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Effect of Shading and Subsequent Sampling days on Eucalyptus Mini-Stump Sprouting and Mini-cutting Rooting

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ABSTRACT

Background: Seedling quality in clone garden handling is associated to root system. Among factors affecting rooting, shading and sampling time interval have great importance. **Objective:** Thus, this study aimed to evaluate shading and subsequent samplings effects on rooting of clone 144 cuttings (Eucalyptus urophylla x E. grandis hybrid) from a clone garden. **Results:** Full sun and shading condition comparison on each sampling day. On the first day, referring to the first evaluation, it was observed that full sun promoted positive effects over all studied variables; and on second and third days, shading had positive effects. For the second evaluation, full sun promoted greater effects than shading on the fourth sampling day and shading on the first day. **Conclusion:** In general, sampling-day analysis under full-sun condition showed a statistically greater effect on the first day for mini-cutting production and rooting. This more intense rooting might be related with the longer times that cuttings remained under greenhouse when compared to the others.

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INTRODUCTION

Focusing on pulp and paper sector demands in the 70's, Brazilian researchers began to study clonal seedling production through conventional cutting technique using plant species from *Eucalyptus* genus. Mini-cuttings came up as an improvement of that technique, which understates a few difficulties in seedling production of some species and clones, especially rooting (Assis *et al*., 2014; Xavier and Silva, 2010). Some results indicated mini-cutting advantages over conventional method for *Eucalyptus* seedling production such as enhanced rooting performance; good root system quality; high root emission speed; reduction of operational activities; and control of water, nutrition and plant health conditions of propagating material plants (Titon *et al*., 2003; Assis *et al*., 2004; Santos *et al*., 2005).

The mini-cutting can be divided into several stages such as sprout production in clone gardens, rooting induction under intermittent mist and high temperature, shade acclimation of saplings, seedling growth and hardening (Xavier; Wendling, 1998; Alfenas *et al*., 2009). A group of mini-stumps from cutting technique composes the clone garden and mini-cuttings are the sprouts obtained from these mini-stumps.

Clone garden can be handled and kept under varied conditions and, irrespective its handling, mini-stumps are submitted to subsequent sprout samplings, which are produced the whole period, for later mini-cutting production. It can be weekly thinned out, removing leaves, sprouts and dead mini-stumps, besides fungicide spraying for pathogen control (Brondani *et al*., 2009).

Final eucalyptus seedling quality is directly related to clone garden management (shading level, defective or surplus irrigations, malnutrition and weed competition) and produced sprout standard (Mônico, 2012; Lopes, 2008). It is expressed through nutritional and morphological characteristics and is one of the essential factors to obtain a high-yield plant stand, which arises from genetics, production procedures (inputs' quality), and seedling delivery means to the field and nursery management (Ciavatta, 2010). Focusing on the later that contributes in several benefits among them fungus incidence control and fertilizer exploitation for production of mini-stumps in good conditions (Xavier and Wendling, 1998; Gonçalves, 2000).

One of the factors that must be taken into account for seedling quality achievement is root system variable analysis. Root malformation hinders water and nutrient absorption in sufficient amounts to meet plant needs, resulting in water and/ or nutritional deficiency as a result of root and shoot imbalance (Mafia *et al*., 2005).

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Bearing in mind the root system importance for seedling quality and assuming that clone garden management, including ideal shading, is one of the main factors for enhanced rooting; in this current study, we aimed to assess the effect of shading and subsequent sampling days on mini-cutting production of a clone garden, as well as the rooting response within this system.

MATERIAL AND METHODS

Location and genetic material:

The experiment was carried out in a nursery belonging to Viaverde Florestal Ltda company, which is located in Abadiânia city, Goiás State, Brazil. The area is at 16º12'31" S and 48º44'26" W geographical coordinates. The city lies in Cerrado area, a savannah-like vegetation; and according to Köppen climatic classification, it has an *Aw* tropical climate type, featuring two distinct seasons one dry period that lasts from five to seven months and another rainy one. Local rainfall varies from 1,300 mm to 2,000 mm. Average temperatures range between 22 °C and 26 °C (Seplan GO, 1994).

The commercial clone 144 was used for this experiment, consisting of a *Eucalyptus urophylla* x *Eucalyptus grandis* (*Eucalyptus* urograndis) hybrid.

Installation:

Clone garden was made of mini-stumps arranged within hanging beds. The mini-stumps were obtained from mini-cutting rooting and were planted into 55-cm³ tubes, which were periodically fertilized and irrigated.

Shading screen cover was made with a 50% shading mesh at the bed ends to compare mini-cutting production per mini-stump under shading and full sun environments. The screen was placed 70 cm above the mini-stumps.

