 2 cm wide ring of bark was extracted between the lines, carefully not to damage the main trunk. In girdling, 100% of the trunk bark circumference was removed (Figure 1a); in semi-girdling, 50% of the bark was removed. In the -10 cm deep treatment the ground

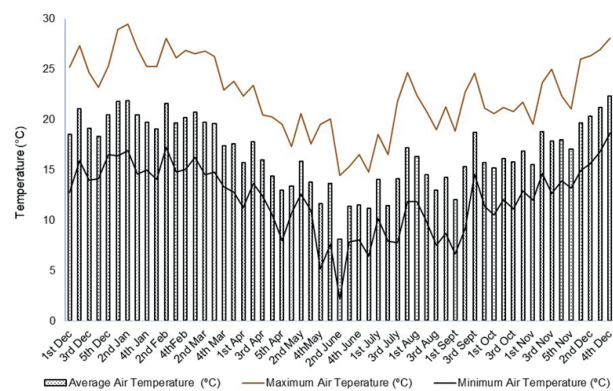
was excavated with a hoe around the circumference to be girdled (50 or 100%) (Figure 1b).

Rescue procedures occurred in September 2014. Evaluations began 90 days after counting the percentage of budded trees (Figure 1c), and the number of shoots per budded tree. The percentage of stock plants that produced shoots, in the four collections, and the average number of shoots, per collections in different stock plants, were also measured. Evaluations were carried out in four periods: 90, 150, 240, and 360 days, after treatment procedures.

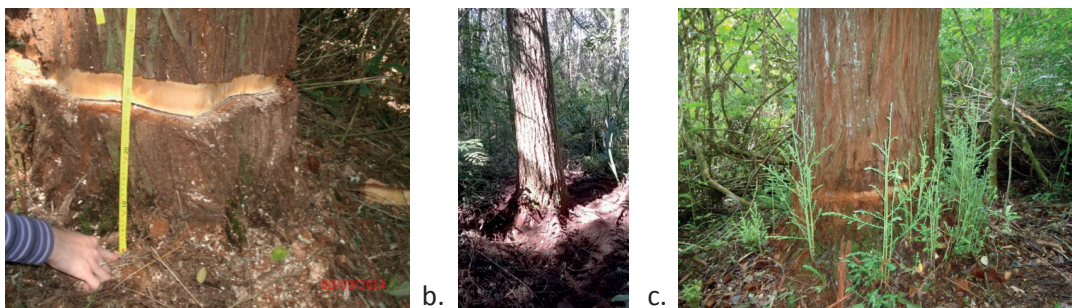
### Rooting of cuttings of different stock plants

In each evaluated period (90, 150, 240, and 360 days), shoots from the previous experiment (rescue) plus the three stock plants (A100, 227 and A228 - applied girdling at 30 cm) were collected and transported, in styrofoam box containing ice in the bottom, covered by sheets of moistened paper with water, to the Forestry Nursery of the Agroveterinarian Science Centre of the State of Santa Catarina University (UDESC).

Figure 2 shows the weekly data for the minimum, medium, and maximum temperature, for the duration of the experiment (early December 2014 to late December



**FIGURE 2** Medium, minimum, and maximum weekly average air temperature, between early December 2014 and late December 2015, in Lages, SC. Data provided by Epagri-Ciram.



**FIGURE 1** a) girdling; b) cleaning the grounds to apply treatment -10 cm deep; c) shoots of *Sequoia sempervirens*, 90 days after girdling, São Francisco de Paula, RS (Brazil).

2015), for the municipality of Lages, SC, according to the meteorological station of EPAGRI-CIRAM.

In the Forestry Nursery, seedlings were prepared with ten centimeters long shoot sections. The basal portion received a bevel cut, and the upper portion was incised crosswise, preserving a couple of acicular leaves, cut in half. For phytosanitary treatment the cuttings were dipped 5 minutes in 2 liters of sodium hypochlorite solution (1.5% active chlorine); followed by immersion in water for 5 minutes; and, then, immersion in fungicide (Benomyl active ingredient at 5%), for 5 minutes.

