Final report

Effect evaluation of Penergetic P® and Penergetic B® on quality and homogeneity of *Eucalyptus spp.* clonal seedlings.

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1. Introduction

The forest production in Uruguay covers an effective area of approximately 875.000 hectares (MGAP, 2018a), what represents over 5 % from national territory. Among these, the *Eucalyptus sp.* commercial plantations occupy about 594.750 hectares, where the species of *Eucalyptus dunnii* and *Eucalyptus grandis* are two of most used in the country (MGAP, 2018a). The main factor that allowed the expansion of forest plantations with these mentioned species was the increase capacity in the annual supply of seedlings from high productive genotypes, which are generated mostly by techniques of mini and micro propagation, allowing the production of cloned plants in a commercial scale (Souza et al., 2013; MGAP, 2018b). The vegetative propagation of seedlings in *Eucalyptus* gender is realized by the confection of mini cuttings generated from tissues of young branches (stem) from donators plants (mother plants), which are cultivated in greenhouses aiming the induction of adventitious roots development from undifferentiated cells (Hackett y Murray, 1993; Xavier et al., 2001; Souza et al., 2013).

In this context, the *Eucalyptus dunnii* is a specie of special interesting due of its high growth capacity, high level of breeding, favorable chemical wood composition for pulp and paper process and its moderated cold resistance (Thomas, 2009). On the other hand, the specie main limitation is the low rooting level, and the high technological nursery structure demanded for obtaining high performance results, what is a risk for increasing the production cost (Brondani et al., 2012).

In mini cuttings vegetative propagation from species of *Eucalyptus*, the formation and development of adventitious roots is a result of complex internal process, dependent specially of genetic constitution, nutritional state, hormonal balance and life time (Ruedel et al., 2013). In this context, the internal conditions of mini cuttings are also influenced by external factors (environment), as temperature and relative air humidity, radiation levels, light quality and, diseases and plagues (Da Costa et al., 2013). In adventitious roots development, Kevers et al. (1997) describe three stages processes: (*i*) induction: stage characterized by scarce visible cellular divisions and the increase of jasmone acid levels, phenolic compounds and auxins concentration in bottom of cells from cuttings, what generates a reprograming of target cells for the formation of new meristems. In the *Eucalyptus* gender this stage generally lasts up to a period of 96 hours by

the moment of cutting abscission, counting with an initial phase (0 – 24 h) and a late phase (24 – 96 h); (*ii*) initiation: phase when the cell division process begins, and meristematic development and primordium root appearance occur, usually accompanied by diminutions in internal auxin concentrations; (*iii*) expression: the root primordial differentiation in radicle starts generally at fifteenth day from mini cutting confection, with strong actuation of ethylene and gibberellins hormonal groups in cellular elongation (Schwambach et al., 2005; Brondani et al, 2012; Da Costa et al., 2013). Currently, the investigation lines emphasis the elucidation of the complex process of adventitious root formation and the interaction between the acting factors, as well as in verifying the results of treatments (hormonal, nutritional and environmental) over process phases (Ruedell, et al., 2013; Negishi et al., 2014; Druege et al., 2019).

In the last years, the evaluation of responses by the use of biostimulators in forest seedlings has been increased, many of them in *Eucalyptus* gender. These kinds of products can be applied through foliar applications or in substrates for improving chemical and physical properties (Melo et al., 2017; Silva, 2017; Goelzer, 2018; Morais et al., 2018; Ozyhar et al., 2019). In this context, the products Penergetic® P, B y K have been used with the objective of improving substrate properties and increasing growth and productivity in plants, constituting an alternative for reducing the use of chemical inputs in the vegetal production (Jakienė et al. 2008; Brito et al., 2012; Cobucci et al., 2015; Souza et al., 2017). According the fabricant (Penergetic, 2015) the referred products correspond to a bentonite clay treated with electromagnetic micro waves, composed by: SiO_2 56%; Al_2O_3 16%; Fe_2O_3 4%; K_2O 2%; Na_2O 0,4%; CaO 4%; MgO 4%; humidity content 13,6%.

The bentonites are constituted by negative charged aluminosilicates, formed in tridimensional arrays, with highly reduced particle size (diameter < 2 µm), which are able to absorb cations from soil/substrate solution (Uddin, 2017; Kontsur et al., 2017). The effects of bentonites addition to solutions are reported as generating an increase in contact surface of absorbents compounds, increasing also the pore space for a fixed volume and the capacity of ion adsorption (Subannaji, 2016). These group of clays can receive specific treatments to modified/improve its properties, specially the adsorption capacity and zeta potential, what can be realized by different physical and chemical techniques (Li et al., 2006; Pandey, 2017). Among the methods of clay mineral treatments usually utilized, the electromagnetic radiation on bentonites presents a high potential

for improving the efficiency of ion adsorption. Usually, the electromagnetic micro wave generator works in frequency ranges between 950 up to 2,450 MHz, what are considered of ultra-high frequency. The literature reports values for these kind of treatments with intensities varying from 50 up to 1,000 W for around 5 to 30 minutes (Li et al., 2006; Subannaji, 2016). Petrovic et al. (2012) verified changes in textural properties of electromagnetic micro wave treated bentonites for periods between 5 and 21 minutes, and intensities varying between 63 and 172 W. The results obtained by the authors showed significative differences in specific surface area (110.80 – 156.50 $m^2\ g^{-1}$ y 63.4 $m^2\ g^{-1}$), total pore volume (0.103 – 0.170 $cm^3\ g^{-1}$ y 0.028 $cm^3\ g^{-1}$) and crystalized size (15.26 – 19.21 µm y 21.49 µm) for minimum, maximum treatments and control, respectively. The same authors reported an increase in the bentonite soluble cation content in the following order: $Ca^{2+} < Na^{+} < Mg^{2+} < Fe^{2+/3+} < Al^{+3} < K^{+} < Si^{4+}$.

Studies conducted in *Eucalyptus urophylla* x *Eucalyptus grandis* hybrid clones have demonstrated positive response by Penergetic® P applications in 4 g L^{-1} doses, reflected in radicular system increasing and seedlings height (Filho et al., 2017). Nevertheless, is scarce the disponible information about the action on *Eucalyptus dunnii*, as well as the interaction between Penergetic P® y Penergetic B® in seedlings production. On the other hand, the use of Penergetic K® for promoting the soil/substrate microbial activity was verified by Muneeswari et al. (2018). The authors obtained increased effects on morphological (root and aerial length, humid and dry weight) and physiological variables (chlorophyll total content, carotenoids, anthocyanins, carbohydrates, foliar nitrogen, amino acids, soluble proteins) on *Abelmoschus esculentus*. The use of the same product on potted seedlings of *Campomanesia adamantium* (Cambess.) resulted in the increase of leaf content for N, P, Ca, Mg, Cu, Mn y Zn at 180 days of cultivate (Goelzer, 2018). In the same study was also evidenced changes in substrates chemical attributes, with elevation of pH, P, Ca, Mg, Cu, Mn, Fe, Zn, SB, cation exchange capacity (CEC) and the relation C/microbial biomass and C/CO_2 breathed, beyond the reduction on the K and organic matter content. According the author, these results indicate a promotion of substrate microbial activity, which tends to decompound the high levels of organic matter present in the utilized hummus, generating the nutrient release.

