EFFECTIVENESS OF ASCORBIC ACID AND PVP IN THE ROOTING OF CLONAL MINI-CUTTINGS OF *Eucalyptus urophylla* x *Eucalyptus grandis*

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ABSTRACT: The objective of this study is to evaluate the effectiveness of the antioxidants ascorbic acid and polyvinylpyrrolidone (PVP) in the rooting of mini-cuttings for three clones of *Eucalyptus urophylla* x *Eucalyptus grandis*. Mini-cuttings were gathered from a mini-clonal hedge which had been cultivated in concrete ducts containing washed sand. Five concentrations of each antioxidant were experimentally tested on each of the three clones (C_1, C_2 and C_3). Assessments were done of mini-cutting survival and rooting rates when leaving the greenhouse and the shade house, as well as seedling survival and growth at age 50 days. Ascorbic acid was found to be beneficial to the mini-cuttings of the clone with a lower rooting percentage (C_3), whereas PVP was found to be unbeneficial to the clones being studied.

Key words: Mini-cutting technique, antioxidants, clonal forestry.

EFICIÊNCIA DO ÁCIDO ASCÓRBICO E PVP NO ENRAIZAMENTO DE MINIESTACAS DE CLONES DE *Eucalyptus urophylla* x *Eucalyptus grandis*

RESUMO: *Objetivou-se, com este estudo, avaliar a eficiência dos antioxidantes ácido ascórbico e polivinilpirrolidona (PVP) no enraizamento de miniestacas de três clones de Eucalyptus urophylla x Eucalyptus grandis. As miniestacas foram coletadas em minijardim clonal, conduzido em canaletas de alvenaria preenchidas com areia lavada. Experimentalmente, foram testadas cinco concentrações de cada antioxidante nos três clones estudados (C_1, C_2 e C_3). Foram realizadas avaliações de sobrevivência e enraizamento de miniestacas na saída das casas de vegetação e de sombra e da sobrevivência e crescimento das mudas aos 50 dias de idade. A utilização do ácido ascórbico foi favorável para as miniestacas do clone com menor porcentual de enraizamento (C_3), porém, a utilização do PVP mostrou-se desfavorável para os clones estudados.*

Palavras-chave: Miniestaquia, antioxidantes, silvicultura clonal.

1 INTRODUCTION

The search for improved effectiveness in the production of nursery seedling has stimulated forestry companies to seek alternative techniques and to improve already established techniques. According to Xavier et al. (2009), knowledge of the finest vegetative propagation techniques in combination with use of substances capable of stimulating rooting can both contribute to make better use of vegetative material and thus lead to productivity gains.

In the Brazilian forest sector, mini-cutting technique is today the most commonly used method for vegetative propagation of eucalyptus. This technique comprises several phases, which include: shoot production in a mini-clonal hedge, induction of adventitious roots in a climatized greenhouse, acclimatization in a shade house, seedling growth and hardening (ALFENAS et al., 2009; ASSIS et al., 2004; HIGASHI et al., 2000; XAVIER et al., 2009; XAVIER; WENDLING, 1998). Optimizing operations in each of these phases will help achieve success in seedling production (FERREIRA et al., 2004).

Countless factors can affect the rhizogenic process, including influence of species/clone, type of cutting, juvenility, hormone balance, presence of root inducers and inhibitors, effect of collection season, rooting environment and influence of nutritional status (XAVIER et al., 2009).

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Certain substances at different concentrations, combined with the time elapsed between collecting and actually planting the mini-cuttings, may impact propagule rooting due to reactions occurring in the vegetative material, for instance oxidation (WENDLING et al., 2000b).

Oxidation on the base of mini-cuttings affects the entire rhizogenic process, yet it can be minimized or even avoided by adopting some procedures, for instance using antioxidant substances, reducing mechanical damage and washing vegetative propagules under running water (WENDLING, 2002). Effects of antioxidants include inactivation of free radicals, complexometry of metabolic ions or reduction of peroxides for products unable to form free radicals with potential to oxidize (ARAÚJO, 2008).

Antioxidative substances include ascorbic acid, polyvinylpyrrolidone-PVP (HANDA et al., 2005), citric acid (HARTMANN et al., 2002) and activated charcoal (MELO et al., 2001). Their use is often reported in micropropagation, however it is still very restricted in the rooting of vegetative cuttings (FACHINELLO et al., 1993).

