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Organic waste and controlled-release fertilizer maximize the growth of *Citharexylum montevidense* in nursery and in the field

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SILVICULTURE

ABSTRACT

Background: The increase in agricultural production requires sustainable alternatives development for reusing the waste generated, such as bovine manure, to obtain ecological, environmental, and economic benefits. In addition, proper fertilization is one of the main steps in the production of forest species seedlings. Thus, this study aimed to characterize the effect of different substrates formulations based on bovine manure and types of fertilizers on the quality of *C. montevidense* seedlings and verify if the field confirms the nurseries' responses. The treatments consisted of three BM-based substrates combinations (S0 - control, no manure; S30 – 30 % BM; and S60 – 60 % BM) and four types of base fertilizers [Control: no fertilizer, FRF - Fast Release Fertilizer (NPK + fritted trace elements), FRF2 - twice as used in FRF and CRF - Controlled Release Fertilizer]. The substrates quality and the seedlings' morphophysiological attributes were determined 160 days after sowing, then conducting the best treatments to the field.

Results: We verified that the use of bovine manure improved the physical and chemical properties of the substrates. For the *C. montevidense* cultivation, 30 % BM plus CRF provided the best seedling development. The substrate used in the seedlings production influenced the post-planting performance of *C. montevidense*, and the bovine manure use allows for more significant plant growth in the field.

Conclusion: For the cultivation of *Citharexylum montevidense*, it is recommended to use substrates composed of 30 % of bovine manure, associated with 6 g L^{-1} of controlled-release fertilizer, as it provides higher quality seedlings in shorter production time in the nursery.

Keywords: Alternative substrates; *Citharexylum montevidense*; Forest species; Morphophysiology.

HIGHLIGHTS

Highest morphophysiological quality was observed with bovine manure
Bovine manure can be used in the production of seedlings in containers
Organic waste with controlled release fertilizer maximizes seedling growth
The use of 30% of bovine manure provides higher quality seedlings

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INTRODUCTION

In recent decades, the Brazilian bovine herd has shown continuous growth and is around 214 million heads (IBGE, 2020). Currently, Brazil is the second-largest producer globally (USDA, 2021), which has resulted in large amounts of cattle waste. Furthermore, according to Sarfaraz et al. (2020) immense quantities of animal and vegetable residues can be found in the country's southern region due to many agricultural enterprises. Thus, it is essential to see possibilities for reusing the waste generated, such as cattle waste, to obtain ecological, environmental, and economic benefits by mitigating the impacts caused by inadequate disposal in the environment.

In this context, Trazzi et al. (2012) emphasize that an alternative for using bovine manure would be the use it as a substrate component for the tree seedlings production because, when stabilized, it adds essential improvements in the physical, chemical, and biological conditions of the input. Benefits include increased water retention capacity, porosity, aggregation, and nutrient availability, such as nitrogen (Lima et al., 2017; Wendling and Gatto, 2002), not to mention the low cost and ease of acquiring. Thus, bovine manure use in the formulation of the substrate for tree seedlings production can increase growth rates and biomass production, ensuring the production of quality plants at a low cost. At the same time, carbonized rice husks also constitute a critical agro-industrial residue in increasing the aeration space in substrates. Besides, several studies worldwide search for sustainable alternatives to reduce or replace components from non-renewable sources in the formulation of substrates (Nhantumbo et al., 2021; Sax and Scharenbroch, 2017; Silva et al., 2020).

Substrate fertility is also a critical factor in forest nurseries, as seedlings, in addition to rapidly depleting the nutrients stored within the seeds, have limited reserves (Jacobs and Landis, 2009). In this sense, identifying the ideal base fertilization in the production of seedlings is extremely important, considering that it is essential to maximize plant growth rates (Klooster et al., 2012). In the production of seedlings in containers, the nutritional needs of each species, the substrate and container used, the irrigation intensity, and frequency direct the fertilizer application (Navroski et al., 2018).

However, in the forestry sector, limitations result from the lack of knowledge and adequate technology for producing many tree species, restricting the sale of seedlings of several native species (Faria et al., 2017). Thus, studies with an emphasis on defining strategies and protocols testing alternative substrates and nutrient sources for seedlings are trending, aiming to contribute to the expansion of efficiency in the production of quality seedlings with a reduction in costs and, consequently, after planting, an increase in survival and initial growth rates.

Among the South American native trees, *Citharexylum montevidense* (Spreng.) Moldenke stands out, which belongs to the Verbenaceae family, occurring naturally in Argentina, Paraguay, Uruguay, and Brazil (Tropicos, 2021). *C. montevidense* is a pioneer species, primarily found in dry and stony soils in early stages of ecological succession, riparian forests, clumps, and open fields of the Pampa and Atlantic Forest Biomes in southern Brazil (Bueno and Leonhardt, 2011; Lorenzi, 2009).

The specie has ecological, medicinal, and ornamental potential, being useful for forest restoration plantations due to its fast growth, good canopy architecture, and large amounts of fleshy fruits, appreciated by the avifauna (Lorenzi, 2009). As for medicinal properties, the fruit treats respiratory diseases, and the infusion made with the bark serves as a diuretic (Sharry et al., 2011). Additionally, due to its vigorous growth, intense flowering, and resistance to pests and diseases, it can be used in urban afforestation (Bueno and Leonhardt, 2011).

The present study hypothesizes that bovine manure utilization as a substrate component maximizes growth and reduces the need for chemical fertilization to produce *C. montevidense* seedlings. Thus, this study aimed to characterize the effect of different substrates formulations based on bovine manure and types of fertilizers on the quality of *C. montevidense* seedlings and verify if the field confirms the nurseries' responses.

MATERIAL AND METHODS

Seedling production

The nursery experiment was carried out from May to October (autumn to spring) in a greenhouse at the Forest Nursery of the Federal University of Santa Maria (29°43' S and 53°43' W) in Santa Maria, Rio Grande do Sul, do Sul, Brazil. According to Köppen's classification, the regional climate is of the primary type Cfa, humid subtropical, characterized by having rainfall well distributed throughout the year (Alvares et al., 2013).

The seeds used in the study came from mature fruits of *C. montevidense*, collected from six trees, with a distance of more than 400 meters from each other, located in remnants of the Seasonal Deciduous Forest in the central region of Rio Grande do Sul. After collection, the fruits were submerged in water for 24 hours to carry out the pulping, being kept on filter paper in a shaded and ventilated environment for approximately 48 hours. Subsequently, the seeds were stored in paper bags and placed inside Kraft paper drums, remaining in a cold chamber, with a temperature of ± 10 °C and relative humidity around 80 %, until the experiment begins. The experimental design used was completely randomized in a 3 × 4 factorial scheme, represented by three substrate formulations and four types of base fertilization (Table 1). Four replicates were used, with 24 plants each.

