iForest
Biogeosciences and Forestry

Research Article
doi: 10.3832/ifor3929-014
vol. 14, pp. 569-575

Composted sewage sludge as an alternative substrate for forest seedlings production

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Received: Jul 15, 2021 - Accepted: Oct 21, 2021

Citation: Gabira MM, Silva RBG, Bortolheiro FPAP, Mateus CMD, Villas Boas RL, Rossi S, Girona MM, Silva MR (2021). Composted sewage sludge as an alternative substrate for forest seedlings production. iForest 14: 569-575. – doi: 10.3832/ifor3929-014 [online 2021-12-16]

Communicated by: Werther Guidi Nissim

The production of forest seedlings with adequate morphological and physiological characteristics is essential for the success of plantations. Substrates and irrigation are the major factors determining seedlings' growth. Substrates made of urban and agricultural residues are a sustainable alternative to peat-based substrates. In this study, we evaluated how composted sewage sludge substrates affect the growth and gas exchange in seedlings of *Cedrela fissilis* Vell. Seedlings were produced under daily irrigation depths of 6, 9, and 12 mm, and on different substrates. The substrates were based on sewage sludge composted with Eucalyptus bark or sugarcane bagasse, and a commercial substrate based on peat, involving a double factorial design with 12 treatments (3 irrigation depths × 3 substrates). Both physical and chemical characteristics of substrates were analyzed, and morphological traits and gas exchanges of seedlings were measured. Sewage sludge-based substrates presented different characteristics according to the material it was mixed. Eucalyptus bark provided higher bulk density (0.19 g cm^{-3}) and lower total porosity (75%) to the substrate, while sugarcane bagasse increased macroporosity up to 60%. Seedlings produced in sewage sludge-based substrates presented a height up to 17.8 cm and stem diameters of between 8.39-10.29 mm. Higher shoot and root dry mass was obtained in sewage sludge-based substrates with irrigation depth of 9 mm, which were 3.71 and 2.01 g, respectively. Photosynthetic carbon assimilation varied between 2.26 and 3.23 μmol CO_2 m^{-2} s^{-1}, and water use efficiency varied from 2.058 to 3.395 μmol CO_2 (mol H_2O)$^{-1}$, with the highest values being obtained in seedlings produced in sewage sludge-based substrates with irrigation depth of 6 mm. Our results demonstrate that sewage sludge-based substrates are an efficient alternative to commercial peat-based substrates for seedling production.

Keywords: Agricultural Residues, *Cedrela fissilis*, Forest Nursery, Gas Exchange, Irrigation, Plant Growth, Silviculture, Solid Wastes

Introduction

Production and use of tree seedlings are important components of forest management. Proper understanding of the relationship between seedling quality and field establishment is the key for new plantations and natural regeneration (Dumroese et al. 2016, Girona et al. 2018). Among the factors affecting seedling growth and quality, the more important ones are cultural practices like irrigation, nutrition, container size, and type, as well as substrate (Kormanek et al. 2020, Madrid-Aispuro et al. 2020). Hardened seedlings, with adequate morphological and physiological attributes, are more likely to reach their greatest growth potential soon after field planting, guaranteeing their survival in adverse environmental conditions (Grossnickle & MacDonald 2018).

Among the substrates available for seedling production, vermiculite combined with peat has been used for a long time in forest nurseries (Raviv 2013). However, its availability is limited, it can be expensive if imported, and harvesting peat causes ecological impacts (Madrid-Aispuro et al. 2020). Several studies have shown that it is possible to obtain substrates made of residues with good physical characteristics, which provides tree seedlings with well-aggregated and quality root systems to be transplanted in the field (Fermino et al. 2018, Fornes & Belda 2019, Gabira et al. 2020a, Sá et al. 2020). In recent years, sewage sludge has also been evaluated to be used as a potential substrate (Kratz et al. 2017, Lopes et al. 2018). Because of its origin, sewage sludge presents a variability in composition, preventing its use at a commercial level based on the actual knowledge on the performance of the product (Nascimento et al. 2020). Nevertheless, the use of sewage sludge reduces the acquisition of commercial substrates and chemical fertilizers, representing a sustainable alternative to landfill disposal (Manca et al. 2020). Research using sewage sludge on seedling production focuses mainly on substrate characteristics and plant morphological responses, but the relationships between these substrates and other production factors on plant growth and gas exchange are still lacking.