The demand for *Eucalyptus dunnii* genotypes that could respond increasing rooting levels and seedling quality by the application of treatments on commercial scales is a priority for the expansion of forest productive capacity and reducing the planting costs.

2. Objectives

2.1. General

Evaluate the effect of Penergetic P® and Penergetic B® on the survival and quality of clonal seedlings from two *Eucalyptus dunnii* genotypes and one inter-specific hybrid of *Eucalyptus grandis* x *Eucalyptus maidenii*.

2.2. Specifics

- Evaluate the effect of the products applications over morphological variables: survival, height, root collar diameter, number of plants with expedition height, leaf area, biomass (roots, stem, leaves and total) and, seedling quality indexes;
- Determinate changes on foliar nutrient concentration (N, P, K, Mg, Ca, Fe, Zn, Mn and Cu);
- Determinate changes on the photosystem II quantic efficiency (Fv/Fm).

3. Material and methods

3.1. Study area characterization

The experiment was conducted at Montes del Plata enterprise nursery, situated at rural area of Fray Bentos city, Río Negro department, with geographic coordinates of 33 º 12’ S y 58 º 19’ O. The experiments were implanted at 11/18/2019 and finalized at 03/06/2020 and 04/16/2020 for *Eucalyptus grandis* x *Eucalyptus maidenii* y *Eucalyptus dunnii* clones, respectively. The greenhouses utilized are destinated to research and experimentation, which possess controlled internal environment by temperature and humidity sensors. These conditions are automatically adjusted, according the weather conditions and objectives of each greenhouse, by opening and closing lateral and superior apertures and using foggers to increase the internal air relative humidity.

In this study, the mini cutting rooting process followed the structure: (*i*) vegetative greenhouse: destinated to promote the formation of adventitious roots, with time of residence approximately to 30 days. The environment is characterized by a range of air temperature between 25 and 30 ºC and air relative humidity over 80 %; (*ii*) growth greenhouse: used for vegetative seedling development of those who had formed roots in the previous stage, counting with major variations on temperature and air relative humidity; (*iii*) acclimation greenhouse: environment with controlled irrigation destined for the last 15 to 20 days of seedlings production process, aiming the acclimation to field conditions. The mean values of temperature and air relative humidity for the study period are presented in the Table 1.

Table 1. Mean temperature and air relative humidity.

Greenhouse	Month	Mean air temperature (°C)	Mean air relative humidity (%)
Vegetative	November	24.66	82.76
Vegetative	December	25.03	90.07
Growth	November	23.54	63.30
Growth	December	22.83	62.34
Growth	January	23.80	51.61
Growth	February	23.72	63.46
Growth	March	22.91	72.79
Growth	April	16.51	77.09

3.2. Experimental design

In this study were evaluated three genetic materials, two of them corresponding to *Eucalyptus dunnii* clones and one of *Eucalyptus grandis* x *Eucalyptus maidenii* inter-specific hybrid. Each material was evaluated as a separated experiment, with a completed random design, counting with three repetitions, and twenty-seven plants per plot, totalizing eight hundred and ten individuals per experiment. The treatments were arranged in a complete factorial design, compound by ten combinations, with Penergetic B® in two levels (0 y 0,5 g L^{-1}) and Penergetic P® with five levels (0, 1, 1,5, 2 y 4 g L^{-1}).

The application of 0,5 g L^{-1} Penergetic B® on the substrate was carried out previously to the mini cuttings staked, with a volume of 10 mL per plant. The doses of Penergetic P® were applied at seventh day since the staked, and then systematically each fifteen days. The foliar applications were realized using a manual sprayer, with 50 mL of solutions per plot. The volume applied was

corrected by the number of survival plants at each aspersion date. The variables height and root collar diameter were evaluated systematically every fifteen days since 01/17/2020. The remain variables were considered at the final evaluation time.

3.3. Seedling quality indexes

The indexes considered were the following: sturdiness quotient (SQ) (1), leaf area / total biomass (Fa/TB) (2), aerial biomass / radicular biomass (AB/RB) (3) and, Dickson quality index (DQI) (4):

$$SQ = \frac{Height\ (cm)}{Root\ collar\ diameter\ (mm)} \textbf{ (1)}$$

$$LA/TB = \frac{Leaf\ area\ (cm^2)}{Total\ biomass\ (g)} \textbf{ (2)}$$

$$AB/RB = \frac{Aerial\ biomass\ (g)}{Radicular\ biomass\ (g)} \textbf{ (3)}$$

$$DQI = \frac{Total\ biomass\ (g)}{\frac{Height\ (cm)}{Root\ collar\ diameter\ (mm)} + \frac{Aerial\ biomass\ (g)}{Radicular\ biomass\ (g)}} \textbf{ (4)}$$

3.4. Chemical analysis

The foliar samples were milled up to a particle size < 0,5 mm. After that, it was added 1 g of this material in a porcelain crucible and mineralized in a muffle furnace at 555 °C during 5 hours. Posteriorly, the ashes were dissolved with HCl at 10 %. The resulting extract was analyzed for P by colorimetric techniques, and for Ca, Mg, Fe, Cu, Mn y Zn by atomic absorption and, K by emission. Finally, the N content was determined by a Kjeldahl distillation, after a mineralization with H_2SO_4 at 350 °C and a mix of catalysts ($CuSO_4$ y K_2SO_4) during 90 minutes.

3.5. Statistical analysis

The variables evaluated along the experiments were analyzed with linear mixed models (LMM) (5):

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + Z_k + \varepsilon_{ijk} \textbf{ (5)}$$

Where: y_{ijk}: is the dependent variable; μ: the general mean; α_i: the fixed effect for application of Penergetic B®; β_j: the fixed effect for application of Penergetic P®; $\alpha\beta_{ij}$: the fixed effect for interaction between the referred treatments; Z_k: the random effect of the plot and; ε_{ijk}: the random error.

In all cases it was verified the assumptions of normality and variance homoscedasticity, modeling the variance structure with varIdent function when it was necessary. Considering the data characteristic, it was included a first order autoregressive (AR 1) due the serial time correlation existing between the measures in the data of each plot. The inclusion of a random effect factor in the model responds that the measures were repeated at the same plot.

The analysis of variables evaluated at the end of the experiment were conducted through an analysis of variance (ANOVA) (6). When the assumption of variance homoscedasticity was not reached it was adjusted a generalized least squares linear model (GLS), modelling the variance by varIdent function. For continuous variables without normal distribution it was adjusted a generalized linear model (GLM) with a Gamma probability distribution and a Log type link function (7). The variable number of plants with expedition height was analyzed by a GLM with a Poisson distribution and a Log link function (7). In the case of survival, the GLM analysis were carried out with a Binomial family distribution and a Logit link function (8).

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk} \ (\mathbf{6})$$

$$y_{ij} = e^{\mu+\alpha_i+\beta_j+\alpha\beta_{ij}} + \varepsilon_{ij} \ (\mathbf{7})$$

$$y_{ij} = \frac{e^{\mu+\alpha_i+\beta_j+\alpha\beta_{ij}}}{1+e^{\mu+\alpha_i+\beta_j+\alpha\beta_{ij}}} + \varepsilon_{ij} \ (\mathbf{8})$$

Where: y_{ijk}: is the dependent variable; μ: the general mean; α_i: the effect for Penergetic B® application; β_j: the effect for Penergetic P® application; $\alpha\beta_{ij}$: the effect for interaction between the referred treatments and; ε_{ijk}: the random error.