While studying the effect of ascorbic acid and PVP on rooting of mini-cuttings in four clones of *Eucalyptus* spp., Goulart (2007) concluded that ascorbic acid was only effective in one clone, whereas PVP was effective in all clones in maximizing the rooting of mini-cuttings.

Ascorbic acid reacts with metals present in the culture medium, preventing them from being available to oxidize (TAIZ; ZEIGER, 2004), and its use has reduced explant oxidation in tissue culture (SOUZA; ABREU, 2007; ZIV; HALEVY, 1983). Similar results were found by Pasqual et al. (2002) using PVP. According to Teixeira (2001), PVP is an adsorbent compound that binds to phenols and prevents oxidation, in addition to adsorbing the products of phenol oxidation. All that considered, the objective of this study is to evaluate effectiveness of the antioxidants ascorbic acid and PVP in the rooting of mini-cuttings of three clones of *Eucalyptus urophylla* x *E. grandis*.

2 MATERIAL AND METHODS

2.1 Experimental material

This work was carried out from September to November 2008, in the forest nursery of Celulose Nipo-Brasileira S.A. - CENIBRA, a company located in Belo Oriente, Minas Gerais. The municipality of Belo Oriente lies in Vale do Rio Doce and has a Cwa climate (subtropical, rainy and temperate) according to Köppen classification, located at geographical coordinates 19°18’23” south latitude and 42°22’46” west longitude, at and altitude of 363 m. The average annual precipitation is 1,233 mm and the average annual temperature is 21°C, with highs of 27°C and lows of 14°C.

Mini-cuttings of three clones of *Eucalyptus urophylla* x *E. grandis* were used, as collected from mini-stumps established in a mini-clonal hedge, following management and nutrition procedures adopted by CENIBRA. The criterion of clone selection was based on average rooting percentages. Selection included one clone with rooting percentage above 90% (C_1), one clone with rooting percentage between 80% and 90% (C_2) and one clone with rooting percentage below 80% (C_3).

2.2 Mini-clonal hedge management

According to the mini-cutting technique described by Alfenas et al. (2009), Assis et al. (2004), Higashi et al. (2000), Wendling et al. (2000a), Xavier et al. (2009) and Xavier and Wendling (1998), and according to management procedures adopted by CENIBRA, the mini-clonal hedge consisted of mini-stumps obtained through the rooting of mini-cuttings introduced in concrete ducts, previously lined with gravel and topped with washed sand.

An automated system of drip fertirrigation was used for plant irrigation and mineral nutrition, according to the operating procedures adopted by CENIBRA. The system was activated every three hours and left to irrigate for six minutes.

2.3 Mini-cutting collection, preparation and planting

The collection, preparation and planting of mini-cuttings took place on September 18, 2008, soon after the start of nursery activities, at 8:30.

Mini-cuttings around six to eight inches in length were collected and prepared, maintaining two pairs of leaves reduced to half the original size. In order to maintain turgidity conditions, the mini-cuttings were placed in polysterene boxes and sprinkled with water by using a manual sprayer, at time intervals of less than five minutes until the planting stage.

Once prepared, the mini-cuttings were treated with the antioxidants ascorbic acid or PVP, prior to being planted and left to root in the climatized greenhouse. The following concentrations were used: ascorbic acid (0, 10, 20, 40 and 80 mg L^{-1}) and PVP (0, 2000, 4000, 8000 and 16000 mg L^{-1}), diluted in distilled water. The base portions of the mini-cuttings (2 cm) were dipped in the antioxidant solution for 15 seconds and then planted in the substrate. The time interval between collecting, preparing, applying antioxidant and planting the mini-cuttings was invariably less than 30 minutes.

The mini-cuttings were planted in a substrate consisting of combined scorched rice husk and vermiculite (1:1), enriched with 8000 g m^{-3} of simple superphosphate, 694 g m^{-3} of ammonium sulfate, 208 g m^{-3} of potassium chloride, 13.9 g m^{-3} of zinc sulfate, 13.9 g m^{-3} of copper sulfate, 13.9 g m^{-3} of manganese sulfate and 27.8 g m^{-3} of boric acid, according to operating procedures adopted by CENIBRA. Tapered plastic tubes were used as containers, each 12 cm in length and with a capacity of 55 cm^3, previously sterilized in hot water at 80°C/30 sec., according to the method described by Alfenas et al. (2009).

