Influence of shadowing in *Sequoia sempervirens* (D. Don) Endl. mini-stumps and mini-cuttings

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ABSTRACT

The aim of the work was to analyze the influence of variables such as shading, temperature and humidity in the production and rooting of mini-cuttings of *Sequoia sempervirens* (D. Dom) Endl. in a clonal mini-garden. The experiment was conducted at the Forest Nursery at the University of Santa Catarina State - CAV/UDESC, Lages - SC. The minicuttings were obtained by vegetative rescue of a single clone (A228). The original *S. sempervirens* individuals studied are located in the National Forest of São Francisco de Paula -RS (FLONA). Sprouts were collected 90 days after the application of girdling rescue techniques. Subsequently, mini-stumps were obtained, which were submitted to three different treatments: T1 - Mini-stumps without shading protection, T2 - Mini-stumps with single layer of shading and T3 - Mini-stumps with double layer of shading. There was no significant difference for humidity and temperature in relation to the production of mini-cuttings by the mini-stumps, while for shading the treatments T2 and T3 showed greater efficiency. It is possible that the shading treatments on *S. sempervirens* mini-stumps allows the vegetative tissue to be more juvenile, obtaining excellent results in cutting.

Keywords: clonal forestry; mini-cutting; illuminance; rooting.

INTRODUCTION

Sequoia sempervirens (D. Dom) Endl. belongs to the Cupressaceae family botany and is native to the United States of America (U.S.A), where it grows along the fog belt from the Pacific coast of southwest Oregon to central California (Cown, 2008). Sequoia wood has the following characteristics: good machinability and good adhesion for paints and varnishes, in addition to not having resins, typical of coniferous wood (Spichinger, 2004). Such qualities make Sequoia wood attractive also for panel production and use in the furniture industry.

However, the species has some characteristics that prevent its sexual reproduction for commercial production purposes, such as, for example, delay in the appearance of seeds, loss of viability in a short period, low seed germination rate (average of 10%) and seedling viability is also considerably low (Ozudogru *et al.*, 2011). In addition, water, temperature, edaphic conditions and especially light are environmental factors that interfere in the development of plants (Pereira *et al.*, 2020).

Alternatively, vegetative propagation via cuttings or mini-cuttings may be an option. These techniques consist of harnessing the juvenile potential of propagules to induce rooting (Gibson *et al*., 2021). However, according to Sukumar *et al.* (2013), the species presents difficulties in rooting and low production of fine roots, in addition to slow rooting, requiring the use of growth regulators framed in the group of auxins that provide as a response a higher percentage of root formation, better quality of the same and uniformity in rooting (Oliveira *et al.*, 2012).

In general, among the main advantages of the vegetative propagation of forest species can be mentioned the formation of clonal plantations of high productivity and uniformity. Because of consecutive selection, there is the improvement of the quality of the wood and its products, the multiplication of genetics materials resistant to diseases

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and pests and adapted specific sites. This can happen from generation to generation, of additive and non-additive genetic components, which results in greater gains within the same generation of selection (Stuepp *et al.*, 2015).

Therefore, based on the information that luminosity has a direct action on the degradation of photolabile compounds such as auxin, light control can be an easy-to-use alternative, leveraging the rooting potential of the species and reducing or eliminating the use of synthetic hormones. Therefore, the aim of the present study was to analyze the influence of light on the production of mini-cuttings in a clonal mini-garden and the rooting of *S. sempervirens*.

MATERIAL AND METHODS

The rescue area of *S. sempervirens* vegetal material occurred in the National Forest of São Francisco de Paula (RS), Brazil. According to the climatic classification of Köeppen, its climate is of the *Cfb* type, mesothermal, super humid, with mild summer and cold winter. The area is part of a planting carried out between 1974 and 1975 using seeds from California, U.S.A. Through observation of the area and considering the spacing used, it is estimated that approximately 50 seedlings were planted, of which 24 trees remained, used for the plant material rescue experiment.