Sugarcane and eucalypt industries are very remarkable in Brazil, producing large amounts of waste. *Eucalyptus* is the most important source of biomass for paper pulp and wood industries, which generate large amounts of waste since the bark is not used as a valuable product (Chemetova et al. 2018). In 2018, the Brazilian forestry sector produced 52 Mt of solid waste, 36.9 Mt of which was composed of barks, branches, and leaves (IBA 2019). Another agro-industrial residue, sugarcane bagasse, is the waste of the sugar and biofuel industries. It represents 30-33% of the weight of sugarcane, and it is mostly used for energy, but remains an important environmental problem (Sahu 2018, Braga et al. 2019). The use of these residues as substrates for forest seedlings is an important alternative, reducing environmental impact and aggregating value for these products (Chemetova et al. 2018, Lopes et al. 2018).

Cedrela fissilis Vell. is a native species from South America. Its wood is known for the high quality, rich extractive compounds, and natural durability, making it a promising species for commercial plantations (Georgin et al. 2019). In addition to its economic importance, it is considered among Brazilian endangered species (Barstow 2018). Both aspects raise the need for developing studies on its distribution, silvicultural treatments, breeding, and genetic selection (Navroski et al. 2016), as well as factors influencing the seedlings' production and establishment after planting.

The aim of this study was to evaluate how substrates based on composted sewage sludge affect the growth and gas exchange of *Cedrela fissilis* seedlings under different irrigation depths. Our hypotheses are that: (i) substrates based on sewage sludge mixed with sugarcane bagasse or eucalyptus bark enable growth and biomass accumulation similar to the commercial substrate; and (ii) seedlings in a substrate with lower water holding capacity need higher irrigation depths to maintain growth and photosynthetic activity.

Material and methods

Experimental design and plant material

The experiment was conducted between November 2016 and April 2017 in a suspended, sectored nursery in Botucatu (São Paulo State), Brazil (22° 51′ S, 48° 25′ W; 780 m a.s.l.). The climate of the region is tropical, with hot and rainy summers and dry winters. The mean annual temperature is 19.1 °C, and annual precipitation is 1324 mm. The region has the typical vegetation of a seasonal semi-deciduous forest under the Tropical Atlantic Forest biome.

C. fissilis seedlings were produced from seeds collected from mother trees in Botucatu and grown in polyethylene tubes (92 cm^3 volume) containing different substrates. Two seeds per tube were sowed. The tubes were placed in polypropylene trays, which were kept in a greenhouse at 50% full solar radiation and irrigated with 200 L h^{-1} flow micro-sprinklers, automatically activated for 20 seconds, every 30 minutes from 9 AM to 4 PM for 30 days. After germination, one plant was maintained in each tube. The experiment was carried out in a completely randomized design with a 3 × 3 factorial scheme (three substrates × three irrigation depths). Each treatment consisted of four replications of 12 seedlings, totaling 48 seedlings per treatment (overall, 432 seedlings).

Treatments

The substrates were produced from domestic sewage sludge resulting from treatment in Up-flow Anaerobic Sludge Blanket (UASB) reactor system and complementary treatment with conventional activated sewage sludge. The sludge was composted with structuring residues (sugar cane bagasse or *Eucalyptus* sp. bark) for 90 days, at a ratio of 1:3 v:v. Sugarcane bagasse and Eucalyptus bark are industrial residues obtained from the sugar and paper industries. In addition to the social and environmental issues, these residuals have a fibrous structure with the potential to improve the physical characteristics of sewage sludge. Mixtures were arranged into piles in a 150-micron plastic-covered composting patio supported by metal frame with open sides. Piles were turned by a composting machine twice a week for the entire composting period.

The resultant materials from the sewage sludge composting process were used as substrates. These consisted of sewage sludge composted with sugarcane bagasse (1:3 v:v – SCB) and sewage sludge composted with *Eucalyptus* bark (1:3 v:v – SEB). As a control, we used a commercial substrate composed of *Sphagnum* peat, vermiculite, and roasted rice husk (2:1:1 v:v:v). Domestic sludge lacks in heavy metals (Nascimento et al. 2020), and no pathogenic microorganism was detected in the material used as substrate after the composting process.

The seedlings trays were placed into suspended beds, covered with light-diffusing plastic in an area under full sunlight in the nursery to start the application of different irrigation depths. In each tray, the density was 120 tubes m^{-2}. Water management involved applying three daily irrigation depths (6, 9, and 12 mm) in two installments, both at 10 AM and 2 PM. Irrigation depths were defined from studies by Silva & Silva (2015).

All treatments received the same amount of growth fertilizer during the application of irrigation depths. Seedlings received 3 mm of a nutrient solution *via* fertigation by the micro-sprinkler system twice a week. The macronutrient solution consisted of purified mono ammonium phosphate, magnesium sulfate, potassium chloride, calcium nitrate, and urea fertilizers at the concentrations of 488, 155.4, 328.1, 312, 72.2, and 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 sulfates, zinc, copper, and iron at the 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. At 120 days after sowing, seedlings started to receive a nutrient solution composed of potassium chloride at a concentration of 750 mg L^{-1} K. We carried out the experiment until at least one of the treatments presented seedlings that were suitable for field planting, *i.e.*, with a well-developed root across the whole substrate.