The process of mini-cutting rooting was conducted in the climatized greenhouse of the forest nursery, set to a temperature of around 27ºC and relative humidity above 80%, for a period of 18 days. The mini-cuttings were then transferred to a shaded area receiving around 50% of light intensity as obtained in an outdoor environment by using a shade screen (remaining there for 10 days to acclimatize), and finally taken to the growth area under sunlight until they reached 50 days of development.

Independent experiments were set up to evaluate the effect of each antioxidant on the rooting of the clonal mini-cuttings of *Eucalyptus urophylla* x *E. grandis*. Both experiments followed a completely randomized experimental design, using a 3 x 5 factorial arrangement, considering all three clones being studied (C_1, C_2 and C_3) and five antioxidant dosages, in five replicates of 32 plants each, to a total of 800 mini-cuttings per clone in each experiment.

2.4 Experimental evaluations

Plants were evaluated as to the percentage of mini-cutting survival when leaving the greenhouse (18 days after planting), percentage of mini-cutting survival and percentage of mini-cuttings with roots longer than 10 cm (with roots sprouting out of the plastic tube base) when leaving the shade house (28 days after planting). At age 50 days, evaluations included percentage of survival, height (cm) and stem base diameter (mm) of seedlings.

In order to measure height and stem base diameter of seedlings at age 50 days, eight randomly picked plants were analyzed per replicate. Height was measured using a ruler marked in mm and stem base diameter was measured using a Mitutoyo 500-144B digital caliper with a resolution of 0.01 mm.

With data at hand for the relevant traits, analysis of variance and regression procedures were performed. For the ANOVA, statistical application SISVAR was used (FERREIRA, 2000), with data analysis following the methodology proposed by Banzatto and Kronka (2006). Regression equations were developed using software application CurveExpert 1.3 (HYAMS, 1997).

3 RESULTS AND DISCUSSION

3.1 Effectiveness of ascorbic acid

The analysis of variance revealed a significant effect, by the F test ($P < 0.05$), of the ‘clone x treatment’ interaction for the traits being assessed, indicating different responses from each clone to various concentrations of ascorbic acid (Table 1).

Table 1 – Analysis of variance results for traits being assessed, as a function of various concentrations of ascorbic acid being used in mini-cuttings of three clones of *Eucalyptus urophylla* x *E. grandis.*

Tabela 1 *– Resultados da análise de variância para as características avaliadas em função das concentrações de ácido ascórbico, utilizadas em miniestacas de três clones de Eucalyptus urophylla x E. grandis.*

Source	DF	Mean Square					
		PSCV (%)	PSCS (%)	PSCS10 (%)	PS50 (%)	ALT (cm)	DC (mm)
Clone (C)	2	1.3835*	1.7389*	2.7742*	1.5208*	291.8152*	0.4741*
Treat. (T)	4	0.0170*	0.0126*	0.0266*	0.0184*	10.4695*	0.0465*
C x T	8	0.0495*	0.0343*	0.0224*	0.0190*	2.9015*	0.0214*
Residual	60	0.0040	0.0031	0.0029	0.0035	0.6294	0.0038
Overall Mean	-	95.2	92.5	86.9	86.9	22.5	2.7
CV (%)	-	4.7	4.3	4.5	4.9	3.5	2.3

Percentage of mini-cutting survival when leaving the greenhouse (PSCV) and when leaving the shade house (PSCS), percentage of mini-cuttings with roots longer than 10 cm when leaving the shade house (PSCS10), survival percentage (PS50), height (ALT) and stem base diameter (DC) of seedlings at the end of the growth period (“*” = significant at the 5 % probability level, by the F test).

Survival rates for clones C_1 and C_2 were found to be above 90% when leaving the greenhouse and the shade house, irrespective of the concentration of ascorbic acid being used, which demonstrates a high percentage of mini-cutting usability in both and thus indicates that applying ascorbic acid in these clones is unnecessary. Survival rates for clone C_3 were found to be below the rates found for the other clones, yet this clone proved positively influenced by dosages of up to 40 mg L^{-1} of ascorbic acid (Figure 1).