We measured the total height (h) in all trees using a Vertex IV hypsometer and the diameter at breast height (DBH) with a tape-measure. The individuals used for the vegetative rescue had an average height of 26.4 m (11.7-37.3 m) and DBH of 52.3 cm (11.6-95.6 cm), with approximately 40 years of age at the time of vegetative rescue. The vegetative rescue was carried out during the spring, and the methods for vegetative rescue were both partial and complete girdling at the base of the trunk, for promoting the epicormics sprouting. The evaluations started 90 days after the application of the girdling techniques, counting the percentage of sprouted trees (%).

We transported the sprouts in polystyrene boxes within ice and water to avoid excessive evapotranspiration of the vegetal material. After transportation, we cut the sprouts into cuttings and placed them in tubes of 110 or 180 cm^3, with commercial substrate composed on pine bark and vermiculite (1:1) with an addition of 6 g L^{-1} of controlled release fertilizer. Five months after cutting, we acclimatized the rooted cuttings in a shade house for 30 days, taking them to acclimatization in a greenhouse for another 30 days afterwards. After this period, we transferred the seedlings with approximately 20 cm high to pots with a capacity of 5 L, filled with substrate based on pine bark (50%) and medium vermiculite (50%). After fifteen days of adaptation of the seedlings to the system, we performed the pruning of the apex of the main sprout at a height of 10 cm (± 2 cm), constituting the mini-stumps for the constitution of the clonal mini-garden. Monthly, for four months, we performed the pruning of the mini-stumps aiming the production of lateral sprouting.

The nutrient solution worn in the clonal mini-garden was the base of the commercial fertilizer. It was composed of 10% N; 10% K_2O (water soluble); 42% P_2O_5 (water soluble); 0.1% Fe; 0.6% Mg and 0.02 Br. We conducted the fertigation once a week, were each mini-stump got 50 ml of the solution composed by diluting 1.5 grams of the fertilizer per liter of water. After the formation of the clonal mini-garden, consisting of approximately 3 months, mini-cuttings were collected for the experiment.

For evaluation, in this study, we used only one clone (A228) with DBH of 68.2 cm and height of 33.9 m. Only one clone was used in order not to show genetic variation between clones, and all the variation obtained was attributed to the treatments tested. We arranged the pots containing the mini-stumps in a completely randomized design composed by 15 replicates per treatment. The three procedures contained in different levels of shadowing: T1 - Mini-stumps without shadow protection; T2 - Mini-stumps with single layer of shading, and; T3 - Mini-stumps with double layer of shading.

We achieved the different levels of irradiance with the use of Sombrite® polyethylene (PE) with Anti-UV stabilizing mesh corresponding to a shading blade in T2 and two blades in T3. We positioned the coverings on metal gratings in order to provide full coverage of the underlying gratings. For the control treatment, we only exposed the mini-stumps to the natural light of the greenhouse. Daily, for 15 weeks, we evaluated the illuminance, temperature and humidity using a Luxmeter with a range of 20000 - reading x 10 (lux) and a data logger measuring the temperature in Celsius degrees (Cº) and the humidity in percentage (%).

After 15 weeks, we performed the pruning of the sprouts by repetition in each treatment, cut those into mini-cuttings containing up to 8±2 cm in length, with one to three pairs of leaves cut in half, counted and put them to root inside a mini-tunnel system. Weekly, after 60 days of conditioning in the greenhouse for up to 95 days, we evaluated the survival of mini-cuttings (%), rooting (%), callus formation (%), number of roots emitted and root origin. We considered alive cuttings those with live wood, old leaves or young sprouts, rooted or not. We considered the percentage of rooted mini-cuttings on the total, not based only on the surviving ones. Was considered rooted mini-cuttings to be those with roots longer than 2 mm.

For root origin, we classified it after callus formation, after woody tissue (which may be from the cortex, cambium, vascular radius or phloem parenchyma) and from both places (Figure 1).