For the rooting of the cuttings, rescue treatments were not kept, because some stock plants hardly produced material (shoots) for rooting. Thus, treatments were composed by different stock plants, variables in each collection depending on the seasonality of production. Only stock plants with a minimal amount of shoots (100 shoots) were selected. A completely randomized design was used, with 10 repetitions of 10 shoots each. The following individuals were selected as stock plants (treatments) by collection: 1st collection - 7 stock plants (A113; A228; A140; A138; A136; A227; and A127); 2nd collection - 10 stock plants (A135; A117; A136; A126; A140; A228; A133; A131; A100; and A129); 3rd collection - 10 stock plants (A127; A113; A228; A140; A100; A138; A115; A116; A117; and A129); and 4th collection - 10 stock plants (A127; A140; A100; A227; A228; A117; A131; A130; A126; A138; A136; and A116).

Shoots were placed in 180 cm<sup>3</sup> tubes of polypropylene, containing medium granulometry vermiculite and commercial substrate (1:1 v/v). The substrate was used (according to the manufacturer), it is composed of peat, expanded vermiculite, pinus bark, and charcoal). The features described on the product packaging are: pH = 6.0 ( $\pm$  0.5); electrical conductivity = 0.7 ( $\pm$  0.3) mS·cm<sup>-1</sup>; density = 500 kg·m<sup>-3</sup>; water holding capacity - WHC (p/p) = 150%; and maximum humidity (p/p) = 50%. The distended vermiculite was of medium granulometry, with pH = 7.0 ( $\pm$  0.5); electrical conductivity = 0.7 ( $\pm$  0.5) mS·cm<sup>-1</sup>; density = 80 kg·m<sup>-3</sup>; water retention capacity - WRC = 60%; and maximum humidity = 10%. No vegetal regulator to induce rooting, of any kind, was used.

Trays containing the tubes with the cuttings were placed in a mini-tunnel, with plastic cover, inside a shadow structure. The temperature inside the mini-tunnel varied between 20 and 32 °C (variable according to the season - there was no temperature control), and relative air humidity remained above 80%, with micro-sprinkling irrigation during 5 minutes, 5 times a day.

For all collections, evaluations were performed 120 days after staking, evaluating survival percentage, the percentage of rooted cuttings, and the number of roots by rooted cutting. Cuttings with live wood, old leaves, or young buds, were considered survivors, rooted or not. The percentage of rooted cuttings was based on the total, and not only on surviving. A cutting was considered rooted with the induction of early roots of at least 1 mm in length.

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

In the evaluations involving stock plants, analysis of REML/BLUP components was performed. The genetic mean and the variance components were computed with Selegen (RESENDE, 2007), based on model 83 for experiments in a completely randomized design, clone test and one plant per plot. The analysis generated estimates of the following genetic parameters:  $V_g$ : genetic variation among stock plant and  $h^2_g$ : average heritability of among stock plant.

## RESULTS

### Rescue of vegetative material

The percentage of trees that exhibited budding did not show significant interaction ( $p=0.99$ ) with the rescue procedure (height and shape of girdling), and with the collection periods. There were only differences between rescue procedures ( $p<0.001$ ), which did not occur between collections ( $p = 0.96$ ). Concerning collections, trees that obtained shoots on the first observation (90 days), also presented the same in all other (Table 1). Rescue procedure influenced the percentage of budded trees, in T2 and T5 treatments it was verified that 100% of trees budded in all collections (Table 1). Treatment T1 obtained the lowest average of budded trees (31%).

There was also no interaction on the average number of shoots per tree ( $p=0.95$ ) among factors, but there was an effect on rescue ( $p=0.0145$ ) and collection procedure ( $p=0.0152$ ). Same result on the percentage of budded trees, treatments T2 and T5 presented the best results, with an average number of 12 to 14 buds by collection for both treatments. Treatment T1 had virtually no budded trees (0.8 shoots) (Table 2).