Sprout number sampling (mini-cuttings), statistical design and analysis:

Twenty-one mini-stumps were selected randomly for each condition (with shade and full sun), in seven replications with three plants each. However, for mini-stumps under shade, we have chosen and selected those located within bed central area, so that there was not a possible edge effect on the clone production evaluation.

It was performed a mini-cutting sampling for three days in a roll at 25th day after screen installation. At 40th day, the sampling lasted for four consecutive days. Each evaluation day, mini-cuttings produced by mini-stumps were collected, counted and taken immediately to a greenhouse.

Considering the first assessment (25th day), we adopted a completely randomized statistical design in simple 3 x 2 factorial scheme. Sampling day factor in three levels (first, second and third sampling day) and light condition factor in two levels (with 50% shading and without - full sun), with seven replications per treatment with three plants each.

For the second assessment (40th day), we also adopted a completely randomized design, however in a 4 x 2 factorial scheme. Sampling day factor in four levels (first, second, third and fourth sampling day) and light condition factor in two levels (with 50% shading and without - full sun), with seven replications per treatment with three plants each.

The data were subjected to variance analysis (ANOVA), using the "F test" at 1% significance level, and the means were discriminated by Scott-Knott test at 5% significance level using ASSISTAT version 7.7.

Mini-cutting sampling, cutting and rooting:

The mini-cuttings with dimensions between 5 to 6 cm, containing one or two leaf pairs were collected from the apical portion of each mini-stump previously marked, in two non-consecutive dates (from 25 and 40 days after screen installation) had the leaves reduced to half the original size.

Sprout sampling in clone gardens was performed with pruning shears, previously sterilized in alcohol (70% v/ v) and was performed in the morning, so that evapotranspiration was reduced. The period between sampling and cutting preparation was shorter than 30 minutes so that the water stress was reduced.

Cuttings were collected and planted into 55-cm³ conical tubes, which were previously sterilized in hot water at 80 °C for 30 s. The cuttings were inserted approximately at two centimeters above substrate basis.

Rooting induction process was conducted under acclimatized greenhouse conditions, in which mini-cuttings remained for 25 days under intermittent mist irrigation system.

Rooting data collection, statistical design and analysis:

All mini-cuttings were removed from the greenhouse on the same day, regardless week sampling day, for rooting evaluations. Mini-cuttings sampled on the first day of the week remained in the greenhouse for 25 days, so those sampled on the second day for 24 days and the ones collected on the third day for 23 days.

Initially, the mini-cuttings were removed from tubes, and then root system was immersed into water, in order to remove substrate. After substrate removal, we performed a cut along sample diameter to evaluate root system.

A completely randomized design in a 3 x 2 factorial scheme was adopted for the first evaluation (25th day). The factors were sampling day in three levels (first, second and third sampling day) and light condition in two levels (with 50% shading and without - full sun), with ten replications of three mini-cuttings/ plot. The same design was used for the second evaluation; however in a 4 x 2 simple factorial, with four sampling days under two light conditions with ten replications of three mini-cuttings / plot.

Mini-cutting roots underwent though the following evaluations root length measure by a scale gauged in centimeters, root number counting and the root dry weight. For the latter evaluation, the roots were placed into paper bags and oven dried at 70 °C for 72 hours. After drying, the material was weighed with the aid of an analytical balance (0.001 g accuracy).

The data were subjected to variance analysis (ANOVA), using the "F test" at 1% significance level, and the means were discriminated by the Scott-Knott test at 5% significance level, using ASSISTAT version 7.7.

Results:

First evaluation (sampling beginning at 25th day):

According to ANOVA (Table 1), all studied variables presented significant interaction in the first evaluation for factors under study (sampling day and light condition).

Table 1: Variance analysis summary for mini-cutting numbers (MN), root length (RL), root number (RN) and root dry weight (RDW) of clone 144, considering the first evaluation.

1st evaluation – sampling beginning at 25th day						
		Mean square value				
SV		DF	MN	RL (cm)	RN	RDW (g)
Condition		1	0.51482^{ns}	11.06822^{ns}	1.06667^{ns}	0.00003^{ns}
Sampling day		2	40.67165**	70.81436**	10.38801**	0.00214**
Interaction		2	18.81529**	280.45816**	11.51667**	0.00328**
Mean			3.22262	8.78483	2.84967	0.02328
VC (%)			29.16	35.29	37.69	31.61

SV: Sources of variation; DF: freedom-degrees; VC: variation coefficient; ** significant at 1% probability ($p < 0.01$); ^{ns} non-significant.

