

EFFECTS OF WATER MANAGEMENT AND COMPOSTED SEWAGE SLUDGE SUBSTRATES ON THE GROWTH AND QUALITY OF CLONAL *EUCALYPTUS* SEEDLINGS

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Resumo

Manejo hídrico e composto de lodo de esgoto como substrato no crescimento e qualidade de mudas clonais de eucalipto. O objetivo deste estudo foi avaliar o efeito de substratos a base de lodo de esgoto compostado and lâminas de irrigação sobre o crescimento and a qualidade de mudas clonais de *Eucalyptus grandis* x *E. urophylla*, a fim de otimizar o manejo hídrico para cada substrato. Os substratos testados foram lodo de esgoto compostado com bagaço de cana-de-açúcar (1:3 v:v); lodo de esgoto compostado com casca de eucalipto (1:3 v:v) and mistura destes substratos na proporção 2:1 (v:v). Como testemunha foi utilizado substrato comercial a base de turfa *Sphagnum*, vermiculita and casca de arroz torrefada (2:1:1 v:v:v). As lâminas diárias de irrigação aplicadas foram de 11, 14 and 17 mm, parceladas em duas vezes. Os substratos foram caracterizados física e quimicamente e, para determinar o crescimento and a qualidade das mudas, foram medidas as variáveis: altura, diâmetro do colo, massas secas aérea, radicular and total, bem como calculado o índice de qualidade de Dickson and avaliada a conformação do sistema radicular. Os substratos obtidos através da compostagem do lodo de esgoto com bagaço de cana ou casca de eucalipto são viáveis para a produção de mudas clonais de eucalipto, no entanto, é preciso considerar a especificidade hídrica de cada um deles. Enquanto na mistura de ambos os substratos (2:1 v:v) é indicada a lâmina de irrigação de 11 mm, no substrato lodo de esgoto compostado com bagaço de cana-de-açúcar (1:3 v:v) é necessário aplicar a lâmina de irrigação de 17 mm.

Palavras-chave: irrigação, viveiro, biossólido, microaspersão, *Eucalyptus*

Abstract

Effects of water management and composted sewage sludge substrates on the growth and quality of eucalyptus cuttings. This study aimed to evaluate the effect of substrates made of composted sewage sludge and irrigation depths on the growth and quality of *E. grandis* x *E. urophylla* cuttings to optimize water management for each substrate. The substrates were obtained from sewage sludge composted with sugarcane bagasse or *Eucalyptus* bark, namely: composted sewage sludge with sugarcane bagasse (1:3 v:v); sewage sludge composted with *Eucalyptus* bark (1:3 v:v) and a mixture of both substrates in a volumetric proportion 2:1 (v:v). Commercial substrate composed of *Sphagnum* peat, vermiculite and rice husk (3:1:1 v:v: v) was used as a control. The irrigation depths tested were 11, 14 and 17 mm, applied in two daily applications. The substrates were physically and chemically characterized and height, stem diameter, shoot, root and total dry mass, Dickson quality index and root system conformation were measured to determine the growth and quality of seedlings. The substrates obtained through the composting of sewage sludge with sugarcane bagasse or *Eucalyptus* bark are fit for producing *Eucalyptus* cuttings, however, the water specificity of each substrate must be considered. Although an 11mm irrigation depth is indicted for the mixture of both substrates (2:1 v:v), a 17mm irrigation depth is needed for the sewage sludge composted with sugarcane bagasse (1:3 v:v) substrate.

Keywords: irrigation, nursery, biosolid, microsprinkler, *Eucalyptus*

INTRODUCTION

Most of the sewage sludge generated in Brazil is destined to landfills, which is costly for public finances and can cause social, environmental and sanitary issues. Agenda 21 (BRASIL, 2004) recommends using sludge in agricultural areas, which has recently encouraged the development of research related to using this material in various activities (GUERRINI; TRIGUEIRO, 2004; OLIVEIRA *et al.*, 2011; CALDEIRA *et al.*, 2012; DELARMELINA *et al.*, 2014; KRATZ *et al.*, 2017). When composted correctly, sewage sludge can be used in agricultural and forestry areas, which was determined by the CONAMA 481/2017 resolution (BRASIL, 2017). However, it is also necessary to maintain the limits of pathogenicity and heavy metals stipulated by related resolutions.