In relation to the stock plants with shoots in the four collections, 70% stock plants with shoots in all the collections, and 16.7% did not sprout (Table 3). It is worth mentioning the good result obtained by stock plants A135, A140, and A138, which produced more than 20 shoots per collection.

**TABLE 1** Percentage of trees that presented budding in different moments of the collection, depending on the rescue technique in *Sequoia sempervirens*, in the municipality of São Francisco de Paula, RS.

Rescue	Collection (days)				Average
	90 days (1 <sup>st</sup> collection)	150 days (2 <sup>nd</sup> collection)	240 days (3 <sup>rd</sup> collection)	360 days (4 <sup>th</sup> collection)	
T1 (-10 cm, girdling)	25	25	25	50	31 c*
T2 (-10 cm, semi-girdling)	100	100	100	100	100 a
T3 (0 cm, girdling)	75	75	75	75	75 b
T4 (0 cm, semi-girdling)	75	100	75	75	81 b
T5 (30 cm, girdling)	100	100	100	100	100 a
T6 (30 cm semi-girdling)	75	75	75	75	75 b
Average	75	79	75	79	-

\* Averages followed by the same low case letter in the column do not differ among themselves by the Scott-Knott test, with a 5% probability of error. CV = 31.2%.

### Rooting of cuttings of different stock plants

The first collection of cuttings (held in December), for the propagation of sequoias, with material from the rescue experiment, showed significant differences, in all analyzed variables of seven observed stock plants (Table 4). Cutting survival was higher than 75% for all stock plants, particularly A113 and A228 with 100%, though not differing from A140 and A138.

Rooting in stock plant A113 also called attention, being the only one with 100%, and with a high number of roots (10.4). With the exception of A227 and A127, which showed low rooting rates (less than 40%); all other presented rooting rates in excess of 80%. As for the number of roots, A127 presented the highest average, despite the lower rate of survival and rooting.

For the second collection, in which 10 stock plants were evaluated, a great variation in cutting survival was present, with values between 16.7% (A129) to 100% (A117 and A135) (Table 4). Different patterns between stock plants were also observed for rooting, ranging from 0% (A129) to 76.9% (A117). The number of roots showed a variation between 1.9 (A117) and 3.9 (A140).

In the third collection, 10 selected stock plants were used, again a great variation was observed among individuals in the assessed variables (Table 4). In general, survival was high, with values varying between 61%

**TABLE 3** Percentage of stock plants that produced shoots in four collections, and an average number of shoots per collection in different stock plants of *Sequoia sempervirens*, in the municipality of São Francisco de Paula, RS.

Stock plant/treatment	% of collection with shoot emissions	Number of shoots
A135/T2	100 a*	51.2 a
A140/T5	100 a	34.2 b
A138/T2	100 a	22.5 c
A117/T3	100 a	18.5 c
A131/T3	100 a	18.0 c
A126/T5	100 a	16.2 c
A129/T4	100 a	14.0 c
A113/T4	100 a	12.2 d
A116/T6	100 a	10.5 d
A132/T6	100 a	9.0 d
A127/T5	100 a	9.0 d
A136/T3	100 a	7.7 d
A115/T6	100 a	7.5 d
A133/T2	100 a	3.0 e
A130/T5	100 a	2.2 e
A125/T1	75 a	2.2 e
A128/T4	100 a	2.0 e
A139/T2	100 a	1.5 e
A118/T1	50 b	1.0 e
A114/T4	25 b	0.2 e
A119/T6	0 c	0.0 e
A137/T1	0 c	0.0 e
A112/T1	0 c	0.0 e
A134/T3	0 c	0.0 e
MS	62.86 <sup>1</sup>	615.10 <sup>1</sup>
Vg	14.82	124.01
h <sup>2</sup> g	0.806	0.510
CV%	24.1	45.6

\* Averages followed by the same low case letter in the column do not differ among themselves by the Scott-Knott test, with a 5% probability of error. MS (mean square) stock plant, Vg: genetic variation among stock plant. h<sup>2</sup>g: average heritability of among stock plant. Significance levels representation: ns for non-significant; <sup>1</sup> for P < 0.05.