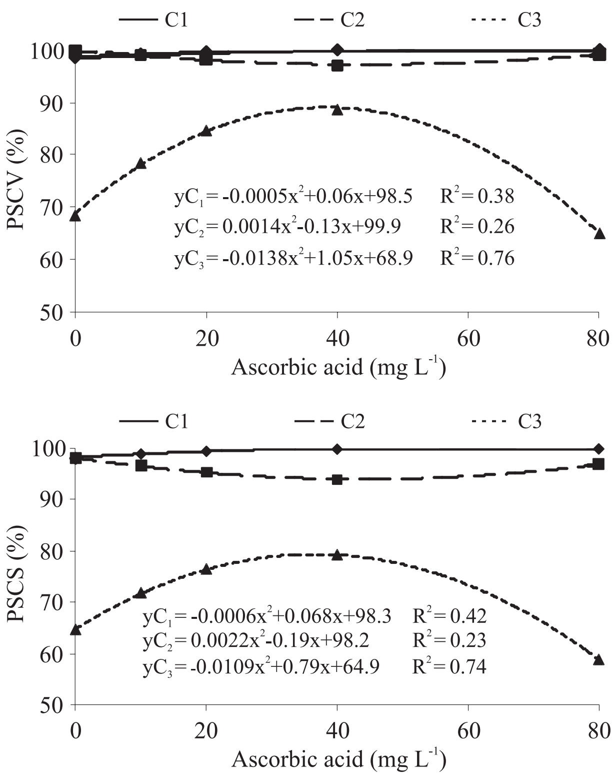

Figure 1 – Percentage of mini-cutting survival when leaving the greenhouse (PSCV) and when leaving the shade house (PSCS), as a function of ascorbic acid use, for the three clones of *Eucalyptus urophylla* x *E. grandis* (C_1, C_2 and C_3).

***Figura 1** – Porcentual de sobrevivência de miniestacas na saída da casa de vegetação (PSCV) e na saída da casa de sombra (PSCS), em função da aplicação de ácido ascórbico, dos três clones de Eucalyptus urophylla x E. grandis (C_1, C_2 e C_3).*

Despite the differences in rooting percentages among clones, the traits mini-cuttings with roots longer than 10 cm (PSCS10) and seedling survival at age 50 days (PS50) followed a similar pattern (Figure 2).

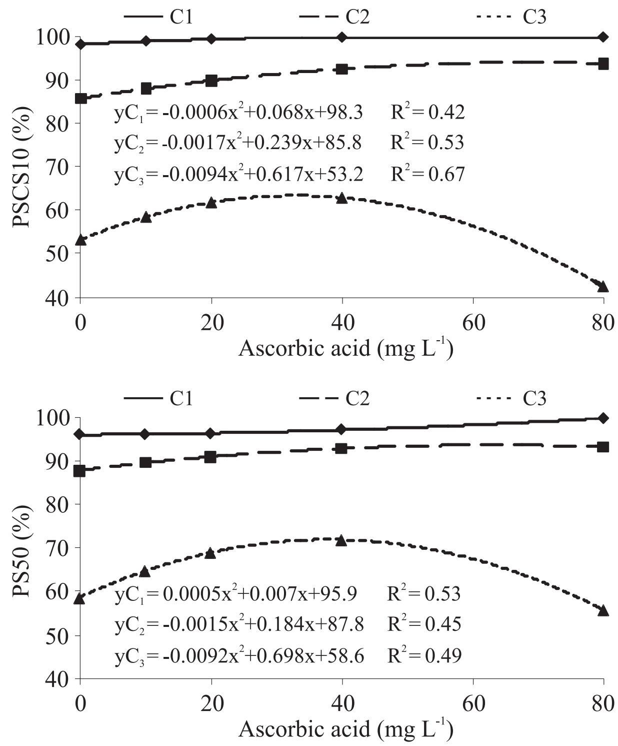

Figure 2 – Percentage of mini-cuttings with roots longer than 10 cm when leaving the shade house (PSCS10) and percentage of seedling survival at age 50 days (PS50), as a function of acid ascorbic use, for the three clones of *Eucalyptus urophylla* x *E. grandis* (C_1, C_2 and C_3).