The substrates used for the seedlings production were composed of different proportions of a commercial substrate (CS) based on *Sphagnum* peat, vermiculite, dolomitic limestone and agricultural plaster (EC ≈ 0.7); tanned bovine manure (BM), and carbonized rice husk (CRH). Bovine manure was obtained from a rural property in the municipality of Faxinal do Soturno/RS. The manure underwent a biological stabilization process in an open place for about 60 days. After being tanned, it passed through a sieve of 3 mm mesh.

The base fertilization tested fast-release (FRF) and controlled-release (CRF) fertilizers. Base fertilization with FRF followed the recommendation of Gonçalves et al. (2005), consisting of urea, simple superphosphate, potassium chloride, and frits (micronutrients). Fertilization with CRF used Osmocote®, which presents: 18% N; 5% P2O5; 9% K2O; 1% Mg, 2.3% S; 0.05% Cu; 0.06% Mn; 0.45% Fe and 0.2% Mo, with nutrient release between five and six months, as per the manufacturer's technical recommendation (Table 1).

For the seedlings production, conical polypropylene tubes with a volume of 180 cm^3 were used, placed in plastic trays suspended one meter from the surface. The substrate was homogenizing with the proper proportions of components and fertilizers and then filled in the containers. Sowing was carried out directly in the tubes, using three seeds per container. Then, the trays were taken to the greenhouse, where they received micro-sprinkler irrigation (8 mm dia^{-1}). The thinning occurred after 60 days, leaving only the most vigorous and central seedling.

Substrates analysis

A control treatment with no addition of fertilization characterized the substrates' chemical and physical properties. The description of wet and dry density (WD and DD, respectively), pH, and electrical conductivity (EC) followed the Brazilian Normative Instructions No. 17 (Brasil, 2007) and No. 31 (Brasil, 2008). Total porosity (TP), aeration space (AS), readily available water (RAW), water retention capacity at 10 cm (WRC10) and 50 cm (WRC50) were determined using the methods described by Kiehl (1979). The substrates macronutrient content determination was made in the Laboratory of Soil and Plant Tissue Analysis of the Regional Integrated University of Frederico Westphalen, in the municipality of Frederico Westphalen, Rio Grande do Sul State, Brazil obtained by the methods of Tedesco et al. (1995).

At the end of the experiment, at 160 days after sowing (d.a.s), three seedlings per treatment were evaluated for root aggregation with the substrate (RAS) and ease of removal of the seedlings from the tube (RT), according to the methodology described by Wendling et al. (2007). These analyzes were performed one hour after irrigation in order to standardize the results obtained. For the RAS, the seedlings without the tubes were released in free fall one meter from the ground under a crushed rock, where scores from zero to ten were assigned, with zero for the wholly chipped clod and ten for the intact clod. As for the RT, the analysis consisted of assigning scores from zero to ten, with zero being the maximum difficulty and ten being the maximum ease of removal of the seedlings, after three taps with a steel rod measuring 0.8 mm in diameter and 25 cm in length at the top of the tube.

Morphological attributes

At 160 days after sowing, the eight central plants of each repetition were measured, obtaining the height (H) and the stem diameter (SD), using a millimeter ruler and a digital caliper (precision of 0.01 mm), respectively. By sectioning the plant into the aerial part and root system and washing the latter part in running water, with the aid of sieves to remove the substrate, it was possible to quantify the leaf area (LA) and root length (RL). Then, the leaves and roots were distributed on white paper and covered with transparent glass, containing a scale reference. The photographs were taken with a digital camera, attached to a fixed structure, and processed by the ImageJ® software.

For the determination of shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM), the material used in the LA and RL evaluations was packed in Kraft paper packages and dried in a circulation oven air force, at a temperature of 65 ± 5 °C to constant weight. Then, the dry matter was weighed, expressed in grams, using an analytical balance (precision 0.01 g). With these data, the Dickson quality index (DQI) was determined (Dickson et al., 1960).

Physiological attributes

A pulse-modulated fluorometer (Junior-Pam Chlorophyll Fluorometer, Heinz Walz GmbH) was used to analyze chlorophyll a fluorescence from 7 am to 11 am. Previously, a fully expanded sheet of the middle third was dark-adapted with aluminum foil for 30 minutes. Then, the reading was performed, obtaining the values of the maximum quantum yield of photosystem II (F_v/F_m). A portable meter (ClorofiLOG, CF 1030, Falker Automação Agrícola, Brazil) determines the chlorophyll leaf index in expanded leaves of the middle third of the plant. One leaf per plant was measured (two readings per leaf, one on each side of the midrib), considering four plants per treatment. The Falker Chlorophyll Index (FCI*a* and FCI*b*) express the results, combining the analyzed light wavelengths.

Table 1. Description of the treatments used in the *Citharexylum montevidense* seedlings production in a nursery.

Substrate % BM	Fertilizer
S0 – 80 % CS + 20 % CRH	Control (no fertilizer)
S30 – 50 % CS + 20 % CRH + 30 % BM	FRF - 100 g L^{-1} of urea, 300 g L^{-1} simple superphosphate, 100 g L^{-1} of potassium chloride e 150 g L^{-1} fritted
	FRF2 - 200 g L^{-1} of urea, 600 g L^{-1} simple superphosphate, 200 g L^{-1} of potassium chloride e 300 g L^{-1} fritted
S60 – 20 % CS + 20 % CRH + 60 % BM	CRF - controlled release fertilizer, Mini Prill, 18-05-09 ($N-P_2O_5-K_2O$) 6 g L^{-1}

Where: CS - Commercial Substrate; CRH - Carbonized Rice Husk; BM- Bovine Manure.

Initial field growth

The planting of seedlings in the field was carried out in November 2015 in Santa Maria (29°47'39"S and 53°40'13"W). Nine of the twelve treatments tested in a nursery were used because the seedlings cultivated in the substrate S0 (0% of bovine manure) without base fertilization or fertilized with a ready-release fertilizer (FRF and FRF2) did not express growth and root development that allowed the seedlings expedition to the field. Therefore, a randomized block design with four replications was used, with the sampling unit consisting of six seedlings, totaling 216 plants.

During the study period (November 2015 to May 2017), precipitation and mean air temperature were 155.8 mm month^{-1} and 20.7 °C, respectively. The soil of the experimental area was classifies as Arrenic Dystrophic Red Argisol (Embrapa, 2013). Furthermore, Berghetti et al. (2020) described the soil's physical and chemical characteristics in the area, which served as a reference for correcting acidity and fertilization. To define the amount of fertilizer needed, the recommendation for *Eucalyptus* spp. cultivation (CQFS-RS/SC, 2016) was adopted, as there is no recommendation for *C. montevidense*.

In the preparation of the area, 30 days before planting, elimination of invasive plants had been carried out using brush cutters, and application Sulfuramide-based baits were applied to control leaf-cutting ants. In addition, dolomitic limestone [$CaMg(CO_3)2$] broadcast liming using a relative neutralizing power of 76 %. Thus, even before planting, a non-selective systemic herbicide chemical (glyphosate) controlled the herbaceous vegetation in the total area. For planting, holes (30 cm in diameter and 30 cm in depth) were dug with a manual digger, with a spatial arrangement of 1 m x 1 m. The seedlings were planted and then irrigated with two liters of water per seedling to avoid the possible formation of air pockets between the clod and the soil and dehydration of the roots.