Measurements and data collection

To determine the substrates' properties, we measured total porosity, macroporosity, microporosity, and water holding capacity of each substrate according to the methodology described by Guerrini & Trigueiro (2004). This methodology consisted in filling polyethylene tubes with the substrates, letting them under the water for 24 h, weighting, let them drain for 1 h, and then weighting again. After, substrates were dried in an oven for 24 h and then weighed again. Physical attributes were calculated using these data. Chemical characterization of available nutrients was performed according to Sonneveld (1988), which assessed the concentrations of N, P, K, Ca, Mg, S, Na, B, Cu, Fe, Mn, Zn, as well as pH and electrical conductivity (EC) of the substrates. It consists of aqueous extraction in a proportion of 1:1.5 (v:v), stirred for 30 minutes, and then filtered through medium filtering paper (Nalgon 3550).

To evaluate seedlings' growth at the end of the experiment (151 days after sowing), we measured height, stem diameter, shoot, root, and total dry mass, and Dickson quality index (DQI). To measure height (cm), we used a millimeter ruler, measuring from the stem to the apical bud. Stem diameter (mm) was measured using a digital caliper positioned horizontally on the seedling stem. Shoot and root dry mass (g) was obtained by sectioning the seedling at stem height. The root systems were washed in running water to remove the substrate and placed in paper bags in a drying oven at 70 °C until reaching a constant mass. Total dry mass and Dickson quality index (DQI) were determined by the combination of the abovementioned growth variables.

The gas exchange was evaluated with an infrared gas analyzer IRGA, model LI 6400® (LI-COR, Lincoln, NE, USA). Gases were evaluated at the end of the experiment, between 9 AM and 11 AM, during a sunny day. The CO_2 concentration used as reference was the one from the environment, which varied between 380 and 400 μmol CO_2 mol^{-1}. Carbon assimilation (A, μmol CO_2 m^{-2} s^{-1}), transpiration (E, mol H_2O m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), and intracellular carbon concentration (C_i, μmol CO_2 mol air^{-1}) were recorded. From this data, we calculated the water use efficiency [WUE, μmol CO_2 $(mol\ H_2O)^{-1}$], determined by the A/E ratio, and the carboxylation efficiency, determined by the A/C_i ratio.

Statistical analysis

Data were submitted to the Shapiro-Wilk test to verify the normality of the variables and to Bartlett test to verify homogeneity of variances; we did not perform data transformation for statistical analysis. Analyses of Variance (ANOVA) was performed for physical and chemical characteristics of the substrates, and for growth and gas exchange data. Tukey test was applied for multiple comparisons. We used the R software to perform analysis (R Core Team 2020).

Tab. 1 - Macroporosity, microporosity, total porosity, water holding capacity (WHC), and bulk density of the substrates used to produce *Cedrela fissilis* seedlings. Averages followed by the same letter in the row do not differ (p > 0.05) by Tukey test. (CS): Commercial substrate of *Sphagnum* peat, vermiculite and 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).

Physical characteristics	Substrate		
	CS	SCB	SEB
Macroporosity (%)	24.0 ^{c}	60.0 ^{a}	32.0 ^{b}
Microporosity (%)	59.0 ^{a}	27.0 ^{c}	43.0 ^{b}
Total porosity (%)	83.0 ^{b}	87.0 ^{a}	75.0 ^{c}
WHC (mL 55 cm^{-3})	29.7 ^{a}	14.0 ^{c}	22.2 ^{b}
Bulk density (g cm^{-3})	0.1 ^{b}	0.1 ^{b}	0.2 ^{a}

Results

Physical and chemical characteristics of substrates

The physical characteristics varied greatly according to substrate composition (Tab. 1). Compared with the other substrates, SCB increased macroporosity up to 60%; water holding capacity decreased to 14.0 mL 55 cm^{-3}, the lowest value among the substrates used in this experiment. The characteristics of SCB differed mostly from CS, which presented the highest microporosity and water holding capacity, 59.0% and 29.7 mL 55 cm^{-3}, respectively. SEB provided intermediate values of macroporosity (32.3%), microporosity (43.0%), and water holding capacity (22.0%), as well as the highest bulk density (0.19 g cm^{-3}) and the lowest total porosity (75.0%).