***Figura 2** – Porcentual de miniestacas com raízes maiores que 10 cm na saída da casa de sombra (PSCS10) e porcentual de sobrevivência de mudas aos 50 dias de idade (PS50), em função da aplicação de ácido ascórbico, dos três clones de Eucalyptus urophylla x E. grandis (C_1, C_2 e C_3).*

Judging by results in Figure 2, once again the use of ascorbic acid proved unnecessary for clones C_1 and C_2, since values for traits being assessed were found to be above 96% and 87% respectively, denoting no significant increment with increasing dosages of antioxidant. Any losses occurring throughout the process for these clones are unlikely due to mini-cutting oxidation.

Under the influence of ascorbic acid, the mini-cuttings of clone C_3 showed increased rooting and survival percentages if compared to the mini-cuttings of other genotypes being studied, which demonstrates antioxidant effectiveness for this clone (Figures 1 and 2). Antioxidant use in the mini-cuttings of clone C_3 was found to have a beneficial effect up to concentrations of 40 mg L^{-1}, with dosages higher than that decreasing trait values.

An analysis of growth traits reveals that once again clones C_1 and C_2 have similarities, especially in that both show higher values of height and stem base diameter of seedlings than clone C_3. As regards seedling height at 50 days, overall all clones were negatively influenced by ascorbic acid use. And as regards stem base diameter, C_1 was the only clone showing increased values for this trait with dosages above 20 mg L^{-1} (Figure 3).

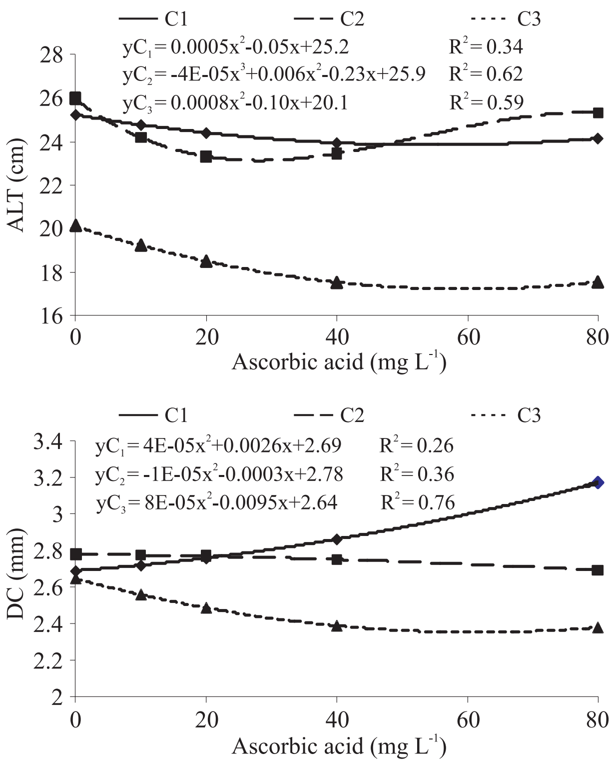

Figure 3 – Height (ALT) and stem base diameter (DC) of seedlings at age 50 days, as a function of ascorbic acid use, for the three clones of *Eucalyptus urophylla* x *E. grandis* (C_1, C_2 and C_3).

Figura 3 – *Altura (ALT) e diâmetro de colo (DC) de mudas aos 50 dias de idade, em função da aplicação de ácido ascórbico, dos três clones de Eucalyptus urophylla x E. grandis (C_1, C_2 e C_3).*

At age 50 days, measurements of height and stem base diameter of seedlings were found to be within minimum quality standards commonly used by the forestry sector (height between 20 cm and 30 cm and diameter above 2 mm). Only in clone C_3 were height measurements below 20 cm (Figure 3). Prior to being transferred to the field, after age 50 days, seedlings are taken to the hardening area of the nursery, a place whose management characteristics do not favor heightwise growth.

Therefore, the seedlings of clone C_3 need to remain longer in the nursery in order to achieve minimum quality standards concerning height, or else they need to be subjected to management procedures capable of boosting height throughout the initial 50 days. According to Carneiro (1995), seedling height is an easily modifiable trait, depending on the management procedure being used in the nursery.