In all cases it was considered a significant p-value lower than 0.05, as well as for differences between the treatments by multiple mean comparison through LSD Fisher. In addition, it was

conducted a comparison by contrasts, following this structure: i) control vs foliar application (Penergetic P®); ii) control vs substrate application (Penergetic B®); iii) control vs the mean of both treatments. The residuals normality was tested through Shapiro-Wilks modified by Mahibbur and Govindarajulu (1997). On the other hand, the variance homoscedasticity was evaluated by Levene test. The mentioned analyses were realized in R® (3.5.3) through the Infostat® Version 9 graphic interface.

4. Results and discussion

4.1. Genetic material 1 (E. grandis x E. maidenii)

4.1.1. Root collar diameter, height, number of plants with expedition height and survival

Considering the temporal analysis for root collar diameter and height, it was verified significative effects in both variables for the interaction between Penergetic B® and evaluation time (LMM, p-valor = 0.0239 y p-valor = 0.0055, respectively). At 96 days since the staked, plants treated with Penergetic B® presented a root collar diameter mean 0.3 mm higher than the plants without the referred treatment (Figure 1). This result indicates a possible precocity in seedlings morphological development, although this difference is diluted to the end of experiment.

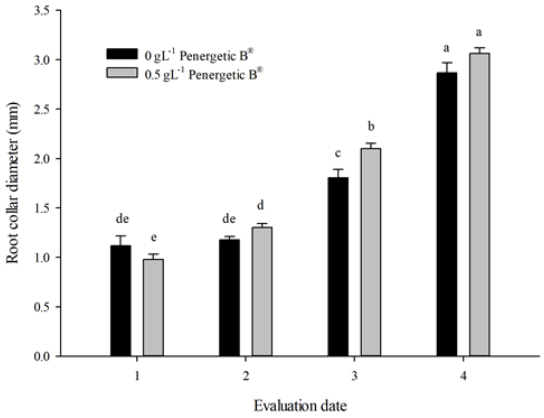

Figure 1. Evolution of root collar diameter at each evaluation date for plants with and without Penergetic B® application. Different letters show significative differences by LSD Fisher (p-value < 0.05).

The mean comparison for height (Figure 2) shows differences at the final date of evaluation (110 days) where plants treated with Penergetic B® were significatively higher (2.23 cm). In addition, in this date, non-treated plants had an equivalent mean to those at 96 days (third evaluation date) who received Penergetic B® application. The result suggests a reduction of 14 days in the seedlings morphological development and a consequent reduction in the production time.

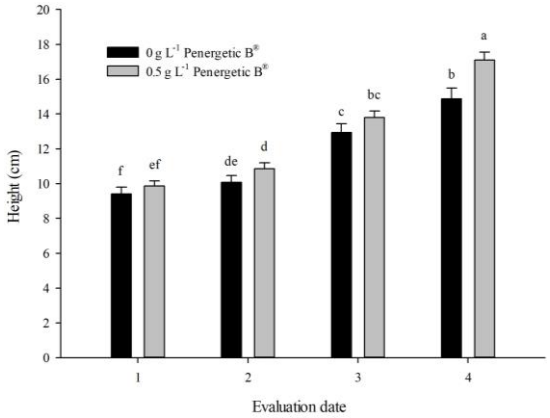

Figure 2. Evolution of height for each evaluation date for plants with and without Penergetic B® application. Different letters show significative differences by LSD Fisher (p-value < 0.05).

Following the previous results, the variable number of plants with expedition height (> 20 cm) at 110 days, showed a significative effect for Penergetic B® application on the substrate (GLM, p-value < 0.0001). The mean comparison presented an increase of 4 plants with expedition height (15 %) due the referred treatment (Figure 3).

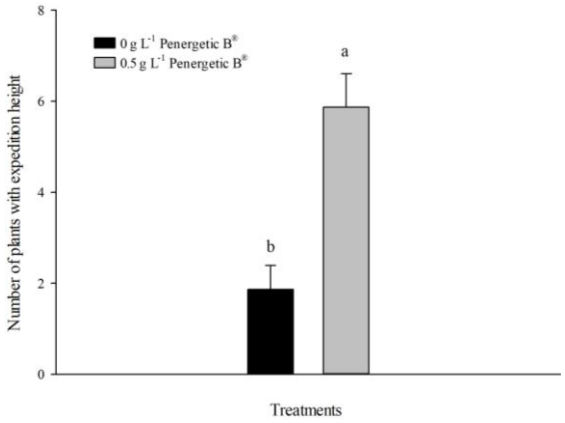

Figure 3. Number of plants with expedition height with and without Penergetic B® application. Different letters show significative differences by LSD Fisher (p-value < 0.05).

The hypothesis test for plant survival showed an effect for interaction (Figure 4) between the Penergetic B® and Penergetic P® applications (GLM, p-value = 0.0142).

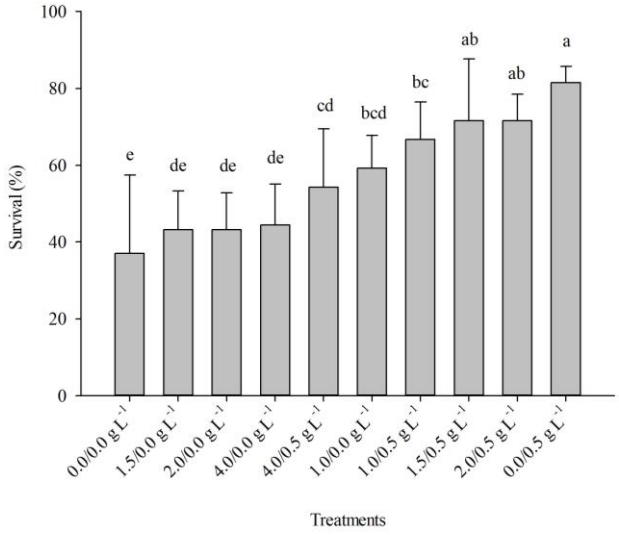

Figure 4. Survival for treatment combinations at the experiment final evaluation. Different letters show significative differences by LSD Fisher (p-value < 0.05).

The comparison between the control versus 0.0:0.5 g L^{-1} for Penergetic P® and Penergetic B®, respectively, indicated an increase of 44 % in survival for this treatment. For the combinations 2.0:0.5 g L^{-1} vs 2.0:0.0 g L^{-1} y 1.5:0.5 g L^{-1} vs 1.5:0.0 g L^{-1}, the mean difference respect to control was about 29 %. These results suggest an effect in the number of live plants at the end of experiment by the Penergetic B® application.

In the sequence, the results obtained are presented for mean comparison through contrasts (Table 2). The root collar diameter did not present significative difference in any case, what is in accordance with temporal analysis. The height evaluation at expedition moment (110 days) presented an effect for Penergetic B® treatment respect to control. Considering the contrast between control (C) and foliar application (FA) it was not verified an effect of this product for the mean of used doses on plants height. The number of plants with expedition height evaluation indicated an effect for substrate treatment (Penergetic B®). In this case the variable was 7,4 % higher than the control.