Using sewage sludge in substrates is important because it presents characteristics that are good for the development of forest seedlings, including a large percentage of organic matter and macro and micronutrients (CALDEIRA *et al.*, 2012). The high concentration of nutrients can provide significant savings in the fertilizer consumption in nurseries, but attention should be paid to nutrient availability and the nutritional needs of the species being produced (GUERRINI; TRIGUEIRO, 2004).

The substrate is one of the most important factors for the development of quality seedlings, as its physical and chemical characteristics influence seed germination and rooting of mini-cuttings (DELARMELINA *et al.*, 2014). There are many materials that can be used as substrates and no one composition is suitable for all cultivation conditions and species, making it necessary to use mixtures of components. Moreover, using alternative substrates requires knowledge about the characteristics of the mixtures, as well as the best conditions for seedling growth (ŞIRIN *et al.*, 2010).

Another relevant factor in forest nurseries is irrigation management, since it influences seed germination, the rooting of mini-cuttings and the growth and rustification of seedlings. Although water is essential for the development of tree species (ARANDA *et al.*, 2012), nursery farmers' lack of knowledge still leads to the production of low-quality seedlings for field planting (DEGRANDE *et al.*, 2012) and overuse of water due to fears of losing production. Water is a limited resource and using it consciously is extremely important for developing long-term, sustainable production systems. Most forest seedling nurseries are located within urban centers, which further demonstrates the importance of responsibly using water resources, whether producing seedlings of native or exotic species, such as *Eucalyptus*.

The total area of planted *Eucalyptus* in Brazil is about 5.7 million hectares, representing 72.3% of the entire planted forest area in the country. Among the hybrids used in the plantations, we highlight the clone commercially known as I-144, produced from *E. grandis* and *E. urophylla*, which is tolerant of high-water deficit and produces good quality wood, among other uses (MÜLLER *et al.*, 2017).

Developing research with new substrates and more appropriate water management for each one in the production of forest seedlings is essential to minimize the negative social and environmental impacts related to water misuse and the inadequate disposal of agro-industrial waste. This study tests the following hypotheses: a) Substrates composed of sewage sludge can be used as alternatives to commercial substrates for the production of clonal *Eucalyptus* seedlings; b) For each substrate with biosolids there is a most appropriate water management that favors growth and quality of clonal *Eucalyptus* seedlings. Thus, the objective of this study was to evaluate the effect of substrates made of composted sewage sludge and irrigation depths on the growth and quality of clonal *E. grandis* x *E. urophylla* seedlings to optimize water management for each substrate.

MATERIAL AND METHODS

The experiment was conducted between December 2016 and April 2017, in a suspended, sectorized nursery, located at the coordinates 22° 51' 03" S and 48° 25' 37" W, with average altitude of 780 m. The climate type is Cwa, according to Wilhelm Köppen classification, with average annual precipitation of 1,524 mm. The *Eucalyptus urophylla* x *E. grandis* mini-cuttings were obtained from a clonal mini-garden and prepared with an average length of 7 to 10 cm, leaving a pair of leaves cut in half. Cuttings were then staked in a circular section of polyethylene tubes with a 50 cm³ volume capacity, containing the tested substrates.

The substrates were produced with sewage sludge from the treatment plant, which was composted with sugar cane bagasse or *Eucalyptus* bark, namely: sewage sludge composted with sugarcane bagasse (1:3 v:v) (SCB); sewage sludge composted with *Eucalyptus* bark (1:3 v:v) (SEB) and mixture of both SCB + SEB substrates in the proportion 2:1 (v:v). Commercial substrate composed of *Sphagnum* peat, vermiculite and roasted rice husk (2:1:1 v:v:v) was used as the control.