On the whole, application of ascorbic acid is not justifiable for the mini-cutting technique in clones C_1 and C_2. In C_3 (Figures 1 and 2), however, which is the clone showing lower rooting and survival percentages, gains from using that antioxidant are expressive, in comparison with the other clones.

Use of ascorbic acid failed to increase usability percentages of mini-cuttings in clones C_1 and C_2 (Figures 1 and 2). Additionally, time spent in treating mini-cuttings with the antioxidant solution should also be computed, as this factor reduces propagule productivity during one day of work.

Results reveal that one of the limiting factors to the mini-cutting technique in clone C_3 is the oxidation of its mini-cuttings, owing to the fact that with concentrations of up to 40 mg L^{-1} of ascorbic acid it was possible to increase the survival percentage of seedlings at 50 days, in comparison with the treatment without the antioxidant (Figure 2).

While investigating the rooting of five hybrid clones of *Eucalyptus grandis*, Melo (2009) noticed the occurrence of oxidation on the base of mini-cuttings in one of the clones and thus concluded that for that reason many mini-cuttings would die before the rooting process was completed, and root formation in those that did survive occurred above the oxidized area, delaying the process of seedling formation.

3.2 Effectiveness of PVP

The analysis of variance revealed a significant effect, by the F test ($P < 0.05$), of the 'clone x treatment' interaction on traits being assessed, indicating different responses from each clone to various PVP concentrations (Table 2).

For clones C_1 and C_2, survival rates when leaving the greenhouse and the shade house were found to be above 90% in the absence of PVP, denoting a high percentage of mini-cutting usability in both. For clone C_2, however, when the antioxidant was used a decrease was observed in the values of relevant traits, particularly at concentrations above 8000 mg L^{-1}. The same occurred with clone C_1

Table 2 – Analysis of variance results for traits being assessed, as a function of various concentrations of the antioxidant PVP being used in mini-cuttings of three clones of *Eucalyptus urophylla* x *E. grandis*.

Tabela 2 *– Resultados da análise de variância para as características avaliadas, em função das concentrações do antioxidante PVP utilizadas em miniestacas de três clones de Eucalyptus urophylla x E. grandis.*

Source	DF	Mean Square					
		PSCV (%)	PSCS (%)	PSCS10 (%)	PS50 (%)	ALT (cm)	DC (mm)
Clone (C)	2	2.3325*	2.2931*	3.1423*	2.7400*	0.7335*	218.3248*
Treat. (T)	4	0.1036*	0.1085*	0.1299*	0.1317*	0.0998*	22.2665*
C x T	8	0.0515*	0.0520*	0.0817*	0.0493*	0.0624*	7.2327*
Residual	60	0.0034	0.0028	0.0038	0.0030	0.0078	0.7879
Overall Mean	-	88.2	82.9	72.6	70.5	2.7	22.0
CV (%)	-	4.8	4.6	6.1	5.5	3.3	4.0

Percentage of mini-cutting survival when leaving the greenhouse (PSCV) and when leaving the shade house (PSCS), percentage of mini-cuttings with roots longer than 10 cm when leaving the shade house (PSCS10), survival percentage (PS50), height (ALT) and stem base diameter (DC) of seedlings at the end of the growth period. ("*" = Significant at the 5% probability level by the F test)

regarding the trait PSCS, yet to a lesser degree if compared to the pattern in clone C_2. In clone C_3, survival percentages were found to be below values found for the other clones and, despite being positively influenced by dosages above 8000 mg L^{-1} of PVP, percentages found up to the maximum dosage were lower than values found in the absence of the antioxidant (Figure 4).

An analysis of the data concerning percentage of mini-cuttings with roots longer than 10 cm when leaving the shade house and percentage of seedling survival at age 50 days (Figure 5) reveals that clone C_2 was negatively influenced by PVP use, showing a more pronounced drop at dosages above 4000 L^{-1}. In clone C_1, on the other hand, the above traits remained stable, up to dosages of 5000 mg L^{-1}, similarly to results found by Schwengber et al. (2000).

The above results validate results found by Bergo and Mendes (2000) and Coutinho et al. (1992), in that they observed that combined use of PVP and IBA was insufficient to stimulate rooting.