After checking the homogeneity by the Bartlett test and normality of the data by means of the Kolmogorov-

Smirnov we performed the analysis of variance (ANOVA). When necessary, the data was transformed by the function $(x + 0.5)^{0.5}$ and compared the means by the Tukey test at 5% probability. The R package "laercio" was used to compute the statistics (Silva, 2010).

RESULTS AND DISCUSSION

Mini-cuttings production

In the production of *S. sempervirens* mini-cuttings, the different light levels did not show statistical difference, varying between 7.5 mini-cuttings for T2 and 6.4 for T3. The control treatment (T1) presented similar average (7.2).

However, although the results do not show significance regarding the amount of mini-cuttings according to the treatments, during the evaluation it was possible to observe visual differences in qualitative variables such as the degree of lignification of the sprouts. The sprouts from T2 and T3 presented a lower degree of lignification and according to Lowe *et al.* (2021) this may favor rooting. Lima *et al*. (2011), studying the rooting capacity of *Maytenus muelleri* Schwacke cuttings, reported that the use of less lignified material results from pruning, can promote rooting rates above 50%.

For relative air humidity (UR%) and temperature (ºC), there was no great variation between treatments (Figure 2). This similarity is probably due to the stability provided by the greenhouse, and the microclimate created by the shading is not significant for these variables. As for the light rate, the different layers of shading in T2 and T3, proved efficient in blocking solar radiation, reducing the luminous flux to values less than 300 lux.

Although there are still no detailed studies on the influence of environmental conditions on the production of propagating material (cuttings or mini-cuttings) of the species under study, knowledge of the ideal conditions of light rate, relative humidity and temperature for the development and growth of plants is extremely important for an efficient propagation of the species worked. Cell division is favored by the increase in temperature and, consequently, aids the formation of roots and the production of sprouts (Hartmann *et al.*, 2011). The majority of studies with conifer rooting achieve better results with lower temperatures during the night (15-18 ºC) and higher during the day (25-27 ºC). Working with the rooting in the seasons of the year in *Sequoia sempervirens*, Pereira *et al*. (2018) got rooting higher than 85% in all seasons, with humidity greater than 80% and average temperatures ranging from 15 to 26 ºC.

Mini-cuttings rooting according to shading levels

After 95 days in the greenhouse, the mini-cuttings obtained a 100% survival rate for all treatments (Table 1). Navroski *et al.* (2015) state that the high survival of the cuttings may be the result of good weather conditions during the experiment, as well as the good ability of the *Sequoia sempervirens* to survive after making into mini-cuttings. According to Cunha *et al.* (2009), the high survival characterizes an adequacy of the cultural treatments used, such as good nutritional, water levels and handling of mini-stumps, which indicates the possibility of using these clonal mini-garden systems to produce sprouts (mini-cuttings) for this species.

Another factor that may have guaranteed the high survival rate was the high presence at the base of the mini-cuttings of calluses (Figure 3). According to Hartmann *et al.* (2011), the presence of calluses at the base of the cuttings, for some time, can perform the function of the roots, that is, absorption of water and mineral salts.

The T2 and T3 treatment presented lower callus formation rates since the start of the evaluations, confirming the fact that a balance in the auxin concentrations supposedly provided by the simple shading condition reduces the formation of calluses at the base of