The substrates with sewage sludge had more acidic pH (5.92 for SEB and 4.93 for SCB) and superior electrical conductivity (3.45 for SEB and 2.93 for SCB) compared to the commercial substrate (6.30 pH and 0.89 dS m^{-1}). Overall, the macronutrient and micronutrient contents of sewage sludge substrates were higher than those of the commercial substrate, especially in N, Ca, Mg, S, and Mn. CS presented higher levels of P and Cu and K, Fe, and Mn compared to SEB.

Seedling growth

Seedling growth was influenced by irrigation depths and substrates, with a significant interaction between the factors for all morphological variables ($p < 0.05$). Seedlings produced in SEB presented superior height when compared to the other substrates in all irrigation depths – 16.7, 16.3, and 17.8 cm for seedlings produced in 6, 9, and 12 mm, respectively (Fig. 1a). The lowest height value was 14.7 cm, observed in seedlings produced in SCB with 6 mm irrigation depth. Stem diameter presented little variation (8.39-10.29 mm). We observed the lowest stem diameter in seedlings produced in SEB with 6 mm irrigation depth, while the highest value was observed in seedlings produced in CS with 9 mm irrigation depth (Fig. 1b). Seedlings in SCB presented the lowest stem diameter when produced with 9 and 12 mm irrigation depth (9.49 and 9.25 mm, respectively).

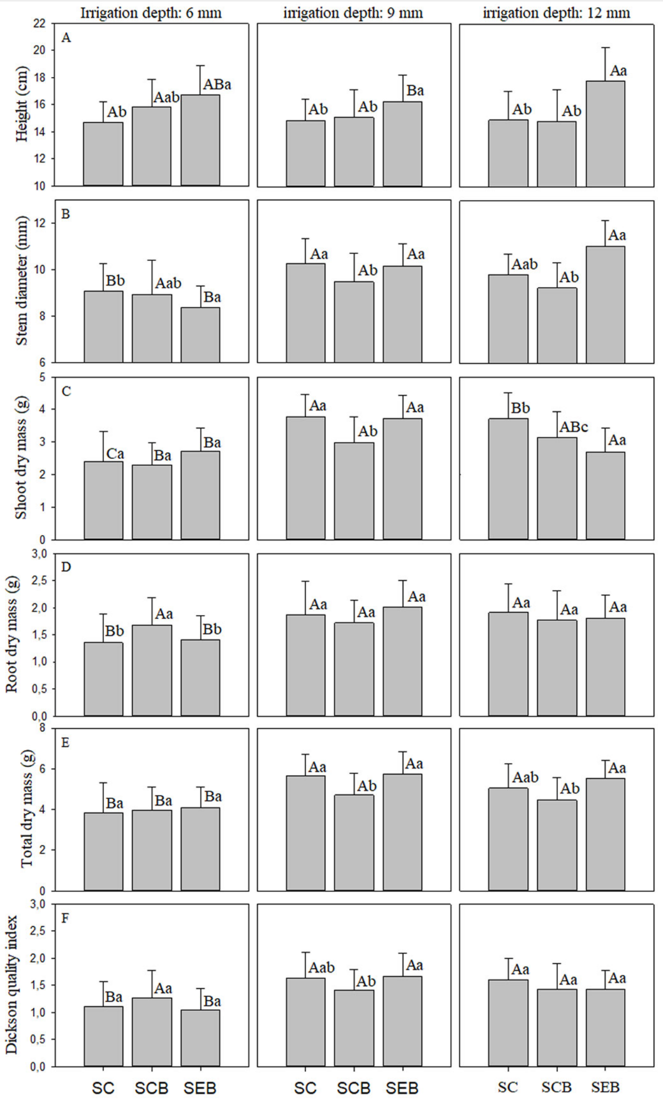

Fig. 1 - Height, stem diameter, shoot dry mass, root dry mass, total dry mass, and Dickson quality index of *Cedrela fissilis* seedlings at 151 days after sowing. (CS): Commercial substrate composed of Sphagnum peat, vermiculite, and rice husk (2:1:1 v:v:v); (SCB): sewage sludge + sugar cane bagasse (1:3 v:v); (SEB): sewage sludge + Eucalyptus bark (1:3 v:v).

We observed higher shoot and root dry mass in seedlings produced in SEB with 9 mm, which were 3.71 and 2.01 g, respectively (Fig. 1c, d). For shoot dry mass, seedlings produced in SCB presented the lowest values in all irrigation depths - 2.28, 2.98, and 2.68 g for seedlings produced in 6, 9, and 12 mm, respectively. For shoot dry mass, we observed the lowest value in seedlings produced in CS with 6 mm irrigation depth. Total dry mass, as a result of the shoot and root dry mass, also had seedlings produced in SEB with 9 mm as the higher value (5.71 g) and the lowest value in seedlings produced in CS with 6 mm (3.82 g) (Fig. 1e). Dickson quality index indicates that SEB and CS under 9 mm irrigation depths provided better growth and mass accumulation of seedlings; in these