The trend of the curves for mini-cutting rooting and seedling survival at 50 days in clone C_3 (Figure 5) was similar to the trend of its curves for mini-cutting survival when leaving the greenhouse and the shade house (Figure 4), validating the claim that PVP application was unfavorable for that clone.

A decreasing trend was observed in growth traits as a function of increasing PVP dosages (Figure 6). Although in clone C_1 height was positively influenced by PVP dosages above 8000 mg L^{-1}, the coefficient of determination of the regression analysis for this clone was low, denoting the unreliability of the regression equation found.

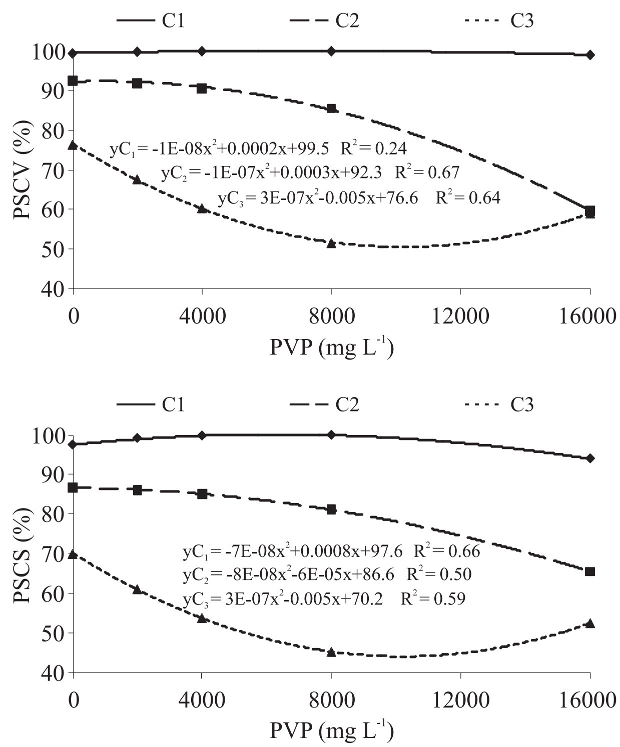

Figure 4 – Percentage of mini-cutting survival when leaving the greenhouse (PSCV) and when leaving the shade house (PSCS), as a function of PVP use, for the three clones of *Eucalyptus urophylla* x *E. grandis* (C_1, C_2 and C_3).

Figura 4 *– Porcentual de sobrevivência de miniestacas na saída da casa de vegetação (PSCV) e na saída da casa de sombra (PSCS), em função da aplicação de PVP, dos três clones de Eucalyptus urophylla x E. grandis* (C_1, C_2 *e* C_3).

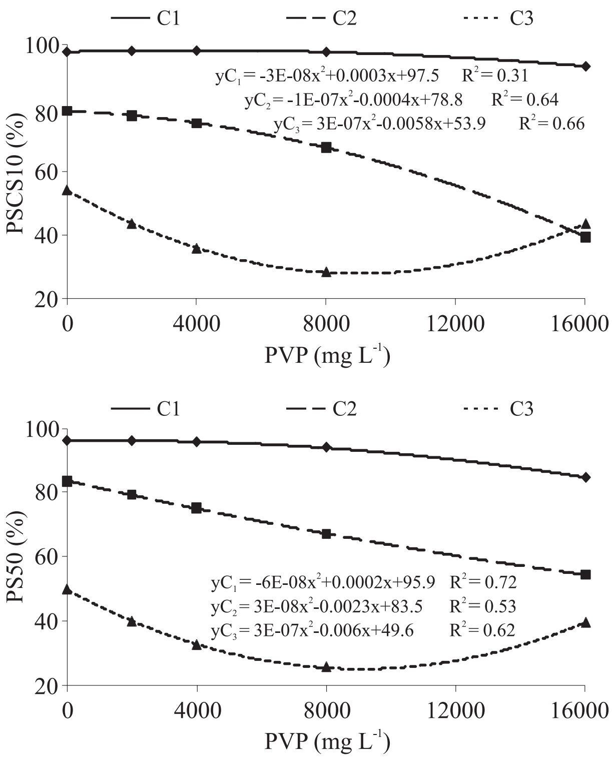

Figure 5 – Percentage of mini-cuttings with roots longer than 10 cm when leaving the shade house (PSCS10) and survival percentage of seedlings at age 50 days (PS50), as a function of PVP use, for the three clones of *Eucalyptus urophylla* x *E. grandis* (C_1, C_2 and C_3).