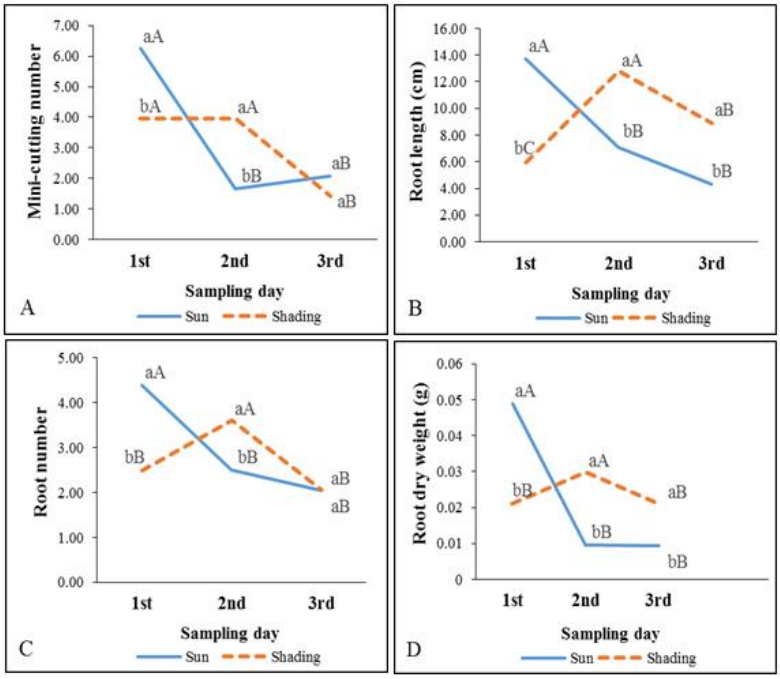

Fig. 1: Breakdown of the interaction between light condition (full sun and shading) and sampling day (1st, 2nd and 3rd) factors for the variables mini-cutting number (A), root length (B), root number (C) and root dry weight (D) in clone 144 (first evaluation). Different lowercase letters indicate statistical significance between light conditions (full sun and shading) for each sampling day; and different uppercase letters stand for statistical significance among sampling days (1st, 2nd and 3rd) within each light condition by Scott-Knott test at 5% significance.

Figure 1 shows the interaction between the two factors for cutting number variable, in which mini-stumps under full sun at the first sampling day stood out for cutting production compared to the others. Under shade conditions, mini-cuttings produced at the first and second sampling days did not differ statistically from each other but they were significantly higher than the third day. Comparing the two conditions within each sampling day, sprouts produced on the first day was statistically greater under full sun. Interestingly, this situation change on the second day, in which sprout production was higher under shading.

Regarding root length, Figure 1B shows that under full sun, root length mean of mini-cuttings from the first day was significantly superior to all other days. Contrarily, under shading, the roots from the second day stood out in length when compared to the other days. Comparing full sun and shading factors in each sampling day, it turns out that the roots of the first day sprouts were significantly higher in length under full sun. In the two subsequent days, these means were higher for shaded plants.

Figure 1C displays root number analysis and it reveals that in the condition without shading, the first day has highlighted when compared to the others. However, when shade is provided, the second day underscored, which featured a root number 63.18% higher than the other day means. Comparison of full sun and shade factors in each sampling day shows similar behavior to root length variable. With respect to root dry weight, as shown in Figure 1D, there was similar behavior to root number.

Second evaluation (sampling beginning at 40th day):

It was verified for the second evaluation as seen in Table 2 (below), the variable number of mini-cuttings per mini-stump and root dry weight have differences between the factors studied separately. Regarding the root number, there was no significant differences among treatments. In contrary, for root length, there was significant interaction between factors, unfolding the interaction.

Tabela 2: Variance analysis summary for mini-cutting numbers (MN), root length (RL), root number (RN) and root dry weight (RDW) of clone 144, considering the second evaluation.

2nd evaluation – sampling beginning at 40th day						
		Mean square value				
SV		DF	DF	DF	DF	DF
Condition		1	9.71111**	28.17938^{ns}	0.02520^{ns}	0.00213**
Sampling day		3	66.28551**	241.18265**	6.65815^{ns}	0.00585**
Interaction		3	1.05853^{ns}	57.15069**	8.49782^{ns}	0.00017^{ns}
Mean			2.19036	10.65825	3.20525	0.04301
VC (%)			38.53	30.70	55.16	35.92

SV: Sources of variation; DF: freedom-degrees; VC: variation coefficient; ** significant at 1% probability ($p < 0.01$); ^{ns} non-significant.