**TABLE 2** Average number of buds per tree, according to collections and rescue technique of *Sequoia sempervirens* shoots, in the municipality of São Francisco de Paula, RS.

Rescue	Collection (days)				Average
	90 days (1 <sup>st</sup> collection)	150 days (2 <sup>nd</sup> collection)	240 days (3 <sup>rd</sup> collection)	360 days (4 <sup>th</sup> collection)	
T1 (-10 cm, girdling)	0.7	0.7	0.5	1.2	0.8 c*
T2 (-10 cm, semi-girdling)	11.7	18.7	8.5	19.3	14.5 a
T3 (0 cm, girdling)	8.8	7.5	7.0	12.0	8.8 b
T4 (0 cm, semi-girdling)	1.0	6.7	7.0	13.8	7.1 b
T5 (30 cm, girdling)	4.5	15.5	15.2	16.5	12.9 a
T6 (30 cm, semi-girdling)	4.0	5.0	6.0	10.0	6.2 b
Average	5.1 C*	9.0 B	7.4 B	12.2 A	-

\* Averages followed by the same capital letter in the line, and lower case in the column, do not differ among themselves by the Scott-Knott test, with a 5% probability of error. CV = 32.7%.

and 100%. In fact, only stock plant A129 presented an average lower than 70%. Stock plant A129 did not produce any cuttings with the presence of roots, similar to what was observed in the 2nd collection, yet average rooting was over 60%, considering all stock plants. Stock plant A100 stood out, with more than 90% rooting. The number of roots ranged from 1.3 to 7.7, providing good root formation for most of the stock plants.

It was possible to evaluate the rooting experiment with a larger number of stock plants (12) in the fourth collection. Although producing shoots (Table 3), some stock plants did not obtain sufficient quantity for use in rooting experiments. Survival in this collection was almost 90%, with a positive highlight for A127, and negative for A116 (Table 4). For rooting, the overall average was 69.0%, with values varying between 15.4% (A126) and 98.0% (A127). The number of roots, following the example of other collections, presented a wide variation between stock plants (1.4 to 8.5).

## DISCUSSION

### Rescue of vegetative material

Depending on the rescue procedure, there was a difference in the percentage of budded trees, with 100% for treatments T2 (-10 cm, semi-girdling) and T5 (30 cm, girdling), and a smaller rate for T1 (-10 cm, girdling). Although there was a significant difference, it is difficult to establish which treatment is superior, since there was no observable trend of increase or decrease of budding related to the height or procedure of rescue (girdling or semi-girdling). Possibly this result is related to the genotypic capacity to issue buds.

It is worth mentioning that, regardless of the treatment, the high number of budded trees (on average 75%) showed the vegetative rescue potential of the species, through girdling or semi-girdling. This result confirms the sequoia capacity to produce shoots (LUNA, 2008). Epicormic shoots are common in some conifers such as *Pseudotsuga menziesii* var. *menziesii* (Mirb.), Franco

**TABLE 4** Survival (%), rooting (%), and a number of roots on cuttings obtained by vegetative rescue (1<sup>st</sup> collection - 90 days, 2<sup>nd</sup> -150 days, 3<sup>rd</sup> -240 days, 4<sup>th</sup> - 360 days) of *Sequoia sempervirens*, for different stock plants, after 120 days after cutting, Lages, SC.