***Figura 5** – Porcentual de miniestacas com raízes maiores que 10 cm na saída da casa de sombra (PSCS10) e porcentual de sobrevivência de mudas aos 50 dias de idade (PS50), em função da aplicação de PVP, dos três clones de Eucalyptus urophylla x E. grandis (C_1, C_2 e C_3).*

At age 50 days, already some seedlings had height and stem base diameter within the minimum quality standards commonly used by the forestry sector (height between 20 and 30 cm and diameter above 2 mm). For seedlings in clones C_2 and C_3, however, height results were below 20 cm with PVP dosages above 4000 mg L^{-1} (Figure 6), validating the claim that PVP application was unfavorable for the clones.

Results reveal that although oxidation may be a limiting factor for the mini-cutting technique (clone C_3), the use of any antioxidant should not be regarded as synonymous with increased rooting percentages (Figures 4, 5 and 6). Additionally, in order for the substance to have a positive effect, it is necessary to define the concentration

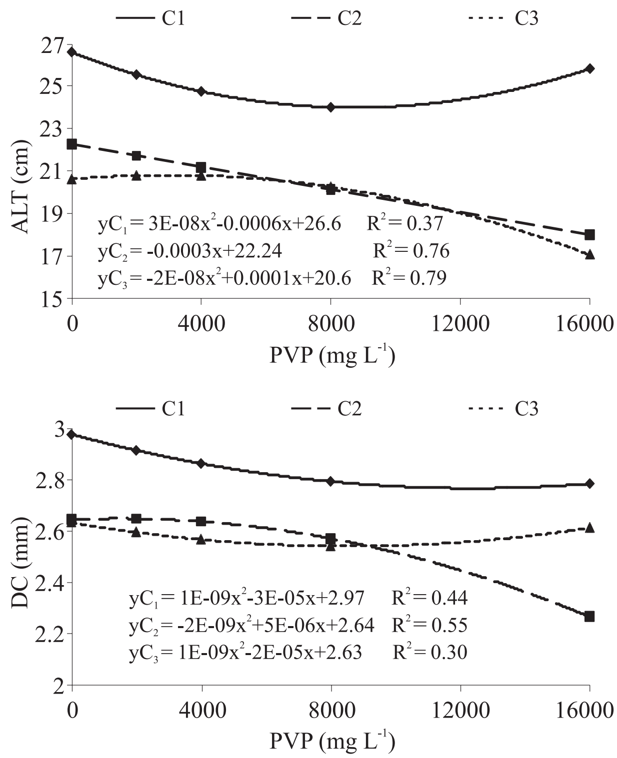

Figure 6 – Height (ALT) and stem base diameter (DC) of seedlings at age 50 days, as a function of PVP use, for the three clones of *Eucalyptus urophylla* x *E. grandis* (C_1, C_2 and C_3).

***Figura 6** – Altura (ALT) e diâmetro de colo (DC) de mudas aos 50 dias de idade, em função da aplicação de PVP, dos três clones de Eucalyptus urophylla x E. grandis (C_1, C_2 e C_3).*

to be used as function of each species/clone, for instance, while a low concentration may produce unfavorable results for a given genotype, a high concentration may conversely have a toxic effect on the tissues and consequently lead to the death of the vegetative propagule (XAVIER et al., 2009).

On the whole, application of the antioxidant PVP proved unfavorable for the clones being evaluated under the specific experimental conditions of this study (Figures 4, 5 and 6), contrary to results found by Goulart (2007) in which PVP proved effective in the mini-cutting technique for all four clones of *Eucalyptus grandis* x *E. urophylla*.

4 CONCLUSIONS

Application of antioxidants had different effects on the rooting of mini-cuttings for the three clones of *Eucalyptus urophylla* x *E. grandis* being studied.

Use of ascorbic acid proved favorable for vegetative propagation of mini-cuttings in clone C_3 but unnecessary for their propagation in clones C_1 and C_2, whereas use of PVP proved unfavorable for all clones being studied.

5 ACKNOWLEDGMENTS

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