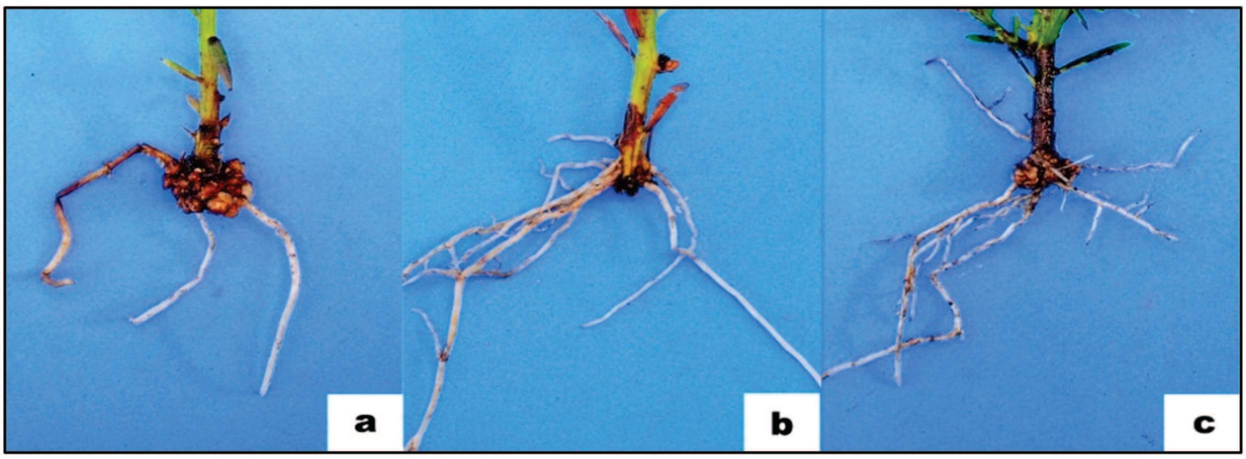

Figure 1: Different origins of roots for mini-cuttings of *S. sempervirens* a: callus; b: woody tissue; c: both places.

the *S. sempervirens* mini-cuttings. The fact that T1 presented greater callus formation at the base of the cuttings may be related to the concentration of auxin. Rasmussen *et al.* (2009) reports that the formation of root callus is favored by the degradation of auxin in the basal region of the propagule and according to Guerra & Nodari (2016), high concentrations of auxin, may increase the formation of calluses.

In this way, it is possible to conclude that simple shading balances the auxin rate in mini-cuttings, thus reducing the formation of callus. The calluses consist of an irregular mass of parenchymal cells in various lignification states, usually developing at the base of the cuttings when placed in favorable environmental conditions for rooting. With reports that the first roots appear frequently through the callus, this leads to the hypothesis that callus formation is essential for rooting in some species, as in *Sequoia sempervirens* (Pereira *et al*., 2019).

For rooting, there was also a difference between treatments, in which, T3 presented a higher percentage (Figure 4). These results agree with Kim & Guak (2014) in all three varieties of blueberries, were the percentage of rooting and growth of the area and roots increased in the treatment with 30% of shading in relation to the treatment without shading (control). However, levels greater than 50% of shading were harmful to rooting hardwood cuttings of highbush blueberries.

This result surpassed the one found by Navroski *et al.* (2015), with 160 days after *S. sempervirens* mini-cuttings put to root with the application of exogenous Indoleacetic

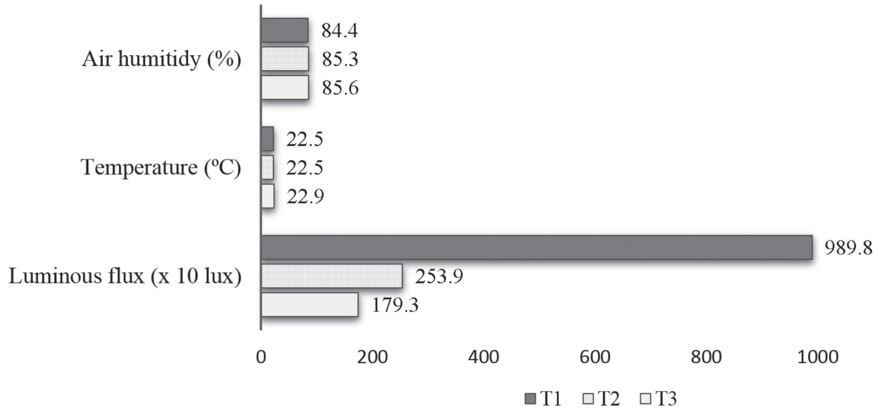

Figure 2: Variation between light (x 10 lux), temperature (ºC) and air humidity (%) averages between the three treatments of shading in mini-stumps of *S. sempervirens*.