Thirty days after planting, all plants were fertilized with 50 kg ha^{-1} of nitrogen, 120 kg ha^{-1} of phosphorus, and 45 kg ha^{-1} of potassium, using urea, triple superphosphate, and potassium chloride as sources, respectively. In addition, a urea application of 30 kg ha^{-1} at 120 days after planting. On both occasions, the application was performed in lateral holes, approximately 15 cm away from the seedling. The applications was performed with moist soil, to facilitate the absorption of nutrients. Maintenance activities consisted of weed control through crowning and semi-mechanized mowing, using brush cutters. The H and SD evaluations were performed at the time of planting and at the end of the experiment (0 and 540 days, respectively), through the height increase (HI) and stem diameter increase (SDI) were calculated. In addition, at the end of the monitoring period, the survival percentage (S%) was evaluated through visual observation.

Statistical analysis

For both experiments, the data were subjected to Shapiro-Wilk and Bartlett tests to verify the assumptions of normality of residuals and homogeneity of variance analysis, respectively. Box-Cox method transformed the data that did not meet the premise. Subsequently, by applying the factorial analysis of variance (ANOVA) (substrate vs. fertilization) for nursery data and One-way ANOVA for field data, the analysis of variance was presented, discarding full factorial. When a significant difference was present, the Tukey test at 5% probability of error (p=0.05) compare,d the means. Complementarily, for the nursery experiment, the Pearson correlation (r) was applied between the seedlings' morphological, physiological, and clod quality variables. All analyzes employed the statistical software RStudio (R Core Team, 2018) with the aid of ExpDes (Ferreira et al., 2021) and Metan (Olivoto, 2021) packages.

RESULTS

Physical and chemical analysis of the substrates

Wet and dry densities improved with the bovine manure increasing proportion in the substrate. Also, total porosity, aeration space, and water holding capacity were higher in substrates S30 and S60 than in substrates without manure (Table 2).

In general, for the chemical characteristics, it was observed that the attributes increased linearly with the addition of bovine manure to the substrate, except for calcium (Ca) and magnesium (Mg). Substrates S30, with 5.7, and S60, with 5.9, had the highest pH values. Regarding electrical conductivity, S60 presented the highest mean (1.0 dS m^{-1}) and S0 (0.5 dS m^{-1}) the lowest (Table 2). Substrates added with 30 % (S30) or 60 % (S60) of bovine manure showed a significant increase in N, P, and K availability. Under these conditions, the P contents were 4.8 and 6.3 times higher than the observed in the control substrate (*Sphagnum peat* and *vermiculite* - S0).

Table 2. Physical and chemical properties of substrates used in the production of *Citharexylum montevidense* seedlings.

Substrate	HD	DD	TP	AS	WEA	WRC10	WRC50	pH	EC	N	P	K	Ca	Mg
S0	185.0	169.0	66.0	14.0	13.0	51.0	39.0	4.9	0.5	0.8	0.1	0.3	1.5	2.0
S30	297.0	273.0	79.0	26.0	13.0	57.0	44.0	5.7	0.7	1.0	0.3	0.4	1.5	1.3
S60	359.0	318.0	80.0	23.0	13.0	58.0	44.0	5.9	1.0	1.1	0.4	0.4	1.2	0.6

Where: HD = humid density (kg m^{-3}); DD = dry density (kg m^{-3}); TP = total porosity (%); AS = aeration space (%); WEA = water easily available (%); WRC10 = water retention capacity from 10 cm (%); WRC50 = water retention capacity from 50 cm (%); EC = electrical conductivity (dS m^{-1}); N = nitrogen (%); P = phosphorus (%); K = potassium (%); Ca = calcium (%); Mg = magnesium (%); S0 = 80 % CS + 20 % CRH; S30 = 50 % CS + 20 % CRH + 30 % BM, and S60= 20 % CS + 20 % CRH + 60 % BM.

Seedling growth in the nursery

It was verified a significant interaction ($p<0.05$) between both the substrates tested and the types of base fertilization concerning the morphological attributes of stem diameter (SD), H/SD ratio, leaf area (LA), root length (RL), and Dickson quality index (DQI). Therefore, for these attributes, the base fertilization to be used will depend on the type of substrate used (Table 3). *C. montevidense* seedlings showed the best SD values when produced in substrate S60 (20 % CS + 20 % CRH + 60 % BM), regardless of the use or not of base fertilization. However, seedlings cultivated on substrates S0 (80 % CS + 20 % CRH) and S30 (50 % CS + 20 % CRH + 30 % BM) obtained the best results when using controlled-release fertilizer (CRF) as a source of nutrients (Table 3). For the attributes LA, RL and DQI, the highest values were obtained when using the substrates S60 and S30, plus CRF in base fertilization (Table 3).

The growth in height (H) and dry matter production (DM) of *C. montevidense* seedlings significantly affected each factor (substrate formulations and types of base fertilization). Seedlings cultivated in substrate containing the highest proportion of bovine manure (S60= 20 % CS + 20 % CRH + 60 % BM) presented the highest values of H (22.9 cm), RDM (1.2 g), SDM (2.8 g), and TDM (3.9 g). In this condition, the growth in H was 72.0 % and 20.1 % higher than that observed in the substrate S0 (80 % CS + 20 % CRH) and S30 (50 % CS + 20 % CRH + 30 % BM), respectively (Figure 1A). Furthermore, it was found that there was a decrease in the accumulation of root and leaf dry matter of 1000% and 612%, respectively, when using the substrate S0 in relation to S30 (Figure 1B).

Table 3. Effect of different substrates and base fertilization on stem diameter (SD), H/SD ratio, leaf area (LA), root length (RL), Dickson Quality Index (DQI) of *Citharexylum montevidense* seedlings, at 160 days after sowing.