After staking in the substrates, the mini-cuttings were kept for 30 days in an automated greenhouse with controlled relative humidity (> 80%, maintained by nebulization system). Then, the rooted plants were taken to suspended microtunnel beds, covered with light diffusing plastic in an area under full sunlight, with tray spacing changed to 50%.

In the beds, seedlings in each substrate were subjected to three different daily irrigation depths (11, 14 and 17 mm) applied at 10:00 and 14:00 H. These irrigation depths were defined by Muller *et al.* (2017).

Thus, the experiment was conducted using a completely randomized design in a 4x3 factorial scheme (four substrates and three irrigation depths), totaling 12 treatments with four plots of 12 seedlings.

Growth fertilization began with the application of irrigation depths, in which seedlings of all treatments received 3 mm of nutrient solution, via ferti-irrigation by the microsprinkler system, twice a week. The macronutrient solution consisted of purified monoammonium phosphate fertilizers, magnesium sulfate, potassium chloride, calcium nitrate and urea in the concentrations of 488; 155.4; 328.1; 312; 72.2; 98.8 mg L⁻¹ of N, P, K, Ca, Mg and S, respectively. The micronutrient solution consisted of boric acid, sodium molybdate and manganese

sulphates, zinc, copper and iron at concentrations of 4.6; 3.9; 1.2; 0.6; 0.3 and 2.5 mg L⁻¹ of B, Mn, Zn, Cu, Mo and Fe, respectively. Water management was applied until at least one of the treatments presented seedlings with well-aggregated root systems in the substrate.

According to the methodology described by Guerrini and Trigueiro (2004), the following characteristics were determined for the physical analysis of substrates: total porosity, macroporosity, microporosity and water retention capacity. The chemical characterization of the nutrients available in each substrate was performed according to the methodology of Sonneveld (1988).

To evaluate the growth at the end of the production cycle in the nursery, the height and the diameter of the stem of all seedlings from each treatment were measured 104 days after the staking. The height (cm) was measured with a millimeter ruler, measuring from the base of the stem to the apical bud. The diameter of the stem (mm) was measured with a digital caliper positioned horizontally on seedling stem. The shoot and root dry mass (g) were obtained by sectioning the stem at collar height of 36 seedlings per treatment. The root systems were washed in running water to remove the substrate, placed in labeled paper bags and taken to greenhouse at 70°C until they reached constant mass. Mass measurements were obtained using an electronic scale. The sum of the aerial and root dry mass resulted in the total dry mass. The Dickson quality index was calculated from the values of dry mass, height and stem diameter.

The same seedlings used to evaluate dry mass were also used to evaluate root system formation, according to the methods by Silva *et al.* (2013).

Data for each variable was submitted to the Shapiro-Wilk test to verify normality. Then, analysis of variance was performed to verify if the treatments affected the variables and, when the value of F was significant, the Scott-Knott test was applied ($p < 0.05$) to compare the differences between treatments.

RESULTS

Physical and chemical characteristics of substrates

The physical and chemical characteristics of each substrate varied according to the materials they were composed of, which may require different management techniques to produce seedlings (Tables 1 and 2).

Tabela 1. Características físicas dos substratos utilizados no experimento.

Table 1. Physical properties of substrate used in this experiment.

Substrates	Porosity (%)			Water retention capacity (mL 55 cm ⁻³)	Bulk density (g cm ⁻³)
	Macro	Micro	Total		
CS	24.2 c	59.3 a	83.5 b	29.7 a	0.13 c
SCB	59.9 a	26.9 c	86.8 a	14.0 c	0.10 c
SEB	32.3 b	42.7 b	75.0 d	22.2 b	0.19 a
SCB+SEB (2:1)	37.4 b	40.3 b	78.7 c	21.2 b	0.15 b

Averages followed by the same letters in the column do not differ statistically by the Scott-Knott test ($p < 0.05$). SC = commercial substrate made of *Sphagnum* peat, vermiculite and roasted rice husk (2:1:1 v:v:v); SCB = Sewage sludge + sugar cane bagasse (1:3 v:v); SEB = Sewage sludge + *Eucalyptus* bark (1:3 v:v); SCB + SEB (2:1 v:v).