Table 1: Survival (%), callus formation (%), rooting (%) and number of roots of *S. sempervirens* mini-cuttings 95 after put to root according to different shading levels of the mini-stumps

Shading levels	Variable			
	Survival (%)	Callus (%)	Rooting (%)	Nº of roots
T1	100.0 a *	100.0 a	77.7 b	5.7 a
T2	100.0 a	71.4 b	80.9 b	3.9 a
T3	100.0 a	78.2 b	93.7 a	5.5 a

*Treatments followed by the same letter do not differ from the average Tukey test at 5% probability.

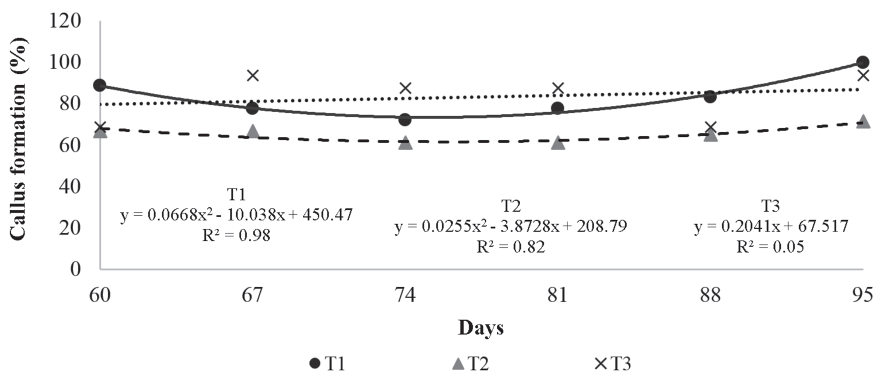

Figure 3: Percentage of callus formation in *S. sempervirens* minicuttings over the period of 60 to 95 days according to shading treatments in the clonal mini-garden.

acid (IAA), with a rooting of 80.9%. It is important to highlight the increasing and linear behavior of T3, surpassing T2 and T1 at the end of the experiment. This indicates that the concentration of endogenous auxin present in this experiment has not yet reached the inhibitory level of root production caused by the phytotoxic effect of auxin (Meneguzzi, 2017).

In addition to rooting, the number of roots on cuttings or mini-cuttings is one of the most relevant variables in plants production through vegetative (Kouakou *et al*., 2016). Regarding the number of roots, it is clear that T2 had a certain similarity during the evaluation days, whereas T1 and T3 presented an increase in quantity over the days (Figure 5). This fact can be associated with the presence of calluses in the base of the mini-cuttings of both treatments.

These variables are very important in this species cutting process, as according to Luna (2008) even with rooting, *S. sempervirens* cuttings can present high mortality in the acclimatization process. Therefore, it is necessary to be careful with environmental conditions, mainly regarding to high temperatures.

When analyzing the origin of the roots, it was possible to notice that those derived from calluses the T1 presented higher averages in relation to T2 and T3. This event is probably due to the elongation provided in T2 and T3, with reduced lignification, and consequently easier rooting. Corroborating with the results of the present study, Kim & Guak (2014) states that the use of shading layers reduces the formation of callus during the rooting process.

Thus, from the results of the origin of the roots in the mini cuttings of *S. sempervirens*, it is possible to observe that T2 and T3 presented a higher percentage of roots from the woody tissue (Table 2). It is worth noting that these roots were also visually more elongated and better distributed at the base of the cuttings, which should favor the development of the seedling. The number of roots that originated from both sites (callus or woody tissue) was also measured, however, it did not show significant variation for the three treatments studied. Grossnickle (2012) states that seedlings that have a more adequate root system tend to survive better than those that have an inferior root system, especially in the first weeks, when adverse conditions can compromise their survival.