Days	Stock plants	Variable			Days	Stock plants	Variable		
		Survival %	Rooting %	Number of roots			Survival %	Rooting %	Number of roots
90	A113	100.0 a*	100.0 a	10.4 b	240	A127	100.0 a*	81.2 a	4.8 b
	A228	100.0 a	85.6 a	5.1 c		A113	100.0 a	50.0 b	3.3 b
	A140	93.8 a	80.9 a	7.7 b		A228	98.5 a	59.7 b	5.0 a
	A138	93.0 a	86.1 a	5.4 c		A140	98.2 a	89.1 a	7.7 a
	A136	85.7 b	85.7 a	7.3 b		A100	94.3 a	91.4 a	3.0 b
	A227	84.4 b	36.2 b	2.4 c		A138	93.9 a	30.3 c	1.8 c
	A127	75.1 b	25.0 b	16.8 a		A115	87.5 a	87.5 a	3.8 b
	MS	0.625 <sup>1</sup>	1.685 <sup>1</sup>	204.158 <sup>1</sup>		A116	85.7 a	28.5 c	3.0 b
	Vg	0.416	6.802	20.372		A117	71.4 b	71.4 a	3.8 b
	h <sup>2</sup> g	0.076	0.317	0.754		A129	61.1 b	0.0 d	-0
	-	-	-	-		MS	0.320 <sup>1</sup>	2.244 <sup>1</sup>	89.174 <sup>1</sup>
	-	-	-	-		Vg	2.351	9.486	31.342
	-	-	-	-		h <sup>2</sup> g	0.056	0.403	0.874
		CV%	23.7	28.4		31.5		CV%	23.1
	General average	94.4%	78.9%	6.5		General average	94.2	62.2	5.1
150	A135	100.0 a*	74.1 a	2.6 b	360	A127	100.0 a*	98.0 a	8.5 a
	A117	100.0 a	76.9 a	1.9 c		A140	95.2 a	95.2 a	7.6 a
	A136	93.7 a	68.7 a	3.4 a		A100	94.7 a	78.9 a	5.4 a
	A126	91.7 a	37.5 c	3.1 a		A227	94.4 a	46.5 c	3.0 b
	A140	81.7 b	67.7 a	3.9 a		A228	89.4 a	63.1 b	3.7 b
	A228	80.1 b	67.4 a	2.5 b		A117	88.9 a	77.8 a	3.9 b
	A133	76.7 b	48.3 b	2.1 c		A131	84.6 a	76.9 a	4.1 b
	A131	66.7 b	50.0 b	2.7 b		A130	83.3 a	83.3 a	5.5 a
	A100	25.7 c	22.8 c	2.4 b		A126	76.9 b	15.4 d	1.4 c
	A129	16.7 c	0.0 d	-0		A138	54.5 c	45.4 c	2.9 b
	MS	1.677 <sup>1</sup>	1.338 <sup>1</sup>	22.125 <sup>1</sup>		A136	44.4 c	44.4 c	2.7 b
	Vg	0.004	1.423	0.378		A116	42.8 c	42.8 c	3.1 b
	h <sup>2</sup> g	0.002	0.069	0.100		MS	0.625 <sup>1</sup>	1.684 <sup>1</sup>	74.263
	-	-	-	-		Vg	0.911	3.582	11.958
-	-	-	-	h <sup>2</sup> g	0.053	0.204	0.429		
	CV%	19.5	17.9	30.6		CV%	34.3	38.5	20.1
	General average	70.1	60.4	3.1		General average	87.7	69.0	4.3

\* Averages followed by the same low case letter in the column do not differ among themselves by the Scott-Knott test, with a 5% probability of error. MS (mean square) stock plant, Vg: genetic variation among stock plant. h<sup>2</sup>g: average heritability of among stock plant. Significance levels representation: for non-significant; <sup>1</sup> for P < 0.05.

(COLLIER and TURNBLOM, 2001); *Picea sitchensis* (Bong.) Carr. (DEAL et al., 2003); *Sequoiadendron giganteum* (Lindl.) Buchh. (O'HARA et al., 2008), among others.

In adults, the spread by vegetative means may be highly influenced by the age of the trees, reflected on the ease or difficulty of rooting, making it necessary to use methods of rejuvenation. The effect of physiological maturity increases with age (cyclophysis), or with the position in the tree (topophysis) (OSTERC, 2009), with greater juvenility in the base of the trees (PIJUT et al., 2011), reinforcing the need for rescue as close as possible to the ground. The maturation process is also variable, depending on the genetic control of each individual (CLIMENT et al., 2013), implying in the process of rescue and budding capacity.