Attributes	Substrates % BM	Base fertilization				Average
		Control	FRF	FRF2	CRF	
SD (mm)	S0	0.6 Bb*	0.9 Bc	0.7 Bb	3.2 Ab	1.4
	S30	4.0 ABa	3.6 Bb	3.9 Aba	4.7 Aa	4.0
	S60	4.5 Aa	4.6 Aa	4.4 Aa	5.2 Aa	4.8
	Average	3.1	3.1	3.0	4.3	
		F(*p*) S*A = 6.8 (0.000)			CV (%) = 12.7	
H/SD	S0 S30	4.7 Aa	4.1 Aa	4.3 Aa	5.0 Ab	4.6
	S30	3.9 Ba	4.3 Ba	3.9 Ba	6.0 Aa	4.6
	S60	4.2 Ba	4.4 Ba	4.2 Ba	6.2 Aa	4.8
	Average	4.3	4.3	4.2	5.8	
		F(*p*) S*A = 2.4 (0.05)			CV (%) = 12.1	
LA (cm^2)	S0	6.7 Bc	5.7 Bc	3.4 Bb	97.0 Ab	28.2
	S30	161.0 Bb	140.3 Bb	143.3 Ba	384.8 Aa	207.4
	S60	251.2 Ba	226.6 Ba	183.8 Ba	421.6 Aa	270.8
	Average	139.6	124.2	110.1	301.2	
		F(*p*) S*A = 5.1 (0.0007)			CV (%) = 21.3	
RL (cm)	S0	1.5 Bb	2.6 Bb	1.5 Bb	7.8 Ac	3.1
	S30	28.6 Aa	28.4 Ba	29.8 Aa	36.6 Ab	30.8
	S60	34.0 Ba	33.3Ba	32.3 Ba	47.8 Aa	36.2
	Average	21.4	21.4	21.2	30.8	3.1
		F(*p*) S*A = 3.0 (0.01)			CV (%) = 16.8	
DQI	S0	0.006 Bb	0.02 Bc	0.009 Bb	0.2 Ab	0.06
	S30	0.4 Ba	0.3 Bb	0.4 Ba	0.8 Aa	0.5
	S60	0.6 Aa	0.5 Ba	0.4 Ba	0.7 Aa	0.6
	Averages	0.3	0.2	0.2	0.6	
		F(*p*) S*A = 4.7 (0.05)			CV (%) = 22.7	

*Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ by Tukey's test (p<0.05). Where: S0 = 80 % CS + 20 % CRH; S30 = 50 % CS + 20 % CRH + 30 % BM, and S60= 20 % CS + 20 % CRH + 60 % BM. Control (without fertilization); CRF = Controlled Release Fertilizer; FRF = Fast-release fertilizer recommended by Gonçalves et al. (2005); FRF2 = Twice as recommended by Gonçalves et al. (2005). CV: Coefficient of variation.

Concerning the fertilizer used in base fertilization, the positive effect was verified of using CRF to produce *C. montevidense* seedlings. When added 6 g L^{-1} of CRF to the substrate, there was an increase in the growth in H of about 100 % in relation to the values observed in the other treatments tested (Figure 2A). The attributes RDM, SDM, and TDM showed the same trend, observing the highest averages with the use of CRF, and under these conditions, root dry matter (1.0 g) and aerial dry matter (3.0 g) were 32 % and 112 % larger than the plants grown in the control treatment (Figure 2B).

About physiological attributes, there was a significant interaction ($p<0.05$) between the substrates tested and the types of base fertilization for the Falker Chlorophyll Index (FCI*a* and FCI*b*) and the maximum quantum yield of the photosystem II (F_v/F_m). *C. montevidense* seedlings produced with CRF as base fertilization had the highest FCI*a* and FCI*b* (Table 4). For the maximum quantum yield of photosystem II, the use of a substrate composed of 30% and 60 % of bovine manure, combined with CRF as a source of nutrients, provided the best results, with values above 0.6 (Table 4).

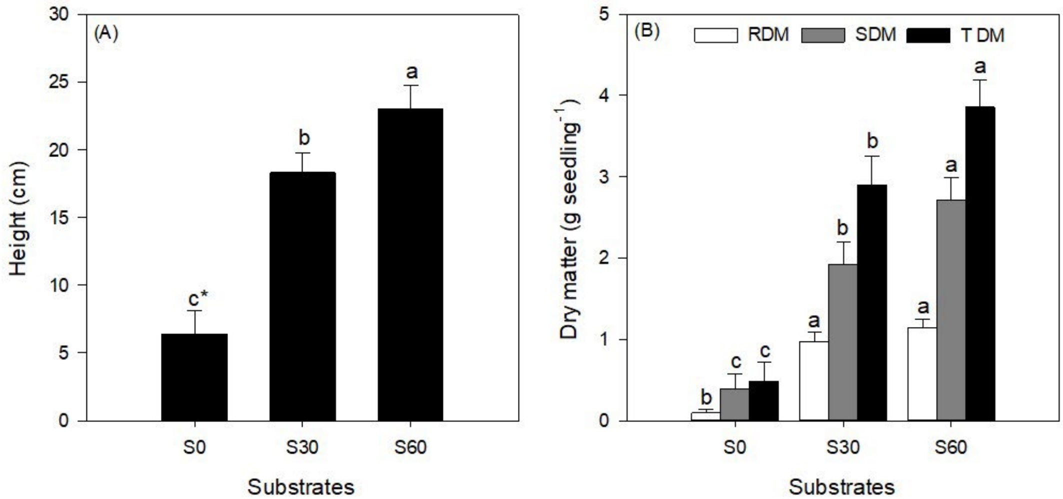

Figure 1. Effect of substrate type on height growth (A) and dry matter accumulation (B) of *Citharexylum montevidense* seedlings at 160 days after sowing. Where: S0 = 80 % CS + 20 % CRH; S30 = 50 % CS + 20 % CRH + 30 % BM; S60 = 20 % CS + 20 % CRH + 60 % BM; RDM = root dry matter; SDM = shoot dry matter (SDM) and TDM = total dry matter. *Comparison of means by Tukey test at 5 % probability of error. Vertical bars indicate standard error.

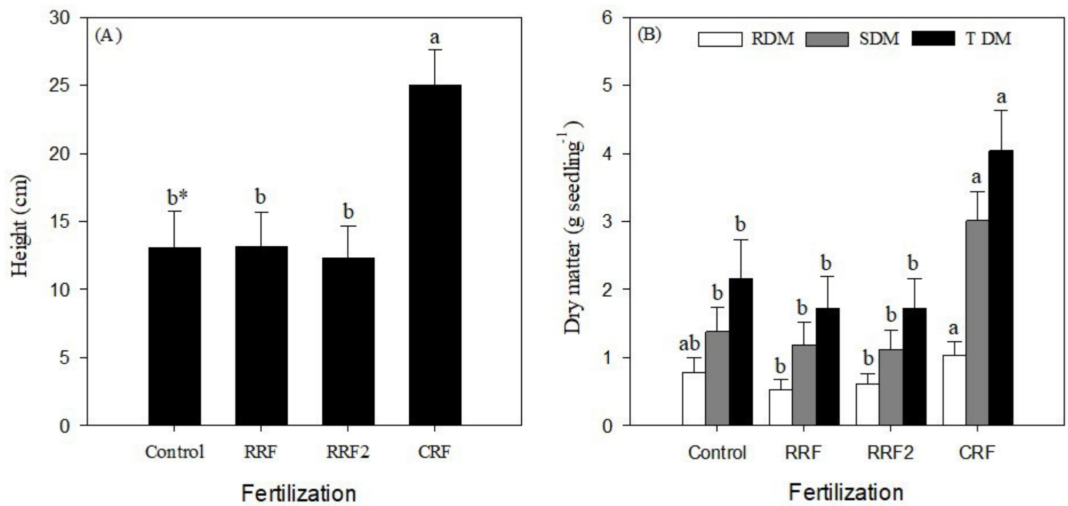

Figure 2. Effect of base fertilization on height (A) and dry matter accumulation (B) of *Citharexylum montevidense* seedlings, 160 days after sowing in the nursery. Of which: Control (without fertilization); FRF = fast-release fertilizer recommended by Gonçalves et al. (2005); FRF2 = Double that used in FRF and CRF = Controlled release fertilizer; RDM = root dry matter; SDM = shoot dry matter and TDM = total dry matter. *Comparison of means by Tukey test at 5 % probability of error. Vertical bars indicate standard error.