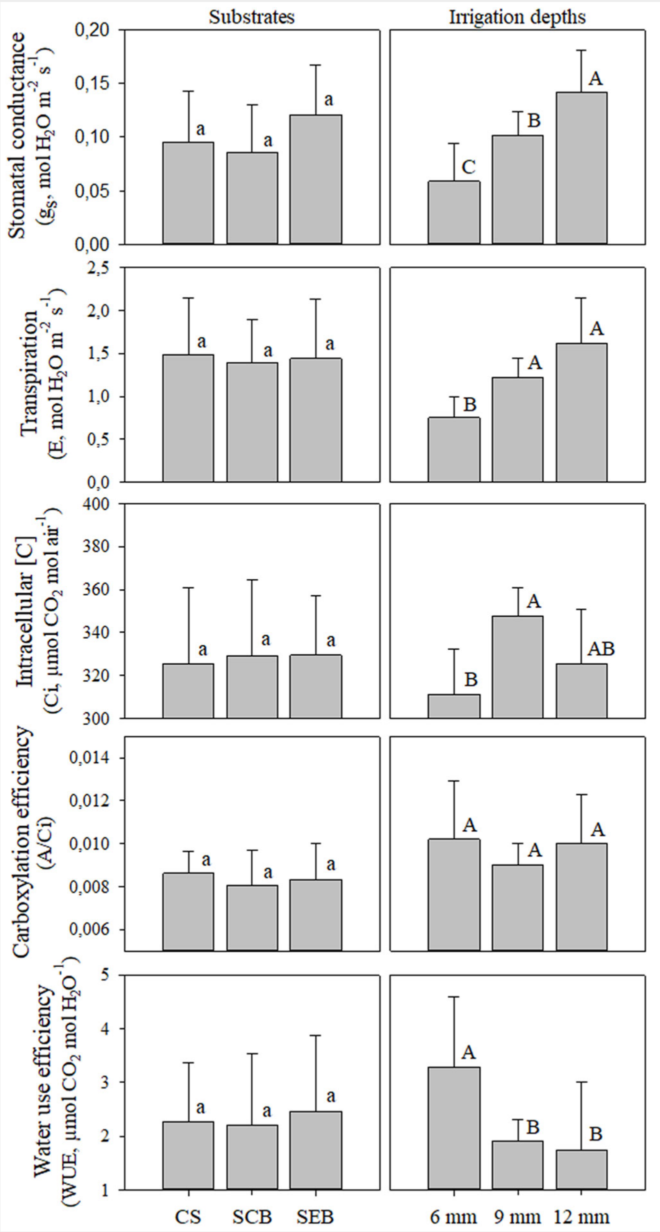

Fig. 2 - Stomatal conductance (g_s), transpiration (*E*), intracellular carbon concentration (C_i), carboxylation efficiency (A/C_i), and water use efficiency (WUE) of *Cedrela fissilis* seedlings at 151 days after sowing. (CS): commercial substrate composed of Sphagnum peat, vermiculite, and rice husk (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).

treatments. Seedlings DQI were 1.70 and 1.68, respectively (Fig. 1f).

Gas exchange

We observed an increase in photosynthetic carbon assimilation (*A*) for seedlings produced in CS associated with the increase in irrigation depth – 2.26, 2.89, and 3.23 µmol CO_2 m^{-2} s^{-1} for 6, 9, and 12 mm (Fig. 2). For seedlings in SEB, a decrease in *A* was associated with the increase in irrigation depth, with values varying from 3.23 to 2.28 µmol CO_2 m^{-2} s^{-1} in seedlings produced under 6 and 12 mm. Seedlings in SCB presented intermediate values of *A*, which varied between 2.45 and 2.75 CO_2 m^{-2} s^{-1}. *A* was the only photosynthetic variable that presented statistical interaction between both substrates and irrigation depths.

There was a similar tendency among seedlings produced in all substrates when regarding these variables, which did not present differences among substrates. Analyzing differences in irrigation depths, the higher value for g_s was obtained in seedlings produced with 12 mm irrigation depth (0.173 mol H_2O m^{-2} s^{-1}), and the lower value was obtained in seedlings produced with 6 mm irrigation depth (0.048 mol H_2O m^{-2} s^{-1} – Fig. 2). Transpiration varied from 0.804 to 1.88 mol H_2O m^{-2} s^{-1} in seedlings produced with 6 mm and with 12 mm irrigation depth, respectively (Fig. 2).