Table 2. Contrasts for genetic material 1.

		C	FA	SA	FA+SA
Root collar diameter (mm)	Mean	2.67	2.91	3.19	3.00
	E. E.	0.31	0.11	0.06	0.06
	p-value		0.4260	0.0990	0.2518
Height (cm)	Mean	13.11	15.30	17.90	16.30
	E. E.	2.06	0.58	0.46	0.40
	p-value		0.1473	**0.0171**	**0.0307**
Number of plants with expedition height (n)	Mean	1	2	7	4
	E. E.	0.88	0.63	0.74	0.62
	p-value		0.5456	**0.0024**	0.0600
Survival (n)	Mean	10	13	22	16
	E. E.	5.51	1.25	1.33	1.07
	p-value		0.0916	**<0.0001**	**0.0001**

C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

Finally, survival presented a clear effect for substrate application (81 %) respect to control (37 %). For the treatments (FA+SA) there was a difference (59 %) when compared to control.

4.1.2. Biomass, leaf area, Fv/Fm and quality indexes

Respect to the hypothesis test for the treatment combinations it was not observed significative differences except to stem biomass by Penergetic B® application (ANOVA, p-value = 0.0391). Comparing the means for substrate product to those without this application, it was found an increase of 20 % in stem weight (Figure 5).

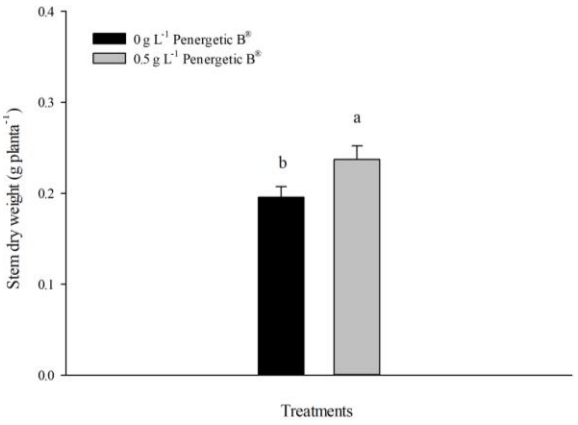

Figure 5. Stem dry weight with and without Penergetic B® application. Different letters show significative differences by LSD Fisher (p-value < 0.05).

In Table 3 it is presented the results for the mean dry weight of considered compartments. The variables did not show differences respect to control.

Table 3. Mean dry weight per plant contrasts.

		C	FA	SA	FA+SA
			g		
LB	Mean	0.58	0.45	0.51	0.49
	E. E.	0.10	0.03	0.03	0.02
	p-value		0.1409	0.4858	0.2863
SB	Mean	0.22	0.19	0.27	0.22
	E. E.	0.01	0.01	0.01	0.01
	p-value		0.3627	0.2783	0.8882
RB	Mean	0.26	0.19	0.22	0.21
	E. E.	0.08	0.03	0.02	0.02
	p-value		0.2046	0.5823	0.3006
AB	Mean	0.80	0.64	0.77	0.71
	E. E.	0.11	0.05	0.04	0.03
	p-value		0.1596	0.8508	0.3910
TB	Mean	1.06	0.83	1.00	0.92
	E. E.	0.16	0.07	0.06	0.05
	p-value		0.1522	0.7429	0.3355

LB = leaf biomass; SB = stem biomass; RB = root biomass; AB = aerial biomass; TB = total biomass; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

The variables leaf area and Fv/Fm were not different for the effects model and contrasts (Table 4).

Table 4. Mean leaf area and Fv/Fm contrasts.

		C	FA	SA	FA+SA
LA (cm^2)	Mean	73.19	68.66	66.79	70.88
	E. E.	12.29	3.82	3.64	2.62
	p-value		0.6394	0.6014	0.7997
Fv/Fm	Mean	0.80	0.75	0.75	0.76
	E. E.	0.02	0.01	0.01	0.01
	p-value		0.1172	0.2012	0.1754

LA = leaf area; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

For the quality indexes, in general, it was not observed differences in hypothesis test, except for sturdiness quotient in which there was an effect due the use of Penergetic B® (ANOVA, p-value = 0.003). The mean values were 5.57 and 5.18 for plants with and without the treatment, respectively (Figure 6).

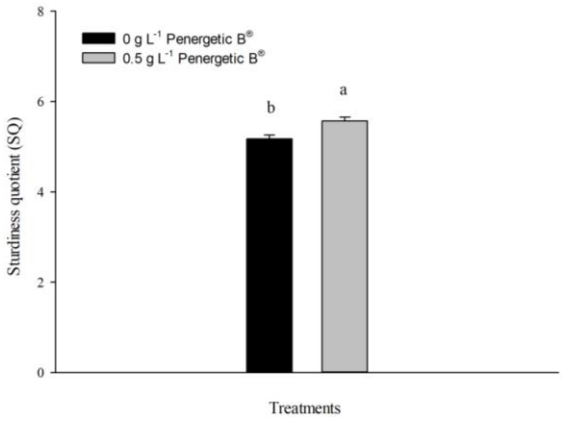

Figure 6. Sturdiness quotient (SQ) with and without Penergetic B® application. Different letters show significative differences by LSD Fisher (p-value < 0.05).

The results showed that the application allowed obtaining plants with sturdiness quotient closer to the adequate range for the variable, which is reported between 6 and 10 (Silveira et al., 2001). Considering that there was a difference for height by the use of Penergetic B® respect to control, and the same effect was not verified for root collar diameter, it can be assumed that the higher value for SQ is due for an increase in height, without a decrease in root collar diameter, obtaining more balanced plants. The same tendency was verified for the contrast's analysis, where the only index that presented differences was the SQ (Table 5). The differences observed were given by Penergetic B® and Penergetic B® in combination with Penergetic P®.

Table 5. Plant quality indexes contrasts.

		C	FA	SA	FA+SA
SQ	Mean	4.85	5.26	5.61	5.43
	E. E.	0.27	0.08	0.08	0.06
	p-value		0.0664	**0.0095**	**0.0078**
LA/TB	Mean	71.23	85.29	67.18	79.86
	E. E.	12.60	3.35	3.17	2.45
	p-value		0.1680	0.7570	0.3721
AB/RB	Mean	3.46	3.81	3.49	3.70
	E. E.	0.74	0.34	0.18	0.18
	p-value		0.5683	0.9721	0.6772
DQI	Mean	0.13	0.09	0.11	0.10
	E. E.	0.03	0.01	0.01	0.01
	p-value		0.1101	0.3788	0.1694

SQ = sturdiness quotient; LA/TB = leaf area/total biomass; AB/RB = aerial biomass/root biomass; DQI = Dickson quality index; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

4.1.3. Leaf nutrient concentration

The results showed no significative differences for the main nutrient concentrations, except for K and Cu. For K there was an effect in concentration resulted by foliar application of Penergetic P® (ANOVA, p-value = 0.0303). For the mean comparison, the doses applied 1, 2 y 4 g L^{-1} had a higher K concentration compared to control, assuming an intermedium value for 1.5 g L^{-1} dose (Figure 7). On the other hand, the Cu concentration presented an effect by substrate product application (GLS, p-value = 0.0381), assuming concentrations of 21.61 mg kg^{-1} and 16.80 mg kg^{-1} with and without the referred treatment, respectively.