Tabela 2. Características físicas dos substratos utilizados no experimento.

Table 2. Chemical properties of substrate used in this experiment.

Substrates	Nutrients (mg L ⁻¹)						
	N	P	K	Ca	Mg	S	Na
CS	0.66 c	33.33 a	93.07 b	23.67 c	49.00 c	146.67 b	24.56 c
SCB	2.53 a	1.33 b	55.73 c	386.67 b	98.00 b	560.00 a	34.90 b
SEB	1.84 b	1.33 b	159.07 a	613.33 a	138.67 a	580.00 a	50.23 a
SCB+SEB (2:1)	1.37 b	2.10 b	99.40 b	580.00 a	128.67 a	606.67 a	43.90 a

Substrates	Nutrients (mg L ⁻¹)					pH	EC (dS m ⁻¹)
	B	Cu	Fe	Mn	Zn		
CS	0.07 b	0.18 a	1.33 b	0.51 d	0.69 c	6.30 a	0.89 b
SCB	0.06 b	0.10 b	0.90 c	4.21 c	2.36 a	4.93 d	2.93 a
SEB	0.15 a	0.11 b	2.47 a	6.07 b	0.44 d	5.92 b	3.45 a
SCB+SEB (2:1)	0.15 a	0.11 b	3.70 a	7.67 a	1.30 b	5.37 c	3.32 a

Averages followed by the same letters in the column do not differ statistically by the Scott-Knott test ($p < 0.05$). SC = commercial substrate made of *Sphagnum* peat, vermiculite and roasted rice husk (2:1:1 v:v:v); SCB = Sewage sludge + sugar cane bagasse (1:3 v:v); SEB = Sewage sludge + *Eucalyptus* bark (1:3 v:v); SCB + SEB (2:1 v:v).

The SCB substrate showed the highest macroporosity and, consequently, lower water retention. In contrast, the commercial substrate presented the highest microporosity and the highest water retention. The SEB substrate and the SCB + SEB mixture (2:1 v:v) were similar regarding macroporosity, microporosity and water retention, differing only in total porosity and dry density.

The pH of the substrates ranged from 4.93 to 6.30. Compared to the control, the substrates composed of sewage sludge presented higher (by at least 3 times) electrical conductivity. In general, substrates with composted sewage sludge presented higher macronutrient and micronutrient contents than the commercial substrate, mainly N, Ca, Mg, S and Mn. Only P and Cu contents were higher in the CS compared to the other substrates, and K, Fe and Mn levels were higher than the SCB substrate.

Growth and seedling quality

The substrates and the irrigation depths used during the seedling production period promoted different growths (5% significance) for all variables (Table 3).

Tabela 3. Efeito da interação entre as lâminas de irrigação and os substratos na altura, diâmetro do colo, massa seca aérea, massa seca radicular, massa seca total, índice de qualidade de Dickson (DQI) de mudas clonais de *Eucalyptus grandis* x *E. urophylla* aos 104 dias após o estaqueamento.