There was little variation in the intracellular C_i (Fig. 2) and C/C_i, indicating that variations in substrate characteristics and irrigation depths did not sufficiently alter these variables. With increased irrigation depths, WUE reduced significantly, indicating that increased irrigation depths promote greater water loss by plants to produce the same amount of biomass. The highest WUE was obtained in seedlings produced with 6 mm irrigation depth [1.94 µmol CO_2 (mol $H_2O)^{-1}$], and the lowest WUE was obtained in seedlings produced with 12 mm irrigation depth [0.90 µmol CO_2 (mol $H_2O)^{-1}$]. For stomatal conductance (g_s), transpiration (*E*), intracellular C (C_i), carboxylation efficiency (C/C_i), and water use efficiency (WUE), there was not interaction between factors, and differences were significant only for irrigation depths (Fig. 3).

Discussion

Physical and chemical characteristics of substrates

The close relationship between substrate porosity and the availability of air and water for plant root system growth highlights the importance of these characteristic, associated with water management, for forest seedling production (Pascual et al. 2018). The addition of *Eucalyptus* bark to sewage sludge increased microporosity, and the addition of sugarcane bagasse increased macroporosity (Tab. 1). Organic compounds, such as sewage sludge, have high density and, consequently, low total porosity (Raviv 2013), but we observed that the addition of structuring materials

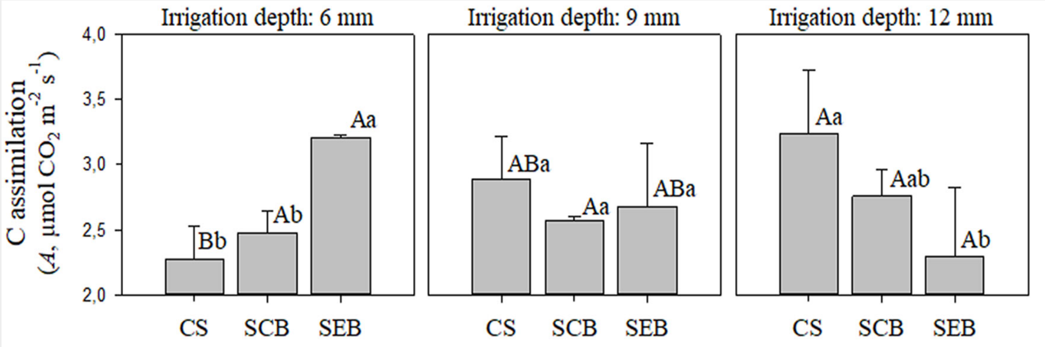

Fig. 3 - Carbon assimilation (*A*) of *Cedrella fissilis* seedlings at 151 days after sowing. (CS): commercial substrate composed of Sphagnum peat, vermiculite, and rice husk; (SCB): sewage sludge + sugar cane bagasse (1:3 v:v); (SEB): sewage sludge + Eucalyptus bark (1:3 v:v).

into the composting process made these materials suitable for seedling production, as displayed in Fig. 1, Fig. 2, and Fig. 3.

In our experiment, substrates with different characteristics were obtained as a function of the constituent materials. The appearance of the particles that compose them, such as size and shape, is one of the main factors that directly affect the arrangement of mixtures (Fermino et al. 2018). The water holding capacity of a substrate is the result of these characteristics and is important for establishing water management in seedling production, as it determines water drainage after irrigation. Among the substrates used in this study, only SBC presented a reduced value for this characteristic, probably due to the fibrous character of sugarcane bagasse. The adequate physical characteristics of substrates to produce forest seedlings can vary according to several factors such as the species needs, type of packaging, propagation form, and management adopted in the nursery must be considered (Fornes & Belda 2019, Gabira et al. 2020b).

The chemical characteristics of substrates were also influenced by the materials of which they were composed. The substrate SEB presented high levels of Ca, Mg, Na, K, B, and Fe (Tab. 2), which can be explained by the high concentration of these nutrients in *Eucalyptus* bark (Salvador et al. 2016). High N contents, observed mainly on SCB, are important to regulate other nutrients uptake and interactions necessary for plants growth (Hu & Chu 2020). Adding sewage sludge to the substrates enhanced nutrients and organic matters content in SEB and SCB according to previous studies (Guerrini & Trigueiro 2004, De Abreu et al. 2017, Gabira et al. 2020a). The higher P content observed in SC (33.33 mg L^{-1}) is due to the addition of simple superphosphate (P_2O_5) by the substrate manufacturer.

Tab. 2 - Nutrient concentration (N, P, K, Ca, Mg, S, Na, B, Cu, Fe, Mn, Zn), pH and electrical conductivity of the substrates used to produce seminal seedlings of *Cedrela fissilis*. Averages followed by the same letter in the row do not differ (p > 0.05) by Tukey test. (CS): Commercial substrate of *Sphagnum* peat, vermiculite, and 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).