Studying singly the mini-cutting number (Figure 2) and the root dry weight (Figure 3), it can be seen a statistically superior effect of light compared to the shading. Shading has provided 47% and 27.3% reductions in mini-cutting number and root dry weight, respectively. Regarding the sampling day, the first had means statistically higher than the others did for both variables.

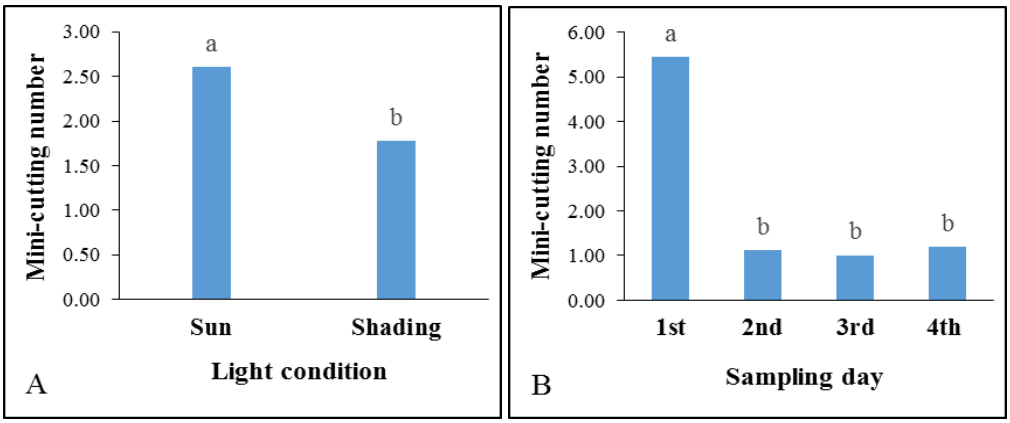

Fig. 2: Mini-cutting number mean of clone 144 (second evaluation) under two light conditions (full sun and shading) – A; and at different sampling days (1st, 2nd and 3rd) – B, according to Scott-Knott test, means statistically differ at 5% significance level.

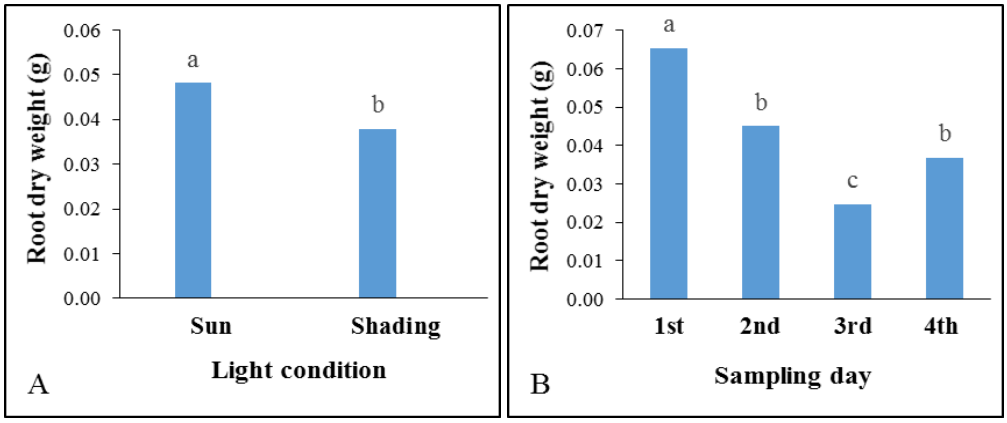

Fig. 3: Root dry weight mean of clone 144 (second evaluation) under two light conditions (full sun and shading) – A; and at different sampling days (1st, 2nd and 3rd) – B, according to Scott-Knott test, means statistically differ at 5% significance level.

It can be seen similar result to the first evaluation in Figure 4, which presents the breakdown of root length. Means of root length under full sun for mini-cuttings collected on the first day was superior to all other days, which did not differ among themselves. Moreover, similar results were observed for shadowing condition. Comparison of full sun and shade factors for each day demonstrated that absence of shading promoted positive effects on root length on the fourth day and, the contrary had effects on the first day.