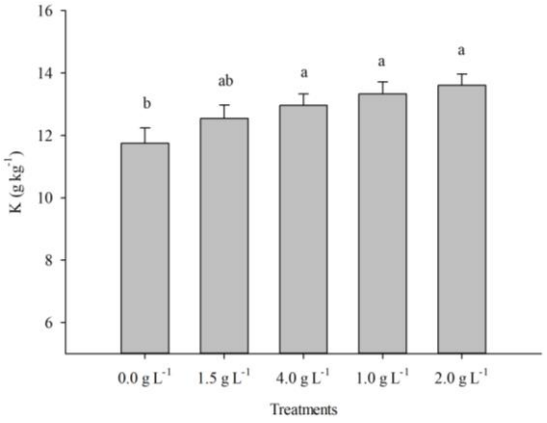

Figure 7. K leaf concentration for doses of Penergetic P®. Different letters show significative differences by LSD Fisher (p-value < 0.05).

Considering the adequate range for K leaf concentration in *Eucalyptus sp.* seedlings at expedition phase (15 – 20 g kg^{-1}), it was observed that all foliar treatments including the control were out of the referred range, while the foliar application moved the concentrations closer to it. In the case of Cu, the adequate range is situated between 10 and 15 mg kg^{-1} (Silveira et al., 2001) what indicates that treated seedlings had concentrations closer to this range. In the sequence it is presented (Table 6) the mean comparison of macro nutrients by contrasts.

Table 6. Macro nutrient concentration contrasts.

		C	FA	SA	FA+SA
			g kg $^{-1}$		
N	Mean	7.59	8.06	7.59	7.98
	E. E.	0.38	0.13	0.17	0.11
	p-value		0.2197	0.9944	0.2800
P	Mean	2.51	2.86	2.94	2.91
	E. E.	0.26	0.06	0.08	0.05
	p-value		0.0737	0.0802	**0.0358**
Ca	Mean	8.15	9.63	9.34	9.69
	E. E.	0.22	0.19	0.20	0.14
	p-value		**0.0055**	0.0612	**0.0026**
Mg	Mean	5.51	6.13	6.09	6.13
	E. E.	0.06	0.10	0.09	0.07
	p-value		**0.0156**	0.0657	**0.0106**
K	Mean	11.06	13.08	12.43	13.04
	E. E.	0.86	0.27	0.25	0.18
	p-value		**0.0046**	0.1015	**0.0034**

FA = foliar application (Penergetic® P); SA = substrate application (Penergetic® B); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3).
Bold values indicate significant differences (p-value < 0.05).

In the comparison by contrasts, P concentration showed significant differences for foliar and substrate means, respect to control. In case of Ca, the concentration was superior for the group of foliar and substrate treatments compared to control. The values obtained for all combinations are in the adequate range reported for this nutrient (8 – 12 g kg^{-1}) (Silveira et al., 2001). For Mg the results presented differences to the mean concentrations of foliar treatments and the mean of substrate and foliar applications together, respect to control. The range for adequate concentrations for this nutrient varies between 3 and 3.5 g kg^{-1} (Silveira et al., 2001). Although, Camargo (1997) obtained similar concentrations from those reported in this study, varying between 5.8 and 6.5 g kg^{-1} for *Eucalyptus grandis* seedlings at 90 days. The elevated concentrations observed is attributed to the high vermiculate contents used in the substrates (Camargo, 1997).

Finally, K concentration showed an increase of 2.02 g kg^{-1} respect to control for plants treated with Penergetic P® and, of 1.98 g kg^{-1} for those treated with foliar and substrate products. In the sequence, Table 7 presents the contrasts for micro nutrients analysis.

Table 7. Micro nutrient concentration contrasts.

		C	FA	SA	FA+SA
		mg kg $^{-1}$			
Fe	Mean	414.54	488.68	460.46	416.24
	E. E.	33.40	13.19	15.22	10.33
	p-value		0.0659	0.3521	0.1014
Cu	Mean	30.43	19.41	16.61	17.96
	E. E.	9.39	1.02	0.56	0.60
	p-value		0.1160	0.1569	0.0777
Zn	Mean	55.46	60.17	56.36	59.02
	E. E.	1.80	0.88	1.25	0.81
	p-value		0.1023	0.7976	0.1849
Mn	Mean	67.27	73.76	77.88	73.10
	E. E.	2.20	2.33	1.90	1.45
	p-value		0.1919	0.0928	0.2057

FA = foliar application (Penergetic® P); SA = substrate application (Penergetic® B); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

4.2. Genetic material 2 (Eucalyptus dunnii)

4.2.1. Root collar diameter, height, number of plants with expedition height and survival

The temporal analysis for root collar diameter and height did not show differences for the first, while the second presented a significant effect (LMM, p-value = 0.0075) for interaction between foliar, substrate and evaluation date. The number of plants with expedition height did not show responses with treatments for hypothesis test. In case of survival a significant effect was verified (GLM, p-value = 0.0266) for the interaction between both products (Figure 8).

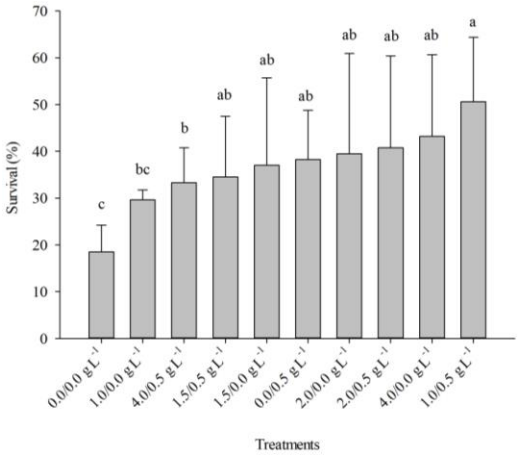

Figure 8. Survival for treatment combinations at the experiment final evaluation. Different letters show significative differences by LSD Fisher (p-value < 0.05).

The control presented a mean value 21 % lower than the mean for the rest of treatments (40 %), excepting the 1.0:0.0 g L^{-1} combination, as presented in the figure above.

In relation to the analysis by contrasts (Table 8) the results indicate no differences for root collar diameter and height, as well as verified in the hypothesis test. Even though, the number of plants with expedition height presented an increase of 22, 15 and 19 % respect to control for foliar, substrate and both applications, respectively.

Meanwhile, for survival the results from contrasts analysis followed the same tendencies reported in hypothesis test, showing a raise of 19 % respect to control for all cases. Even though, it was not verified differences for the mean root collar diameter and seedling height.

Table 8. Contrasts for genetic material 2.