The analysis of variance denoted interaction ($p<0.05$) between the tested factors (substrate vs. base fertilization) for the aggregation of roots to the substrate (RAS) and ease of removal of seedlings from the tube (RT). The highest root aggregation occurred in the substrate S60 (S60 = 20% CS + 20% CRH + 60% BM), regardless of the fertilization used (Table 5; Figure 3). Using substrate S0 (80% CS and 20% CRH), combined with the use of ready-release fertilizer (FRF and FRF2), showed the lowest means. There was no grade for seedlings produced without the use of fertilizer in the S0 substrate.

Table 4. Effect of different substrates and base fertilization on Falker Chlorophyll Index (FCI*a* and FCI*b*) and maximum quantum yield of photosystem II (F_v/F_m) of seedlings produced in substrates by different proportions of bovine manure and types of fertilization, to 160 days after sowing.

Attributes	Substrate	Base fertilizer				Average
		Control	FRF	FRF2	CRF	
FCI*a*	S0	9.6 Bb*	12.6Bb	14.2 Bb	36.1 Aa	18.9
	S30	29.4 Ba	28.1 Ba	29.5 Ba	37.1 Aa	30.8
	S60	31.8 ABa	30.4 Ba	33.2 ABa	39.3 Aa	33.2
	Average	23.6	23.7	25.7	37.5	
		F(*p*) S*A = 13.9 (0.000)			CV (%) = 12.0	
FCI*b*	S0	1.6 Bb	2.0 Bb	1.9 Bb	18.0 Aa	1.6
	S30	7.1 Ba	6.7 Ba	7.3 Ba	13.1 Ab	8.6
	S60	8.8 Ba	8.0 Ba	9.2 Ba	14.1 Ab	10.0
	Average	5.9	5.6	6.1	15.07	
		F(*p*) S*A = 11.9 (0.000)			CV (%) = 21.0	
F_v/F_m	S0	0.4 Ab	0.3 Bc	0.6 Aa	0.5 Ab	0.4
	S30	0.6 Ba	0.5 Bb	0.5 Ba	0.6 Aa	0.5
	S60	0.6 ABa	0.6 ABa	0.5 Ba	0.7 Aa	0.6
	Average	0.5	0.5	0.50	0.6	
		F(*p*) S*A = 3.9 (0.008)			CV (%) = 7.2	

* Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ by Tukey's test (p<0.05). Where: S0 = 80 % CS + 20 % CRH; S30 = 50 % CS + 20 % CRH + 30 % BM and S60 = 20 % CS + 20 % CRH + 60 % BM. Control (without fertilization); CRF = Controlled Release Fertilizer; FRF = Fast-release fertilizer recommended by Gonçalves et al. (2005); FRF2 = Twice as recommended by Gonçalves et al. (2005). CV: Coefficient of variation.

Table 5. Root aggregation with the substrate (RAS) and ease of removal from the tube (RT) of Citharexylum montevidense seedlings produced in substrates with different proportions of bovine manure and types of fertilization, 160 days after sowing, in a nursery.

Attributes	Substrates	Base Fertilizer				Average
		Control	RRF	RRF2	CRF	
RAS	S0	0,0 Cc*	0.3 Cc	1.3 Bc	5.8 Ab	7.4
	S30	6.8 Cb	6.4 Cb	8.0 Bb	10.0 Aa	7.8
	S60	10.0 Aa	10.0 Aa	9.0 Aa	10.0 Aa	9.8
	Average	5.6	5.6	6.1	8.6	
		F(*p*) S*A = 31.0 (0.019)			CV (%) = 5.42	
RT	S0	0,0 Bb	0.7 Bc	1.1 Bc	8.0 Aab	2.4
	S30	6.5 Ba	5.9 Bb	7.0 Bb	10.0 Aa	7.4
	S60	8.3 Aa	9.0 Aa	9.1 Aa	6.3 Bb	8.2
	Average	4.9	5.2	5.7	8.1	
		F(*p*) S*A = 25.1 (0.03)			CV (%) = 15.7	

* Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ by Tukey's test (p<0.05). Where: S0 = 80 % CS + 20 % CRH; S30 = 50 % CS + 20 % CRH + 30 % BM and S60 = 20 % CS + 20 % CRH + 60 % BM. Control (without fertilization); CRF = Controlled Release Fertilizer; FRF = Fast-release fertilizer recommended by Gonçalves et al. (2005); FRF2 = Twice as recommended by Gonçalves et al. (2005). CV: Coefficient of variation.

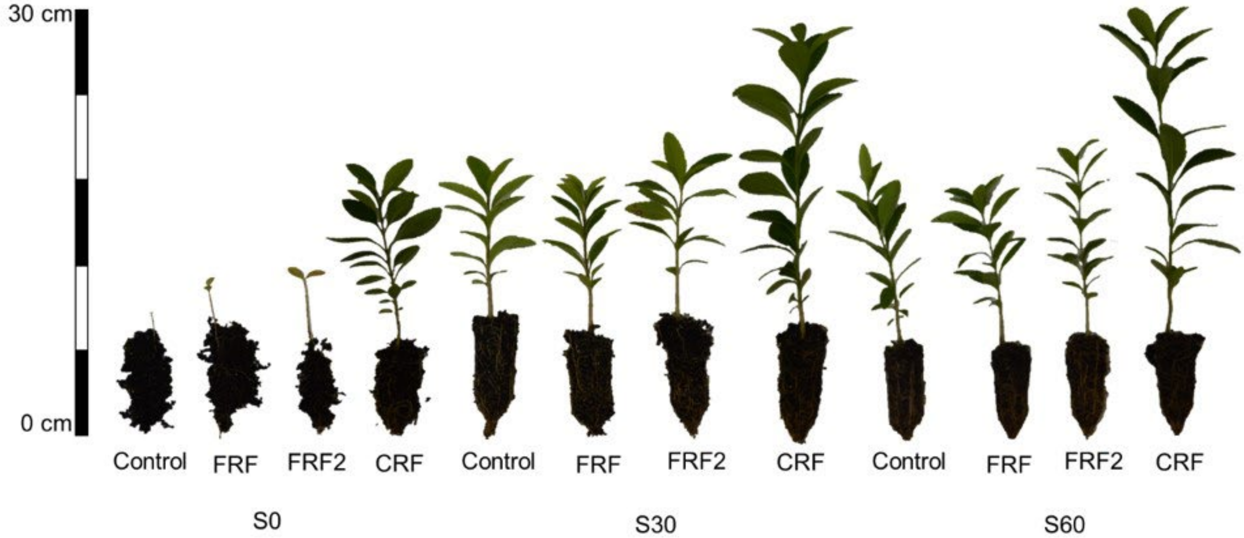

Figure 3. General aspect of root aggregation with substrate for *Citharexylum montevidense* seedlings produced on substrates with different proportions of bovine manure and base fertilization, 160 days after sowing. Where: S0 = 80 % CS + 20 % CRH; S30 = 50 % CS + 20 % CRH + 30 % BM and S60 = 20 % CS + 20 % CRH + 60 % BM. Control (without fertilization); CRF = Controlled Release Fertilizer; FRF = Fast-release fertilizer recommended by Gonçalves et al. (2005); FRF2 = Twice as recommended by Gonçalves et al. (2005).