Nutrients	**Substrates**		
	CS	**SCB**	**SEB**
N (mg L^{-1})	0.66 ^{c}	2.53 ^{a}	1.84 ^{b}
P (mg L^{-1})	33.33 ^{a}	1.33 ^{b}	1.33 ^{b}
K (mg L^{-1})	93.07 ^{b}	55.73 ^{c}	159.07 ^{a}
Ca (mg L^{-1})	23.67 ^{c}	386.67 ^{b}	613.33 ^{a}
Mg (mg L^{-1})	49.00 ^{c}	98.00 ^{b}	138.67 ^{a}
S (mg L^{-1})	146.67 ^{b}	560.00 ^{a}	580.00 ^{a}
Na (mg L^{-1})	24.56 ^{c}	34.90 ^{b}	50.23 ^{a}
B (mg L^{-1})	0.07 ^{b}	0.06 ^{b}	0.15 ^{a}
Cu (mg L^{-1})	0.18 ^{a}	0.10 ^{b}	0.11 ^{b}
Fe (mg L^{-1})	1.33 ^{b}	0.90 ^{c}	2.47 ^{a}
Mn (mg L^{-1})	0.51 ^{c}	4.21 ^{b}	6.07 ^{a}
Zn (mg L^{-1})	0.69 ^{b}	2.36 ^{a}	0.44 ^{c}
pH	6.30 ^{a}	4.93 ^{c}	5.92 ^{b}
EC (dS m^{-1})	0.89 ^{b}	2.93 ^{a}	3.45 ^{a}

The pH of organic substrates used for seedling production should be around neutrality, between 5.0 and 6.0, for better availability of nutrients (Taiz et al. 2014). Thus, all the substrates used in this experiment match the recommendations. Substrate pH directly affects the mobility and availability of ions and, when outside the indicated range, nutrient deficiency or toxicity may occur (Pascual et al. 2018). The electrical conductivity (EC) results were similar to those obtained by Guerrini & Trigueiro (2004) for substrates with different proportions of sewage sludge. EC is related to the total amount of soluble salts in the saturated extract of the substrates; high EC values are expected in organic composts and can affect root growth (Raviv 2013). However, values outside this standard may not impair the growth of seedlings of certain species, as observed in this study with *C. fissilis*.

Seedling growth

Studies conducted in nurseries are based on morphological or physiological variables to determine seedling quality, however, it is important to relate seedlings characteristics to its survival after planting in the field (Grossnickle & MacDonald 2018, Stuepp et al. 2020). The variables most used to determine forest seedlings' quality are height and stem diameter because the measurement is simple, fast, and non-destructive (Ivetić et al. 2016). The correlation with Dickson Quality Index also shows height and stem diameter as important indicators of seedling quality (Madrid-Aispuro et al. 2020). In this study, we obtained satisfactory values of these variables for all treatments, especially for seedlings produced in SEB.

Bortolini et al. (2017) recorded a maximum height of 22.2 cm in seedlings growing in substrates with sewage sludge 90 days after sowing. This result agrees with those obtained in our study (23.0 cm in seedlings produced in SEB under 12 mm irrigation depth). Taller plants have an advantage in areas with vegetation competing for light; however, tall seedlings can have a higher sensitivity to wind and rain when growth is not accompanied by an increase in stem diameter (Grossnickle & MacDonald 2018). Also Bueno et al. (2020) produced taller plants under higher irrigation depths. However, we observed that seedling height depended mainly on substrate characteristics, which was not evaluated by the authors.

Stem diameter was also influenced by substrates, although all the substrates allowed the growth of seedlings with an adequate diameter for outplanting, similar to those obtained by Navroski et al. (2016). Stem diameter is considered the best morphological attribute to forecast field growth, especially because it correlates with seedling weight and root system size (Stuepp et al. 2020). Ivetić et al. (2016) highlight that this morphological characteristic well correlates with initial growth in the field, although it is unable to predict seedlings' survival.

Regardless of irrigation depths, SEB provided seedlings with the largest amount of dry mass, although seedlings produced using 6 mm irrigation depth presented lower values than seedlings produced in SCB. Other studies using sugarcane bagasse and sewage sludge as substrate obtained better morphological characteristics of *Eucalyptus* and *Peltophorum dubium* seedlings growing under adequate irrigation depths (Gabira et al. 2020a, Manca et al. 2020, Silva et al. 2020a). The lower total porosity values of SEB may have hindered the growth of the root system of seedlings under a low irrigation depth, making it difficult for water to go beyond the superficial region of the tube. Maximum growth is achieved when the allocation to photosynthetic tissues is maximized, although it must be considered that dry mass importance lies not only in the photosynthetic process but also in nutrient storage and during photosynthesis (Bravo Baeza et al. 2019, Kumarathunge et al. 2019).