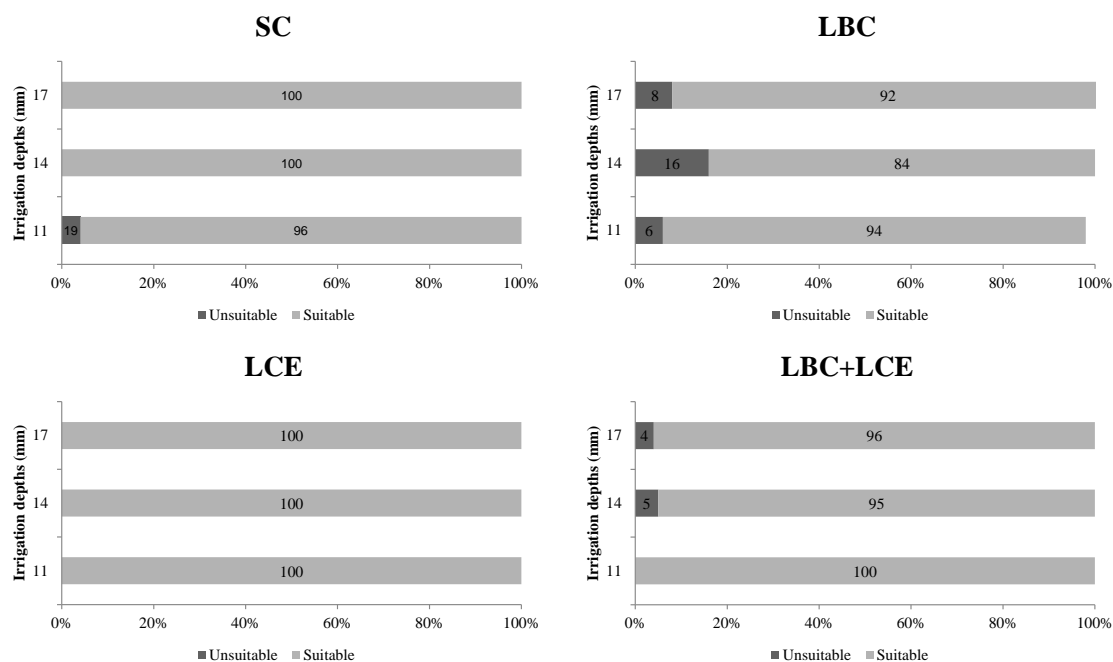
Table 3. Effects of the interaction between irrigation depths and the substrates on height, stem diameter, shoot dry mass, root dry mass, total dry mass and Dickson quality index (DQI) in *Eucalyptus grandis* x *E. urophylla* cuttings 104 days after staking.

Irrigation depths (mm)	Height (cm)				Stem diameter (mm)			
	Substrates				Substrates			
	CS	SCB	SEB	SCB+SEB (2:1)	CS	SCB	SEB	SCB+SEB (2:1)
11	25.5 Ba	20.7 Bc	23.7 Bb	25.8 Aa	3.40 Bb	3.04 Bc	3.30 Bb	3.62 Aa
14	28.5 Aa	22.3 Ac	26.5 Ab	25.1 Ab	3.65 Aa	3.12 Bb	3.54 Aa	3.54 Aa
17	27.9 Aa	22.8 Ac	27.3 Aa	24.6 Ab	3.57 Aa	3.32 Ab	3.55 Aa	3.44 Ab
Irrigation depths (mm)	Shoot dry mass (g)				Root dry mass (g)			
	Substrates				Substrates			
	CS	SCB	SEB	SCB+SEB (2:1)	CS	SCB	SEB	SCB+SEB (2:1)
11	2.93 Ba	2.11 Ab	2.50 Bb	2.95 Aa	1.00 Ba	0.91 Ba	0.83 Ca	0.98 Aa
14	4.01 Aa	2.21 Ac	3.26 Ab	3.07 Ab	1.22 Aa	0.78 Bc	1.05 Bb	1.01 Ab
17	4.28 Aa	2.58 Ac	3.28 Ab	3.27 Ab	1.39 Aa	1.04 Ab	1.05 Aa	1.15 Ab
Irrigation depths (mm)	Total dry mass (g)				DQI			
	Substrates				Substrates			
	CS	SCB	SEB	SCB+SEB (2:1)	CS	SCB	SEB	SCB+SEB (2:1)
11	3.95 Ba	3.05 Bb	3.36 Bb	4.07 Aa	0.38 Ba	0.34 Bb	0.34 Cb	0.42 Aa
14	5.26 Aa	3.02 Bc	4.33 Ab	4.14 Ab	0.49 Aa	0.29 Bc	0.41 Bb	0.41 Ab
17	5.46 Aa	3.73 Ad	4.82 Ab	4.46 Ac	0.53 Aa	0.39 Ab	0.49 Aa	0.45 Ab