Regarding RT, the best results were obtained in S0 (8.0) and S30 (10.0) when CRF was used as a nutrient source, as well as in the S60 substrate without base fertilization (8.3) or with the use of FRF (9.0 and 9.1) (Table 5; Figure 3). On the other hand, S0 without the addition of fertilizer, FRF, or FRF2 presented the lowest values for this attribute and when the highest proportion of bovine manure combined with CRF was used (6.3).

The physiological attributes evaluated (FCI*a*, FCI*b*, F_v/F_m) were positively correlated (r>0.51; $p<0.01$) with all growth variables of *C. montevidense* seedlings (Figure 4). The same trend was observed for RAS (r>0.7; $p<0.001$) and RT (r>0.6; $p<0.001$), with the higher values of the morphophysiological attributes. The better quality of the seedling clod, that is, more significant aggregation of roots to the substrate and, consequently, ease of removal of seedlings from the container.

Initial field growth

The survival rate of *C. montevidense* plants at 540 days after planting in the field was 100 %, regardless of the treatments tested. IH and SDI presented a difference in the function of the treatments used in the seedlings production in nurseries ($p<0.05$). The mean values of IH and SDI ranged from 193.8 cm to 239.1 cm (Figure 5A) and 37.8 mm to 47.4 mm (Figure 5B), respectively. Plants of *C. montevidense* cultivated in a substrate composed of 60 % of cattle manure (S60) without base fertilization expressed the most significant increases in height and diameter of the stem in the field. However, they did not differ from treatments composed of substrates based on bovine manure, regardless of the fertilization used. Plants grown on the commercial substrate with carbonized rice husk (S0) and fertilized with CRF had the lowest values for these attributes.

DISCUSSION

Physical and chemical analysis of the substrates

The study proves that bovine manure is efficient when used as a component of substrates for the seedlings production of *C. montevidense*. Furthermore, we verified that the use of bovine manure improved the physical (SD, AS, TP, and RAW) and chemical (pH, CE, and N, P, and K) properties of the substrates. Substrates composed of 30 % (S30) and 60 % (S60) of bovine manure presented adequate DD and TP (Table 2), according to the range proposed by Fermino et al. (2018), which indicate ideal values between 240 to 320 Kg m^{-3} and 77 to 80 % for DD and TP, respectively. Under these conditions, the substrate showed better aeration, allowing gas exchange, avoiding the lack of oxygen to the roots (Kratz et al., 2013), and greater drainage capacity and water availability (water:air ratio).

Aeration space (AS) and water retention capacity (WRC) are also critical physical properties and should be considered in the composition of substrates (Fermino and Mieth, 2018; Jayasinghe et al., 2010) as they guarantee the availability of oxygen and water adequately for the roots (Heiskanen et al., 2013). According to Regan (2014), the ideal range for AS is 10 % to 30 %. In this study, all substrates are within this range (Table 2). However, substrates formulated using bovine manure showed the highest values of AS (S30 = 26 % and S60 = 23 %) and WRC (Table 2). The benefits of bovine manure concerning AS and WRC can be attributed to the arrangement (shape and size) and porosity of the particles as observed, being irregular and rounded, which favors the formation of macropores and the water storage capacity, thus being beneficial for the seedlings production of forest species in containers. Thus, these characteristics allow the forester to adopt water regimes that provide irrigation with less depth and less frequency.

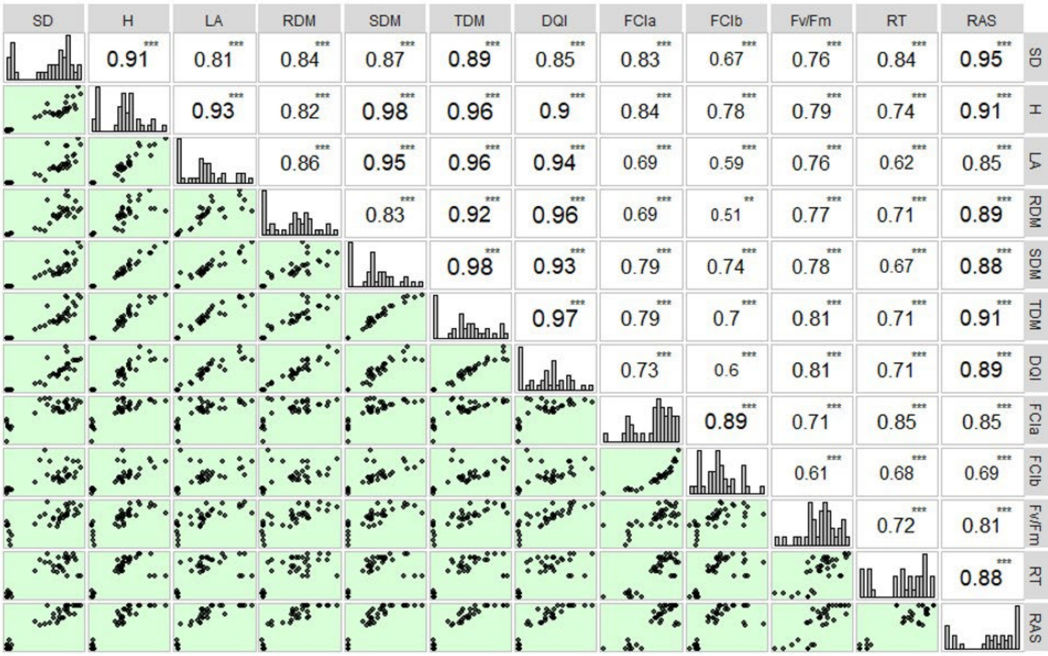

Figure 4. Correlation between the attributes stem diameter (SD), height (H), H/SD ratio, leaf area (LA), root dry matter (RDM), shoot dry matter (SDM), total dry matter (TDM), Dickson Quality Index (DQI), Falker Chlorophyll Index (FCI*a* and FCI*b*), the maximum quantum yield of photosystem II (F_v/F_m), aggregation of roots to the substrate (RAS) and ease of removal from the tube (RT) of *Citharexylum montevidense* seedlings produced in different and base fertilization, 160 days after sowing. Where: *, **, *** significant at 5 %, 1 % and 0.1 % probability of error, respectively.