A similar behavior was observed in the number of shoots in relation to the percentage of budded trees. In regard as to the number of budded trees, despite differences between rescue treatments, it is difficult to establish a relationship between the best height for girdling or semi-girdling.

This result is possibly attributed to the genetic effect since the heritability between the stock plants for the number of shoots was high (0.510). The dendrometric variables did not show significance in the Pearson correlation between the variable response total number of buds and DBH ( $p=0.855$ ) and height ( $p=0.932$ ). This same effect (genetic cause) could be proved by the results of shoots per tree stock plant, where these patterns correspond to those reported by Meason et al. (2016), which concluded there is high genetic diversity in *Sequoia sempervirens*, which include influencing epicormic budding. In the same sense, Burrows et al. (2010) mention the genetic effect, in obtaining epicormic buds, which is usually caused by responses to stress or luminosity effect.

Between the stock plants that presented budding in all the collections and with an average number higher than 10 (A135 stock plant through A116), with the exception of T1, all treatments have a stock plant with a high number of buds. O'Hara and Berrill (2009) reported that there is a clonal effect on the budding capacity in sequoias, influencing the development of these buds, which corroborates the results of this study. The same authors also comment that environmental conditions, such as incident light and competition with nearby individuals, affect the vigor of the bud.

The results obtained demonstrate the potential for rescue by methods employed and, for many stock plants, with a high number of shoots, as noted in A135, A140,

and A138. These three individuals, besides producing many shoots, also presented good dendrometric features: A135 (dbh = 59.6cm; h = 36.5m); A140 (dbh = 67.5cm; h = 28.1m); and A138 (dbh = 51.9cm; h = 25.5m). However, it is necessary to check the rooting of these materials to verify their potential for clone fixation, and, later, experimental planting in the field aiming to verify the adaptation and growth of the sequoia in Brazil.

### Rooting of cutting of different stock plants

In relation to rooting of cuttings, there was a high variation between stock plants (25 - 100%), with an average close to 80%. Similar results were observed by Ramos-Vilches (2004), with differences in rooting (20.5 to 63.5%) in different stock plants of 2.5-year-old *Sequoia sempervirens*, using apical cuttings. Navroski et al. (2015) working with sequoia cutting obtained variation between 40 and 80% of rooting, however without considering the effect of stock plants.

An important point to be highlighted in this study is the use of cuttings from trees over 40 years old, in which the morphogenetic capacity for rooting is reduced by age (WADE et al., 2014). Thus, one can consider the rooting results satisfactory, mainly for specific clones.

Concerning the number of roots, with the exception of stock plant A227, all others presented a well-formed root system. In stem cuttings, besides rooting percentage, the number of roots formed per cutting is one of the most important variables in quality seedling production (LIMA and OHASHI, 2016). Better results for this variable indicate that seedlings formed later will have a better development since a better root system will result in greater chances of survival, when transplanted to the field (REIS et al., 2000).

Adventitious rooting is a feature with significant correlation with the genetic component (BORGES et al., 2011); this can be verified in this study with values of heritability ( $h^2g$ ) close to 0.40 in some collections for rooting percentage and 0.80 for root numbers. Corroborating these data, Dantas et al. (2016) comment that the genotype is one of the factors that strongly influence rooting and that there is a great variation among species, cultivations, and clones in relation to the greater or lesser natural ability to form roots.

Good rooting results also demonstrate the feasibility of the vegetative rescue technique, through invigoration by means of girdling or semi-girdling at the tree base. There is a greater success in rooting with the use of juvenile material, in comparison to more adult material (MCMAHON et al.,

2014). This difference may be related to different auxin transport mechanisms and less lignification of tissues (OSTERC and ŠTAMPAR, 2011). Luna (2008), working with sequoia individuals, less than 10 years old, and shoots collected from the apex and apical buds, obtained 30 to 35% rooting rates, lower than this study.