		C	FA	SA	FA+SA
Root collar diameter (mm)	Mean	3.69	3.57	3.03	3.36
	E. E.	0.16	0.13	0.19	0.12
	p-value		0.7825	0.2285	0.4191
Height (cm)	Mean	24.58	26.72	24.01	25.09
	E. E.	2.64	0.73	1.18	0.77
	p-value		0.4955	0.8881	0.8677
Number of plants with expedition height (n)	Mean	3	9	7	8
	E. E.	0.63	1.83	1.48	1.14
	p-value		**0.0048**	**0.0387**	**0.0050**
Survival (n)	Mean	5	10	10	10
	E. E.	1.53	1.96	1.44	1.16
	p-value		**0.0019**	**0.0061**	**0.0007**

C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

4.2.2. Biomass, leaf area, Fv/Fm and quality indexes

The variables related to biomass did not show significant differences for the treatments, as well as obtained by contrasts analysis (Table 9).

Table 9. Mean dry weight per plant contrasts.

		C	AF	AS	AF+AS
			g		
LB	Mean	1.05	1.38	0.91	1.35
	E. E.	0.17	0.29	0.26	0.19
	p-value		0.3763	0.6189	0.2888
SB	Mean	0.47	0.67	0.40	0.65
	E. E.	0.45	1.07	1.04	0.73
	p-value		0.3031	0.5027	0.1818
RB	Mean	0.44	0.60	0.31	0.57
	E. E.	0.04	0.13	0.11	0.08
	p-value		0.4626	0.4146	0.5678
AB	Mean	1.52	2.05	1.31	2.00
	E. E.	0.21	0.45	0.41	0.30
	p-value		0.4250	0.7304	0.4875
TB	Mean	1.96	2.65	1.62	2.57
	E. E.	0.24	0.58	0.52	0.38
	p-value		0.4309	0.6532	0.5024

LB = leaf biomass; SB = stem biomass; RB = root biomass; AB = aerial biomass; TB = total biomass; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

The variables leaf area and Fv/Fm did not show differences for the treatments in the hypothesis test and for contrasts either (Table 10).

Table 10. Mean leaf area and Fv/Fm contrasts.

		C	AF	AS	AF+AS
LA (cm^2)	Mean	73.19	68.66	66.79	70.88
	E. E.	12.29	3.82	3.64	2.62
	p-value		0.6394	0.6014	0.7997
Fv/Fm	Mean	0.80	0.75	0.75	0.76
	E. E.	0.02	0.01	0.01	0.01
	p-value		0.1172	0.2012	0.1754

LA = leaf area; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

On the other hand, the Table 11 contains the results obtained for seedling quality indexes considered. The Penergetic B® application on the substrate resulted in a significative response for sturdiness quotient. This result is consistent with the obtained for genetic material 1 (*E. grandis* x *E. maidenii*). The values are found in the adequate range for the variable (6 – 10) (Silveira et al., 2001).

Table 11. Plant quality indexes contrasts.

		C	FA	SA	FA+SA
SQ	Mean	6.64	7.57	8.14	7.55
	E. E.	0.47	0.25	0.16	0.14
	p-value		0.1051	**0.0463**	0.0949
LA/TB	Mean	22.96	23.73	27.14	23.35
	E. E.	1.63	1.46	1.02	0.84
	p-value		0.7292	0.0967	0.8382
AB/RB	Mean	3.41	3.51	4.96	3.64
	E. E.	0.26	0.15	0.24	0.15
	p-value		0.8984	0.1500	0.7596
DQI	Mean	0.19	0.25	0.13	0.24
	E. E.	0.02	0.06	0.04	0.04
	p-value		0.4401	0.2816	0.4229

SQ = sturdiness quotient; LA/TB = leaf area/total biomass; AB/RB = aerial biomass/root biomass; DQI = Dickson quality index; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

4.2.3. Leaf nutrient concentration

The nutrient concentration results did not present significant differences for the contrasts considered (Table 12 and 13) and for hypothesis tests either.

Table 12. Macro nutrient concentration contrasts.

		C	FA	SA	FA+SA
				g kg^{-1}	
N	Mean	6.71	6.22	6.09	6.17
	E. E.	0.09	0.12	0.17	0.11
	p-value		0.1304	0.1098	0.0747
P	Mean	1.46	1.44	1.43	1.43
	E. E.	0.12	0.05	0.03	0.03
	p-value		0.8834	0.8262	0.8329
Ca	Mean	8.50	8.30	8.80	8.27
	E. E.	0.17	0.41	0.12	0.19
	p-value		0.7895	0.6604	0.7396
Mg	Mean	5.17	5.12	5.26	5.10
	E. E.	0.10	0.19	0.05	0.09
	p-value		0.8788	0.7719	0.8267
K	Mean	14.89	14.80	13.87	14.54
	E. E.	0.76	0.42	0.30	0.25
	p-value		0.9251	0.3851	0.6895

FA = foliar application (Penergetic® P); SA = substrate application (Penergetic® B); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

Table 13. Micro nutrient concentration contrasts.

		C	FA	SA	FA+SA
		mg kg^{-1}			
Fe	Mean	425.42	484.97	478.33	487.7
	E. E.	7.21	22.15	10.29	11.14
	p-value		0.0860	0.2192	0.0589
Cu	Mean	20.49	23.52	20.11	22.58
	E. E.	0.83	1.59	1.09	0.93
	p-value		0.1305	0.8751	0.2553
Zn	Mean	70.42	72.08	70.33	71.91
	E. E.	1.88	2.82	1.30	1.41
	p-value		0.5721	0.9756	0.5598
Mn	Mean	66.19	68.4	72.64	70.82
	E. E.	5.96	3.11	2.87	2.11
	p-value		0.7605	0.4839	0.4993

FA = foliar application (Penergetic® P); SA = substrate application (Penergetic® B); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

4.3. Genetic material 3 (Eucalyptus dunnii)

4.3.1. Root collar diameter, height, number of plants with expedition height and survival

In temporal analysis for root collar diameter and seedling height were verified, for the first, a significant effect for Penergetic B® application in interaction with evaluation time (LMM, p-value = 0.0106), while the second did not show effect. The mean comparison for the referred effect presented differences between treated and non-treated plants, at the second and third measure date, 67 and 81 days respectively, converging at the final of the experiment (Figure 9).

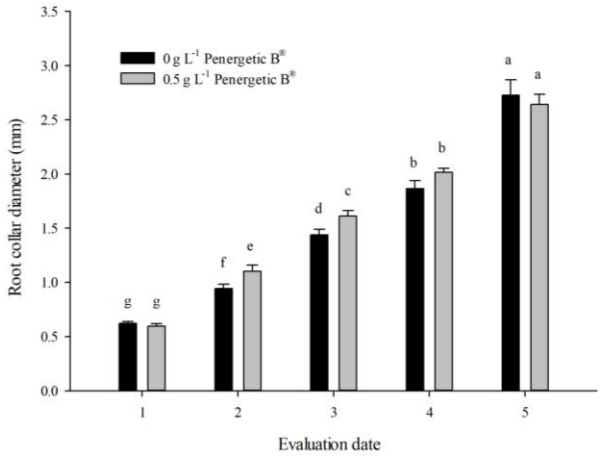

Figure 9. Evolution of root collar diameter for each evaluation date for plants with and without Penergetic B® application. Different letters show significative differences by LSD Fisher (p-value < 0.05).

The variable number of plants with expedition height presented significant effects for the foliar and substrate applications (GLM, p-value = 0.0227 and p-value = 0.0270, respectively), without effect for the interaction. The mean comparison (Figure 10) shows that foliar application, in the dose of 4 g L^{-1}, has generated a response of 13 % in the number of plants with expedition height, respect to treatments with no application. The application of Penergetic B®, in the dose of 0.5 g L^{-1}, increased the variable around 8 %.