Bueno et al. (2020) observed that increasing water supply can fail in improving seedlings development, which suggests the importance to know the requirements of the species during seedling production. If low water volumes are applied diregarding the proper maintenance of water in the container, stomata may close at higher temperatures, impairing photosynthesis and, consequently, plants growth and biomass accumulation (Taiz et al. 2014). Higher irrigations can increase nutrients leaching, and reduce water and nutrient use efficiency, as observed by Silva et al. (2020b) in tropical species.

Root system quality determines seedlings' ability to produce new roots quickly, defining the velocity with which they fix to the soil and grow soon after planting (Khanal et al. 2018). Root dry mass is related to higher survival and growth potential because they are required for the absorption and transport of water and nutrients from the soil to the shoot (Grossnickle & MacDonald 2018). The appearance of particles that compose the substrates, such as size and shape, is one of the main factors that affect mixture arrangement and determines the space that the root system has to grow, especially in restricted volume containers (Fermino et al. 2018).

Gas exchange

Despite the practical importance of morphological measurements, physiological attributes have a major influence on planting performance. Physiological processes are strongly influenced by environmental conditions, and understanding these processes is central to predict adaptive responses to field conditions, producing seedlings capable of establishing and growing in the field (Salmon et al. 2020). We observed that irrigation depths provided different plant responses in each substrate regarding gas exchange. Photosynthesis is a balance of light energy absorbed and consumed by plant's metabolic sinks and is therefore very sensitive to environmental changes (Zhang et al. 2020).

The higher assimilation rates (A) allow species to maintain a competitive advantage over other plants at the post-establishment stage, as described by Le et al. (2019). Furthermore, the availability of water and air to the root system is very important for seedling growth, and substrates characteristics may provide these resources (Taiz et al. 2014). A reduction of A in seedlings produced under 9 and 12 mm in SEB may be associated with the bigger size seedlings reached at the production cycle end, indicating the need for a larger container or immediate planting.

With lower irrigation depths, stomatal conductance (g_s) and transpiration (E) decreased. Bravo Baeza et al. (2019) obtained lower values of g_s and E in *Cedrela odorata* subjected to dry periods, which reflected in a higher WUE efficiency, as confirmed by our results. These authors also state that g_s is an important predictor of water use and carbon assimilation, limiting plant growth, as observed in our results. Stomatal closure is a common response to water stress, as well as high vapor pressure deficit values (Taiz et al. 2014). It justifies the higher E in plants with higher water availability detected in this study.

We observed that carbon assimilation (C_i) values did not coincide with the alterations of g_s and E. It indicates that, despite the stomatal opening, there was no increase in CO_2 input in leaves, which can be explained by changes in the photosynthetic apparatus due to water or nutrient availability for plants (Baron et al. 2013). Photosynthesis is not only related to stomatal limitations but also nonstomatal limitations, such as the diffusion of CO_2 from intercellular spaces to the chloroplasts and biochemical limitations to photosynthetic efficiency (Salmon et al. 2020). The availability of C within the mesophyll cells is one of the factors that regulate the C/C_i ratio, increasing or reducing its carboxylase activity and A (Taiz et al. 2014).

Reflecting A and E values, water use efficiency (WUE) generally decreased as irrigation depths increased. We emphasize that not always the greatest WUE results in the greatest growth of seedlings, given that the assessment of gas exchange was carried out at the end of the production cycle. The increase in WUE is a preventive mechanism and immediate effect of water deficit related to g_s, as also reported in *C. odorata* subjected to dry periods (Bravo Baeza et al. 2019). Water availability and its use is an important factor determining growth and photosynthesis because it relates to temperature in leaves, which directly affects gas exchange (Kumarathunge et al. 2019). That is why this parameter is very important for defining irrigation management in nurseries, and it is clear from our results that an increased amount of water applied to seedlings does not always proportionally result in seedlings' growth and gas exchange.

Conclusion

In this study, we evaluated the use of sewage sludge-base substrates for seedlings production to increase the sustainability of seedling production process. Our results of morphological and physiological characteristics demonstrated that the substrates used in our study was able to provide conditions needed to produce high-quality seedlings of *Cedrela fissilis* Vell. The daily irrigation depth must be adequate to species needs and substrates characteristics, increasing irrigation depth in substrates with reduced water holding capacity.

Acknowledgements

Funding was provided by CAPES - Coordination of Improvement of Higher Education Personnel, Brazil (financing code 001), FAPESP - São Paulo State Research Foundation, Brazil (process: 2013/50413-0), and the *Ministère des Relations Internationales et de la Francophonie – Coopération Québec-Brésil* from Québec (Canada) obtained by MMG.

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