The use of shoots obtained from rescue, through girdling or drastic pruning, generates basic juvenile morphological and physiological characteristics (for the recovery of cell competence, and consequent rooting) such as reduced lignification and high exchange activity, resulting from the most active growth phase (LIMA et al., 2011; WENDLING et al., 2013), thus ensuring the expression of genetic potential of the selected material.

The good rooting rate, for shoots of the first collection in most stock plants, is also related to the propitious period for cutting (December), with high luminosity, an average air temperature close to 20 °C (Figure 2), average daytime temperature in the mini-tunnel between 25 and 32 °C (summer), and relative air humidity greater than 90% (readings performed inside the propagation box without defined periodicity).

Rooting observed in the second collection was lower than in the first, probably due to the decrease in temperature and luminosity for the period (late March, April and May) (Figure 2) when the greatest cell differentiation begins to occur (after 30 days cutting). However, good rooting rates were achieved by cuttings from some stock plants (>65%) for the season, mainly A117. Stock plant A129 did not present any rooted cuttings, reinforcing the probable genetic effect on rooting. Note that stock plant A117 exhibited the highest percentage of rooting and the lowest formation of roots. The negative relationship between rooting percentage and root number might be observed in the first collection, where the stock plant with a lower percentage of rooting showed the highest number of roots. Therefore, it is necessary to verify the development of the sapling, so as not to impair its quality and survival, during acclimatization and transplant.

Despite the rooting process of the third collection having occurred during the cold period (May-August) (Figure 2), rooting rates similar to those of the second one (close to 60%) with good root formation were observed for most of the stock plants. This demonstrates that the species has the potential to take root during colder periods, and, depending on the stock plant, with practically the same success rate compared to spring/summer, a more favorable period for root production and growth (MARANGON and BIASI, 2013). According to Trueman et al. (2013), the temperature is one of

the most important factors for rooting of cuttings, especially in species of the genus *Eucalyptus*, which require temperatures between 25 and 30 °C. However, for sequoias, this influence can be minimized since the species is native to a colder region. This feature allows working stem cuttings with lower environmental control of the rooting area (no heating).

On the fourth collection, the overall survival average was lower than the autumn/winter collection, this may be related to the period of higher temperatures (Figure 2) and, consequently, greater loss of stakes. As well as in the second collection, there was great mortality in some stock plants, reducing the overall average. In this evaluation rooting was close to 70%, with a highlight for stock plant A127 with the greater survival rate, rooting, and a number of roots (not differing from other stock plants) (Table 4), similar to what occurred in the third collection (Table 4). Despite this, in the first collection, this stock plant presented the lowest survival rate and rooting. The differences observed are likely to occur because of hormonal changes occurred after the rescue process, due to stress caused to the tree (WENDLING et al., 2014, STUEPP et al., 2017), and also by genetic interactions with the environment, resulting in differences in rooting caused by the period of collection.

The induction of new shoots, by felling trees, or girdling, is one of the main techniques used for the partial reversal of maturation in juvenility in cultivated forest species. Continuous pruning of shoots, in clonal mini gardens, is the common base for clonal forestry of some species, mainly of the genus *Eucalyptus* (XAVIER et al., 2013). The same technique can be used for sequoias since the species possess the potential for rescue through girdling, and good levels of rooting of cuttings. The high increase in timber production in some countries, with a similar climate to southern Brazil, presents an encouraging scenario for the cultivation of the species. These results will be tested in the field planting with clones rescued in this experiment.

## CONCLUSIONS

The vegetative rescue of *Sequoia sempervirens*, through girdling or semi-girdling, is effective to produce shoots; it is not possible to define the best method, mainly due to the genetic effect between stock plants.

The rooting of cuttings of the vegetative rescue material presents good results (average >65%), also with a great difference between stock plants.



Different periods for stem cutting alter the rooting potential of cuttings; however, good rooting rates were obtained in all seasons of the year, with a higher rate in summer - first collection (79%).

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