Averages followed by identical lowercase letters in rows and uppercase letters in columns do not differ by Scott-Knott test ($p < 0.05$); CV (%) Height = 14.9; Stem diameter = 13.0; Air dry mass = 29.9; Root dry mass = 31.1; Total dry mass = 30.2; DQI = 29.8. CS = commercial substrate made of *Sphagnum* peat, vermiculite and roasted rice husk (2:1:1 v:v:v); SCB = Sewage sludge + sugar cane bagasse (1:3 v:v); SEB = Sewage sludge + *Eucalyptus* bark(1:3 v:v); SCB + SEB (2:1 v:v).

Of the substrates made of sewage sludge, the SCB + SEB (2:1) substrate and 11 mm irrigation depth favored greater or equal growth of seedlings in all variables. The CS and SEB substrates and the 14 mm depth, which did not differ from the 17 mm depth in most variables, promoted higher growth. In the SCB substrate, it was necessary to apply the 17 mm depth to promote higher growth in most variables.

When evaluating root system conformation, all substrates and irrigation depths formed between 95 and 100% of seedlings fit for planting, except the SCB substrate and the irrigation depths tested which provided the highest percentages (between 6 and 16%) of unfit seedlings (Figure 1).



SC = Commercial substrate made of *Sphagnum* peat, vermiculite and roasted rice husk (2:1:1 v:v: v); SCB = sewage sludge + sugarcane bagasse (1:3 v:v); SEB = sewage sludge + *Eucalyptus* bark (1:3 v:v); SCB + SEB (2:1 v:v).

Figura 1. Avaliação da conformação do sistema radicular de mudas clonais de *Eucalyptus grandis* x *E. urophylla* aos 104 dias após o estaqueamento.

Figure 1. Evaluation of root system conformation in *Eucalyptus grandis* x *E. urophylla* cuttings 104 days after staking.

DISCUSSION

Physical and chemical characteristics of substrates

The porosity of the substrate and air and water availability for plant root systems are closely connected (SIGUINO *et al.*, 2013). Regarding total porosity (Table 1), all substrates used in this study presented adequate or high values, according to Pascual *et al.* (2018). Adding *Eucalyptus* bark to sewage sludge increased microporosity, while adding sugarcane bagasse increased macroporosity. Mixing these two substrates, represented by the substrate SCB + SEB (2:1), balanced the macro and microporosity.

Regarding water retention capacity, only the SCB substrate was below the appropriate value (20-30 mL 50 cm⁻³). The lower water retention values are associated with a greater amount of macropores inherent to sugarcane bagasse. This physical characteristic may have negatively influenced seedling growth, since water drained more easily after irrigations. In addition, the SCB substrate showed higher zinc concentration at the beginning of the experiment. Pinto *et al.* (2009) found that increased Zn concentration in the nutrient solution also caused a decrease in seedling height for *Eucalyptus urophylla* seedlings.

According to Silva *et al.* (2012), other values of physical characteristics of the substrates, different from those mentioned in the literature, may also be considered adequate. Such adequacy varies according to the species, type of packaging, propagation form and management adopted in the nursery.

The materials added to the sewage sludge influenced the chemical characteristics (Table 2). The substrates with *Eucalyptus* bark (SEB and SCB+SEB 2:1) presented higher levels of Ca, Mg, Na, B and Fe. The SEB presented higher K content, while the SCB substrate presented higher N and Zn levels than the others. According

to Kratz *et al.* (2017), materials with biosolids present nutrients in their organic forms, which are gradually released and more easily used by plants when used as substrate.