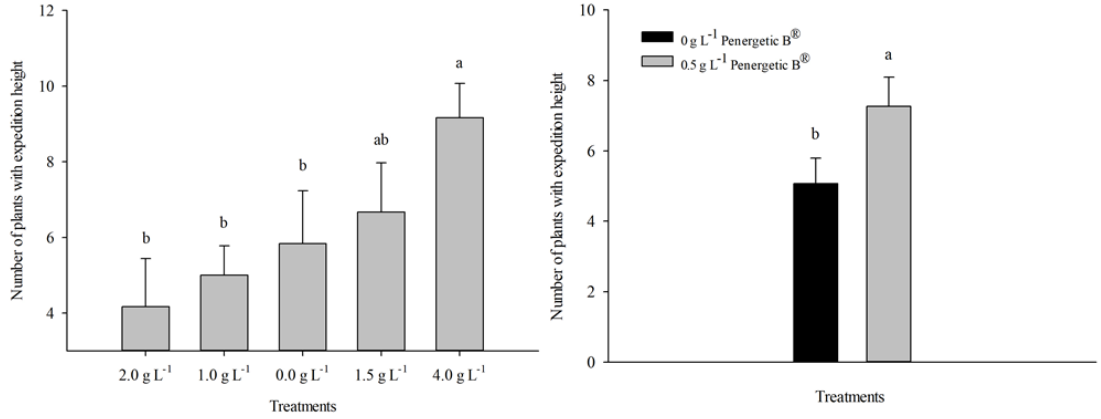

Figure 10. Number of plants with expedition height with and without Penergetic B® application (right) and for the different utilized doses of Penergetic P® (left). Different letters show significative differences by LSD Fisher (p-value < 0.05).

For survival it was obtained a significant response by the interaction of both treatments (GLM, p-value = 0.033), where the 4 g L^{-1} Penergetic P® dose was superior than control (Figure 11).

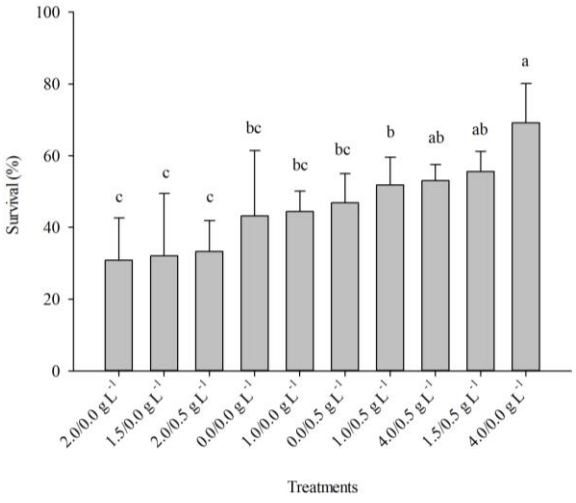

Figure 11. Survival for treatment combinations at the experiment final evaluation. Different letters show significative differences by LSD Fisher (p-value < 0.05).

The comparison by contrasts evidenced no differences between the treatments and the control (Table 14).

Table 14. Contrasts for genetic material 3.

		C	FA	SA	FA+SA
Root collar diameter (mm)	Mean	2.72	2.73	2.91	2.68
	E. E.	0.37	0.16	0.09	0.09
	p-value		0.9799	0.6500	0.8987
Height (cm)	Mean	20.86	20.16	21.40	20.98
	E. E.	3.23	1.03	0.72	0.61
	p-value		0.7592	0.8528	0.9578
Number of plants with expedition height (n)	Mean	4	5	7	6
	E. E.	0.67	0.90	0.83	0.63
	p-value		0.6947	0.1326	0.2745
Survival (n)	Mean	12	12	13	13
	E. E.	4.91	1.88	0.92	0.96
	p-value		0.9051	0.6358	0.6090

C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

4.3.2. Biomass, leaf area, Fv/Fm and quality indexes

The variables related to biomass did not present significant differences for evaluated treatments, as well as for the contrast analysis (Table 15).

Table 15. Mean dry weight per plant contrasts.

		C	FA	SA	FA+SA
			g		
LB	Mean	0.74	0.79	0.73	0.76
	E. E.	0.13	0.13	0.05	0.06
	p-value		0.8140	0.9617	0.9387
SB	Mean	0.34	0.34	0.32	0.33
	E. E.	0.07	0.04	0.02	0.02
	p-value		0.9821	0.8593	0.9326
RB	Mean	0.15	0.20	0.20	0.20
	E. E.	0.02	0.04	0.01	0.02
	p-value		0.1686	0.1462	0.1034
AB	Mean	1.08	1.14	1.05	1.09
	E. E.	0.20	0.17	0.07	0.08
	p-value		0.8442	0.9417	0.9641
TB	Mean	1.23	1.34	1.26	1.29
	E. E.	0.20	0.20	0.08	0.10
	p-value		0.7450	0.9491	0.8388

LB = leaf biomass; SB = stem biomass; RB = root biomass; AB = aerial biomass; TB = total biomass; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

The leaf area and Fv/Fm did not show differences for proved treatments either (Table 16).

Table 16. Mean leaf area and Fv/Fm contrasts.

		C	FA	SA	FA+SA
LA (cm^2)	Mean	30.33	35.77	34.28	36.79
	E. E.	5.68	4.75	3.47	2.81
	p-value		0.5883	0.7555	0.4966
Fv/Fm	Mean	0.79	0.77	0.78	0.75
	E. E.	0.01	0.01	0.03	0.02
	p-value		0.7685	0.9134	0.4970

LA = leaf area; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

The Table 17 presents the results obtained for the seedling quality indexes considered in this study. These indexes did not show significant differences for hypothesis test, as well as for the evaluated contrasts.

Table 17. Plant quality indexes contrasts.

		C	FA	SA	FA+SA
SQ	Mean	7.68	7.44	7.31	7.90
	E. E.	0.52	0.16	0.27	0.18
	p-value		0.6676	0.5936	0.6672
LA/TB	Mean	24.50	27.79	26.45	28.65
	E. E.	0.63	2.18	1.49	1.26
	p-value		0.4445	0.7179	0.3088
AB/RB	Mean	7.39	6.38	5.18	5.82
	E. E.	1.81	0.73	0.25	0.36
	p-value		0.4190	0.1708	0.1913
DQI	Mean	0.08	0.10	0.10	0.10
	E. E.	0.01	0.02	0.01	0.01
	p-value		0.4624	0.5961	0.5697

SQ = sturdiness quotient; LA/TB = leaf area/total biomass; AB/RB = aerial biomass/root biomass; DQI = Dickson quality index; C = control; FA = foliar application (Penergetic P®); SA = substrate application (Penergetic B®); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

4.3.3. Leaf nutrient concentration

In the macro nutrient (N, P, Ca, Mg y K) hypothesis analysis did not show treatment effects, although the evaluation by contrast (Table 18) evidenced differences for Mg and Ca. For the first, the mean of foliar treatments (5.55 g kg^{-1}), substrate application (5.58 g kg^{-1}) and the mean of both treatments (6.67 g kg^{-1}) were superior respect to control. All the obtained values are over the adequate range (3 – 3.5 g kg^{-1}) referred for *Eucalyptus* seedlings at expedition phase (Silveira et

al, 2001). In a similar study, Camargo (1997) verified Mg concentrations in between 5.8 – 6.5 g kg^{-1}, attributing the values due the high contents of vermiculate in the substrate. For Ca concentration was obtained higher levels by foliar applications (9.82 g kg^{-1}) and substrate and foliar applications (10.27 g kg^{-1}) respect to control (12.70 g kg^{-1}). The values for the treatments were found inside the adequate range reported (8 – 12 g kg^{-1}) (Silveira et al., 2001), although the control had a value slightly over this range.