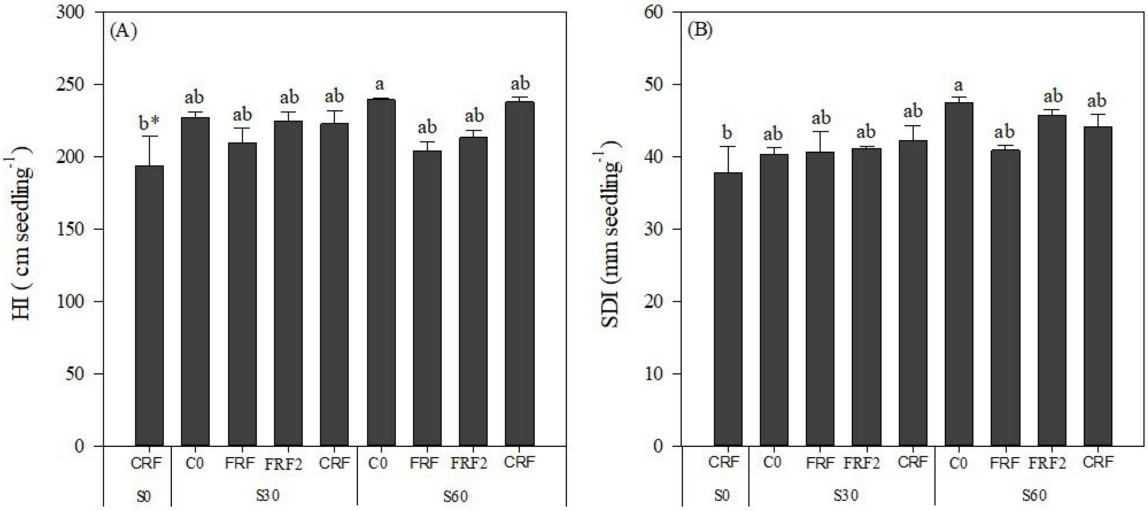

Figure 5. Increase in height (A) and stem diameter (B) of *Citharexylum montevidense* plants, at 540 days after planting in the field, as a function of substrates and basic fertilizers used in the production of seedlings in the nursery. *Averages followed by lowercase letters indicate the comparison between treatments by the tukey Test at 5 % probability. Vertical bars indicate standard error. Where: S0 = 80 % CS + 20 % CRH; S30 = 50 % CS + 20 % CRH + 30 % BM and S60 = 20 % CS + 20 % CRH + 60 % BM. Control (without fertilization); CRF = Controlled Release Fertilizer; FRF = Fast-release fertilizer recommended by Gonçalves et al. (2005); FRF2 = Twice as recommended by Gonçalves et al. (2005).

As for pH, once again the substrates S30 (50 % CS + 20 % CRH + 30 % BM) and S60 (20 % CS + 20 % CRH + 60 % BM) presented values within the range proposed by Fermino (2014), which considers the ideal pH for cultivation in containers between 5.5 and 6.5 (Table 2). Values below or above this limit interfere in the availability of elements provided by fertilization, especially micronutrients, denoting problems in the formation of seedlings due to the unavailability of some nutrients and/or plant toxicity (Malavolta, 2006). However, Regan (2014) highlights that the ideal value can vary depending on the species to be cultivated and must be adjusted and monitored since it can change due to the irrigation water's alkalinity or the decomposition of organic materials in the substrate.

Concerning the fertility of the substrates evaluated, we found that the increase in the availability of N, P, and K (Table 2) is associated with increasing the proportion of bovine manure to the substrate. The greater availability of N, P, and K in the substrate tends to denote more significant growth and accumulation of biomass. It can be expected since the greater the supply of N, P, and K in the substrate, the greater the approximation to the roots and the probability of absorption and the concentration in plant organs. After accumulation in an organ such as leaves, such nutrients directly influence carbon synthesis, being constituents of several enzymes and proteins, in addition to participating in processes such as photosynthesis and cell multiplication and differentiation (Berghetti et al., 2020; Navroski et al., 2018; Taiz et al., 2017).

Similar results to the present study were obtained by Trazzi et al. (2012), studying the physical and chemical characteristics of substrates formulated with bovine manure and by Jayasinghe et al. (2010), evaluating the potential of substrates developed from bovine manure compost for the production of ornamental plants, as an alternative to peat replacement. Faria et al. (2017), evaluating the potential of using organic residues in the formulation of substrates, recommended using 20 to 60% of bovine manure to produce *Moquiniastrum polymorphum* (Less.) G. Sancho seedlings in containers.

Seedling growth in the nursery

In the nursery, the highest averages for the morphological attributes SD, H/SD, LA and RL and DQI in seedlings cultivated on substrates S60 (20 % CS + 20 % CRH + 60 % BM) and S30 (50 % SC + 20 % CRH + 30 % BM), combined with the use of controlled-release fertilizer (6 g L^{-1}) as base fertilizer. In addition, it is noteworthy that when using ready-release fertilizer or in the absence of base fertilization (control), the addition of bovine manure to the substrate contributed to the increase in growth rates and improved the quality of the produced seedlings (Figure 3).

The positive results of using bovine manure as a substrate component for the production of forest species are confirmed by observing the height growth and biomass accumulation of *C. montevidense* plants (Figure 1 The height obtained at 160 d.a.s. increased by 258 % with the use of 60 % of cattle manure in relation to the control treatment (0 % bovine manure). Additionally, the biomass accumulation (total dry matter) was approximately 700 % higher in seedlings cultivated in the S60 substrate (20 % CS + 20 % CRH + 60 % BM), compared to the control substrate (0 % bovine manure).

The highest growth rates of forest species cultivated in containers filled with the substrate based on bovine manure are directly related to improving its physical (SD, AS, TP, and RAW) and chemical (pH, CE, and N, P and N contents) properties. However, it is noteworthy that these characteristics may vary according to the primitive composition of the organic remains that gave rise to it and the degree of decomposition in which the manure is found (Gomes and Paiva, 2011). Thus, when the proper preparation and tanning process occur, bovine manure becomes a sustainable component, which can reduce the use of *Sphagnum* peat in the production of forest species seedlings, especially in regions with high availability of this material, providing cost reduction with the acquisition of chemical substrates and fertilizers.

The use of controlled-release fertilizers demonstrated an adequate supply of nutrients to *C. montevidense* seedlings. Recently, several studies have highlighted the advantages of using CRF in the production of seedlings of forest species (Aimi et al., 2019; Menegatti et al., 2016; Mezzomo et al., 2018; Turchetto et al. 2019). The use of CRF as base fertilization allows the gradual efficient supply of nutrients to seedlings (Navroski et al., 2018), mediating the balance between plant demand and availability in the substrate. This condition reduces nutrient losses through leaching, in addition to preventing possible damage to the root system due to toxic concentrations. Thus, the higher initial cost with the CRF can be mitigated by the higher quality of the seedling, less time needed for production, reduction of labor with top dressing, and, at the same time, reduction of groundwater contamination.