The pH of organic substrates used for seedling production should be between 5.0 and 6.5 for greater nutrient availability. All substrates used in this study fall within this pH range. Regarding electrical conductivity, the highest values observed in sewage sludge substrates were expected due to the higher intrinsic nutrient concentration of these materials, differing from commercial substrates. In CS, the higher phosphorus content was caused by the addition of simple superphosphate (P_2O_5).

Growth and seedling quality

Shoot height and stem diameter are the most widely used variables for large scale quality classification of seedlings in nurseries and, in many cases, have been correlated to seedling survival and growth after planting (ZIDA *et al.*, 2008). In this study, despite the differences in growth height (Table 3), all substrates and depths produced seedlings that met the quality standard established for *Eucalyptus* spp., which varies from 20 to 35 cm in height and at least 2.5 mm stem diameter, which occurred in all substrates and irrigation depths.

In this study, we found that the greatest growth in the SCB substrate occurred with the application of the largest depth (17 mm). This could be related to the lower water retention capacity of this substrate, since, according to Sammons and Struve (2008), substrates with higher water retention capacity (a function of physical properties) can increase irrigation efficiency.

Considering the substrates with sewage sludge, the SCB+SEB (2:1) substrate presented greater or equal growth of seedlings in all variables with the application of the smallest irrigation depth (11 mm). This result can be explained from a physical point of view, as this substrate presented balanced macro and microporosity, as well as adequate water retention and intermediate density.

The higher growth of seedlings cultivated in the CS and SEB substrates, in most of the variables, occurred with the 14 mm depth, which did not differ from the 17 mm depth. In the CS substrate, which presented the highest water retention, the smaller depth may have been insufficient to moisten the whole root clump. According to Warren and Bilderback (2005), if adequate water maintenance is not considered and low levels of water are applied, stomata can close, and photosynthesis and plant growth can reduce. In the SEB substrate, which is physically and chemically similar to the SCB+SEB (2:1) substrate, the need for 14 mm of water may be due to its higher density, that is, the larger size and mass of the particles reduced the total porosity of this substrate.

Mañas *et al.* (2009) stated that seedling quality depends on the plants's ability to produce new roots quickly, the speed at which seedlings fix in the soil and begin to grow after planting, the development of the root system, the sun-adaptation of leaves, large stem diameter, good carbohydrate reserves and optimum mineral nutrition. In this experiment there was no difference between the percentages of fit and unfit seedlings in the treatments (Figure 1). The SCB substrate and the irrigation depths provided the highest percentages of seedlings unfit for planting, probably due to the low water retention capacity. Just as this substrate did not fully favor root system formation, Kratz and Wendling (2016) observed that the aggregation of the root system showed a significant positive correlation with several growth variables for *Eucalyptus camaldulensis* seedlings. Grossnickle (2012) states that cultural practices in the nursery that alter the morphological characteristics of the seedlings can limit the susceptibility to planting under water stress conditions. Therefore, the growth variables, such as larger stem diameter of the seedlings and the root system conformation may provide higher chances of survival.

CONCLUSIONS

- The substrates obtained through composting sewage sludge with sugarcane bagasse or *Eucalyptus* bark are viable to produce *Eucalyptus* clonal seedlings, however, water specificity of each substrate must be considered.
- Considering reduced water use, the use of sewage sludge residue as substrate and the adequate growth and quality of the seedlings, the sewage sludge composted with bagasse sugar cane + sewage sludge composted with *Eucalyptus* bark (2:1 v:v) substrate and the 11 mm irrigation depth are recommended.
- The other substrates can also produce seedlings with adequate growth and quality, however, with higher water expenditures. For the sewage sludge composted with sugar cane bagasse (1:3 v:v) substrate, an 17mm irrigation depth should be applied. For the sewage sludge composted with *Eucalyptus* bark (1:3 v:v) and the commercial (peat *Sphagnum* + vermiculite + roasted rice husk (2:1:1 v:v: v) an 14mm irrigation depth is needed.

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