Table 18. Macro nutrient concentration contrasts.

		C	AF	AS	AF+AS
				g kg^{-1}	
N	Mean	8.71	7.88	8.48	7.88
	E. E.	0.28	0.15	0.25	0.15
	p-value		0.5970	0.6456	0.5793
P	Mean	1.84	1.72	1.78	1.68
	E. E.	0.15	0.08	0.08	0.06
	p-value		0.5188	0.7948	0.3768
Ca	Mean	12.70	9.82	10.10	10.27
	E. E.	0.16	0.52	0.36	0.31
	p-value		**0.0129**	0.0828	**0.0296**
Mg	Mean	6.67	5.55	5.58	5.59
	E. E.	0.05	0.18	0.14	0.11
	p-value		**0.0096**	**0.0424**	**0.0083**
K	Mean	10.32	11.14	11.82	11.24
	E. E.	0.13	0.49	0.30	0.27
	p-value		0.8005	0.4173	0.6942

FA = foliar application (Penergetic® P); SA = substrate application (Penergetic® B); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

For the hypothesis test of micro nutrients, it was verified effects only for Fe, with an interaction between foliar and substrate treatments (ANOVA, p-value = 0.0130). The mean comparison indicates lower levels of this nutrient for the major part of treatments respect to control (Figure 12).

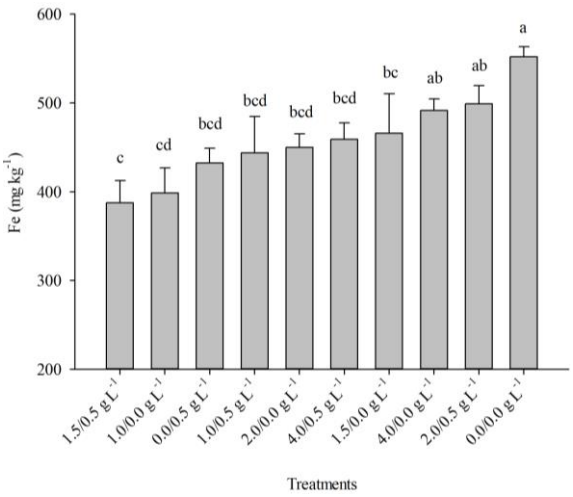

Figure 12. Fe leaf concentration for all treatments at the end of the study. Different letters show significative differences by LSD Fisher (p-value < 0.05).

The comparison by contrasts presented significant differences for Fe leaf concentrations, Cu and Zn (Table 19). All treatment combinations presented higher Fe values respect to the adequate range for *E. grandis* seedlings (80 – 130 mg kg^{-1}) (Silveira et al., 2001), as well as for those obtained by Navroski et al. (2015) in *E. dunnii* seedlings (137.82 mg kg^{-1}) and by Fernandes (2010) for *E. urophylla* (129.43 mg kg^{-1}). The concentrations obtained for this nutrient with foliar, substrate and with both applications, 451.62, 432.26 and 447.56 mg kg^{-1}, respectively, were all significative lower than the value verified to control (552.09 mg kg^{-1}).

Table 19. Micro nutrient concentration contrasts.

		C	FA	SA	FA+SA
		mg kg^{-1}			
Fe	Mean	552.09	451.62	432.26	447.76
	E. E.	11.36	15.79	13.81	10.22
	p-value		**0.0024**	**0.0038**	**0.0010**
Cu	Mean	17.64	31.14	30.41	30.31
	E. E.	0.91	1.74	2.74	1.69
	p-value		**0.0013**	0.0873	**0.0024**
Zn	Mean	76.73	68.45	71.18	69.13
	E. E.	1.10	1.63	1.65	1.15
	p-value		**0.0430**	0.2664	**0.0483**
Mn	Mean	57.24	55.36	56.86	55.73
	E. E.	0.06	2.63	1.80	1.51
	p-value		0.7106	0.9498	0.7507

FA = foliar application (Penergetic® P); SA = substrate application (Penergetic® B); FA+SA = foliar and substrate applications; E. E. = standard error (n = 3). Bold values indicate significant differences (p-value < 0.05).

The Cu concentration showed differences for foliar applications (31.14 mg kg^{-1}) and for the mean of all treatments (30.31 mg kg^{-1}), respect to control (17.64 mg kg^{-1}). All the obtained values were over the adequate range for this nutrient (10 – 15 mg kg^{-1}) (Silveira et al., 2001).

Finally, the Zn concentration for the control (76.73 mg kg^{-1}) was superior of those verified for foliar applications (68.45 mg kg^{-1}) and both treatments (69.13 mg kg^{-1}), where all values were over the reported adequate range (30 – 40 mg kg^{-1}) (Silveira et al, 2001).

5. Conclusions

The results obtained in this study allowed to conclude that the treatments had a positive influence on the seedlings survival, verifying effects for at least one of them in all genetic materials considered. Additionally, it can be assigned a high proportion of these responses by the use of Penergetic B® applied on the substrate, in the dose of 0.5 g L^{-1}, at the moment of staking the mini cuttings. The increases on seedlings survival varied between 19 and 44 % for the treatments, depending on the doses of each product and genetic material.

In relation to root collar diameter, the use of Penergetic B® produced an anticipated growth in two out of the three clones, even though, the mentioned effects were diluted at the end of the experiment. On the other hand, the seedlings height was positively influenced in the hybrid clone of *E. grandis x E. maidenii*. The plants of this material treated with Penergetic B® were higher in height, and presented an acceleration of growth, reducing around 15 days the production period.

For the number of plants with expedition height it was verified an effect for both treatments, Penergetic P® (for the *E. dunnii* clones) and Penergetic B® (for all genetic materials), and for the interaction between them (for genetic material 2). The results obtained suggests increments for this variable between 8 and 22 %.

The variables related to biomass, fluorescence and leaf area were not influenced by the treatments. Although, there was a response in the sturdiness quotient for the genetic material 1 (*E. grandis* x *E. maidenii*) and 2 (*E. dunnii*), resulting in plants more balanced at the center of the adequate range for the variable.

Respect to the nutrient leaf concentrations, the results were variables according to the treatments and genetic material considered, suggesting an interaction between them.

Finally, it is possible to conclude that the treatments produced consistent results for survival and number of plants with expedition height. For the rest of the evaluated variables, the responses depended on the treatment combinations and genetic material. These results indicate consistent responses for some variables and the existence of an interaction between the applications and the evaluated clones for others, what imply in doses responses no clearly determined being a potential objective for future evaluations.

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