The results show that plants grown in the presence of bovine manure and CRF had higher values of chlorophyll index a and b and maximum quantum yield of photosystem II (F_v/F_m), providing seedlings with a high standard of quality. Possibly, it is associated with greater availability of nutrients, such as N, P, and K in the substrate and less leaching during the seedling production process, which enables more excellent absorption and accumulation of these nutrients in organs such as leaves and, consequently, more remarkable synthesis of photosynthetic pigments Berghetti et al. (2019). In response, plants use the process of converting light energy into chemical energy, which contributes to the rapid growth and development of seedlings and, consequently, more outstanding biomass production (Berghetti et al., 2019; Taiz et al., 2017).

Additionally, the highest values of maximum quantum yield of photosystem II (F_v/F_m) indicate that the seedlings have adequate physiological conditions for growth due to the higher efficiency of light energy absorption by the PSII antenna complex (Baker 2008). Furthermore, the increase in F_v/F_m indicates that a greater amount of energy absorbed by the plant through pigments is used to transport electrons and produce dry matter (Tiecher et al., 2017). Thus, for *C. montevidense* seedlings, after interpreting the F_v/F_m

ratio, it is evident that values above 0.59 can be considered good growth predictors, as the higher value of this variable corroborates the higher chlorophyll indices (Table 4), growth and dry matter production (Figure 2 and 3).

In the present study, the addition of bovine manure improved the root system aggregation of the produced seedlings, expressing the highest scores for the RAS (Table 5; Figure 1). Thus, the RAS indicates the quality of the clod formed, and quality substrates should favor the aggregation of the roots, thus avoiding losses during handling for removal of containers, making rolls and transport, and field planting. Wendling et al. (2007) also emphasize that the roots aggregation to the substrate is essential, as it prevents drying out, contributing to more remarkable seedling survival after planting in the field. On the other hand, the substrate must not be too cohesive, leading to disruption of the root system when removing the container (Kratz et al. 2013; Wendling et al., 2007).

In this sense, the combination of 60 % of bovine manure plus CRF presents one of the lowest scores for RT (Table 5). In this condition, the seedlings of *C. montevidense* expressed vigorous root growth with an emphasis on the rusticity of the species. However, the higher density of the substrate S60 (318 kg m^{-3}) made it difficult to remove the containers, requiring a lot of strength and, consequently, breaking the roots. RT was also compromised in substrates with no bovine manure and without fertilization, or even combined with ready-release fertilizers, due to the inexpressive development of the root system (Figure 3).

The results obtained indicate a high correlation between RAS and RT for all morphophysiological attributes evaluated ($r > 0.62$), except for the H/SD ratio ($r \approx 0.24$). Furthermore, Mieth et al. (2019) evaluating the use of crushed stone and peach in the formulation of alternative substrates for the production of seedlings of *Eucalyptus dunnii* Maiden, and Kratz et al. (2013) studying the technical feasibility of using renewable components in the production of *E. benthamii* Maiden & Cambage, also found a high positive correlation between RAS and RT and seedling growth, as well as a negative correlation with the density of the evaluated substrates. Thus, the RAS and the RT are two attributes simple to measure, with practical application, which allow the estimation of the substrate's quality and the seedling formed in the nursery (Figure 4). Therefore, grades above eight can be considered adequate for these variables, as they denote an intact clod, well aggregated, and without damage to the roots during removal.

Initial field growth

The 100 % survival observed in *C. montevidense* plants at 540 days after planting, regardless of the treatments tested, was favored by the edaphoclimatic conditions during the establishment phase, especially about adequate rainfall levels recorded. According to Berghetti et al. (2020), the intensity and regularity of rainfall in the initial post-planting period are essential to maintain soil moisture and replenish water to the seedlings, preventing dehydration of the root system and thus ensuring high survival in forest plantations.

It is essential to mention that the high survival of the species can also be associated with its rusticity and adaptability to different site conditions. Rorato et al. (2018) indicated *C. montevidense* as a priority species for the formation of the initial structure in plantations to restore degraded areas. In addition, we observed that this pioneer species present rapid growth, rusticity, and early fruiting 15 months after planting in the field. In this context, our results reinforce the potential of the species to compose plantations destined for the recovery of altered areas in regions of its natural occurrence.

When observing the increase in height and diameter of the collection of plants conducted in the field, we verified that the nursery results were partially confirmed. Plants produced in substrate S60 (20 % CS + 20 % CRH + 60 % BM), without base fertilization, expressed the highest values of HI (≈ 240 cm) and SDI (≈ 48 mm), despite the quality of seedlings in the nursery has been minor. In addition, we emphasize that the treatments composed by the addition of bovine manure in the formulation of substrates used for the production of seedlings in nurseries did not show a significant difference in field growth. Thus, the potential for using bovine manure as a substrate component is confirmed, in proportions of 30 %, since it guarantees high rates of establishment and growth of plants in the field and cost reduction in the production of seedlings.

The growth responses of *C. montevidense* plants in the field are directly related to the quality standard of the seedlings used, with H ≥ 18.3 cm and SD ≥ 3.6 mm, as well as a rustic and well-formed root system (Figure 4 and Table 3). According to Berghetti et al. (2020), the seedling tends to explore a greater volume of soil with a well-developed root system and absorb more significant amounts of nutrients. As a result, there is a reduction in the dependence on the fertilized substrate from the seedlings production (Griebeler et al., 2021).

The results emphasize the importance of complementary field trials as a strategy for validating the results obtained in nurseries since the best results in nurseries do not always equate to better responses in the field (Jacobs and Landis, 2009). Similar results to the present study were verified by other authors when they tested the responses of treatments applied in the nursery phase in the field (Gasparin et al., 2014; Mieth et al., 2019). This fact can be explained by the influence of several factors (weather conditions, applied cultural treatments, genetic and herbivorous variability) that act as covariates on plant growth, which tend to present performance own of the species.

CONCLUSIONS

For the cultivation of *Citharexylum montevidense*, it is recommended to use substrates composed of 30 % of bovine manure, associated with 6 g L^{-1} of controlled-release fertilizer (18-05-09), as it provides higher quality seedlings in shorter production time in the nursery. The post-planting performance of *C. montevidense* is influenced by the substrate used in the production of seedlings, and the use of tanned bovine manure allows for more significant plant growth in the field, partially confirming the results obtained in the nursery.

AUTHORSHIP CONTRIBUTION

Project Idea: AMG, MMA
Database: AMG, FT
Processing: AMG, ÁLPB, MHF
Analysis: AMG, FT, MHF
Writing: AMG, FT, MMA
Review: ÁLPB, MHF, DGR, SCA, CC

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