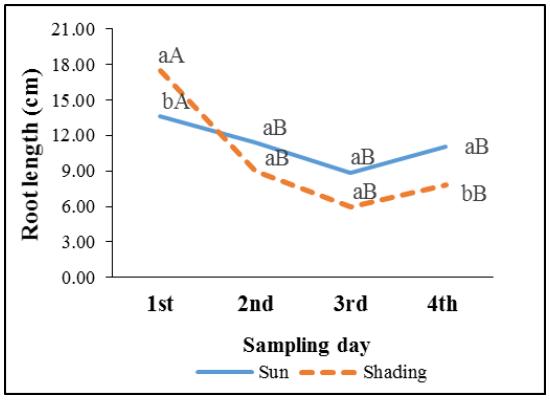

Fig. 4: Breakdown of the interaction between light condition (full sun and shading) and sampling day (1st, 2nd and 3rd) factors for the variable root length of the clone 144 (second evaluation). Different lowercase letters indicate statistical significance between light conditions (full sun and shading) for each sampling day; and different uppercase letters stand for statistical significance among sampling days (1st, 2nd and 3rd) within each light condition by Scott-Knott test at 5% significance.

Discussion:

The comparison of full sun and shading on each sampling day, for the first evaluation, shows that although full sun has been favorable for all variables on the first day, shading was beneficial for the same variables during the second day. By contrast, the results changed for the second evaluation with full sun conditions favoring root growth (root length) on the fourth sampling day and the shading on the first. Regardless sampling day, full sun was better then shading also in the second evaluation, providing increases in mini-cutting number and root dry weight.

This shading positive effect corroborates with findings of Borges (1978). According to this author, the decrease in natural light exposure of clone gardens has highly significant effect on root formation. On the other hand, Cunha *et al*. (2009), studying different eucalyptus clones, found that light intensity increase in clone garden places has promoted positive effects on both mini-cutting rooting and production. Shading positive effect on mini-cutting production observed at the first evaluation is also in disagreement with the findings of Martins *et al*. (2012) assessing *Dipteryx alata* Vogel (Baru) seedlings and Diogenes *et al*. (2013) studying *Myyracrodruon urundeuva* Fr. All. (aroeira).

The positive light influence on studied variables can be explained as cited by Alfenas *et al*. (2009), who claim that light influences indirectly rooting, since the photosynthetic products, in particular carbohydrates and

growth regulators, are essential for initiation and root development. Assis *et al*. (2004) complement stating that the light intensity can greatly influence cutting production and rooting for reduction or enhancement of endogenous phenolic substances, which may act as inhibitors or promoters, depending on tissue concentrations and species.

Sampling day analysis for full sun condition shows statistically superior effect on the first day compared to the other for all variables under study, even when the day factor is analyzed regardless lighting (mini-cutting number and root dry weight – 2nd evaluation). These results show that mini-cutting sampling in clonal under full sun, when performed within several consecutive weekdays promotes a decrease in mini-cutting number per mini-stumps. Thus, for a better mini-stump yield, it is suggested that samples should be held at bigger day intervals.

The highest root system development (longer roots, higher root number and dry weight), found on that first day, might be related to the increased rooting time under those conditions. The cuttings collected on the first day from mini-stumps grown under full sun, due to the sampling logistics, remained one and two days more inside acclimatized greenhouse, compared to those sampled on the second and third days, respectively. It is also noteworthy the outstanding effect of few hours (24 to 48 hours) on rooting. Contrarily, concerning sampling day breakdown for shading condition, it was possible to observe a different root system behavior from above describe. The second day sampling performed in mini-stumps under shade provided greater root length and number.

According to Ferreira *et al*. (2004), mini-cutting length of stay under greenhouse directly influences rooting and subsequent survival under a shade house. Melo *et al*. (2011) highlighted the importance of optimizing the time required for rooting of mini-cuttings. This period depends on a number of factors, among which are species, sprout and leaf presence, region and time of the year and is generally lower than that used in conventional cutting (Cunha *et al*., 2003; Ferriani *et al*., 2010; Brondani *et al*., 2012;. Guidotti *et al*., 2013).

Some researches indicate an average time of 30 days, as seen in findings of Wendling *et al*. (2000) and Wendling & Xavier (2003) for *Eucalyptus* spp. and *Eucalyptus grandis*, respectively. Titon (2001), studying *E. grandis* suggested a shorter period of stay of around 21 days. However, Brondani *et al*. (2012), who studied the hybrid *Eucalyptus benthamii* x *Eucalyptus dunnii*, pointed out as the ideal length of stay a period between 35-42 days but may vary depending on the genetic material. In this research, the period of 25 days under greenhouse for mini-cuttings collected from mini-stumps grown under full sun provided better rooting performance compared to the other two times (23 and 24 days).

Conclusions:

It was sampled a higher number of mini-cuttings under full sun on the first sampling day, with gradual decrease along consecutive days. Thus, for a better mini-cutting yield per mini-stump, we suggest that samplings in a clone garden must be performed with longer intervals.

In general, the greater the length of stay in rooting house of cuttings from mini-stumps, grown under full sun, the better the root system development.

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