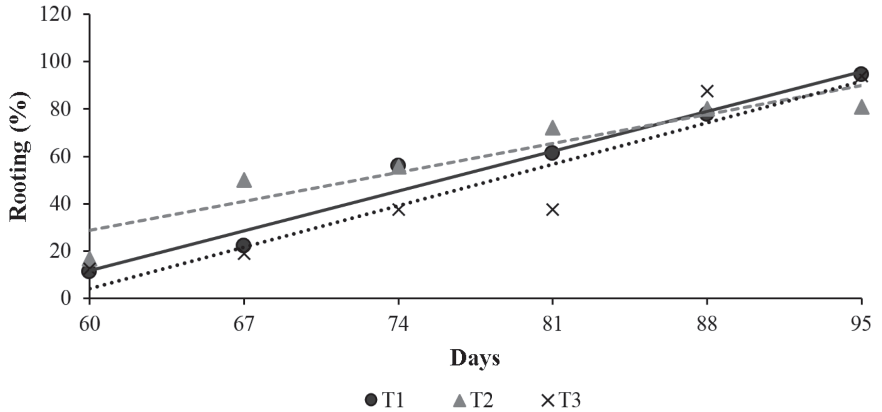

Figure 4: Rooting percentage of *S. sempervirens* mini-cuttings over the period of 60 to 95 days as a function of shading treatments in a clonal mini-garden.

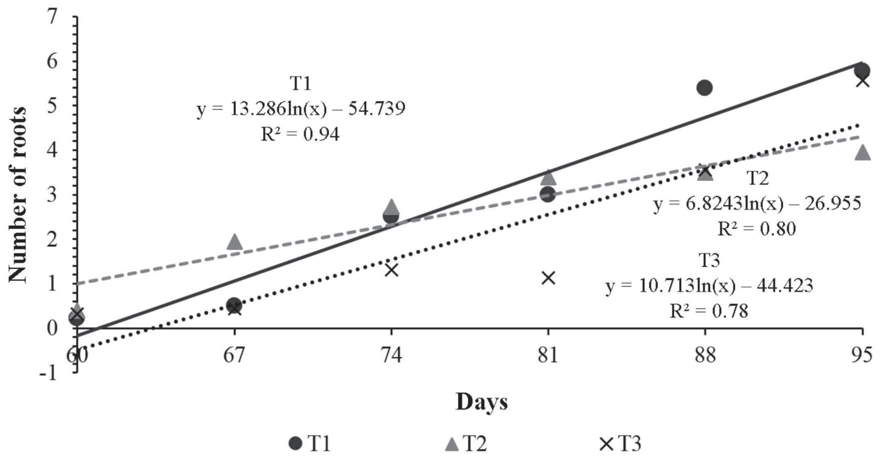

Figure 5: Number of roots in mini-cuttings of *S. sempervirens* over the period of 60 to 95 days as a function of shading treatments in a clonal mini-garden.

Table 2: Roots origin – % (callus, woody tissue or both places) in *S. sempervirens* mini-cuttings during rooting process

Shading levels	Root origin (%)		
	Callus	Woody tissue	Callus/Woody tissue
T1	63.3 a *	26.5 b	10.2 a
T2	29.4 b	61.8 a	8.8 a
T3	39.1 b	45.6 a	15.2 a

*Treatments followed by the same letter do not differ from the average Tukey test at 5% probability.

CONCLUSIONS

In the production of *S. sempervirens* mini-cuttings, the different levels of shading had no influence.

For the rooting of the mini-cuttings, a difference was found between the treatments, in which two blades of Sombrite® with 95 days after cutting presented a higher percentage of rooting. As for the origin, the roots derived from calluses and no shadowing presented higher averages in relation to any shadowing levels.

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