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Articles

Substrate composition and container management influence the production of *Handroanthus impetiginosus* seedlings for urban afforestation

Substratos e manejo de recipientes afetam a produção de mudas de *Handroanthus impetiginosus* para a arborização urbana

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ABSTRACT

Container size and substrate composition are among the main factors affecting seedling quality and, consequently, successful establishment in urban forestry programs. This study evaluated the use of sewage sludge-derived biosolids as an alternative substrate and different container management systems for the production of *Handroanthus impetiginosus* (ipê-roxo) for urban afforestation purposes. Two types of substrates were used: biosolids (BIO) and a formulated substrate (FS) consisting of subsoil soil, manure and sand in a 5:4:1 ratio. The experiment followed a 2 × 2 factorial design, comprising four treatments with five replicates of four seedlings each. Two container management systems were evaluated: TC, in which seedlings were initially grown in 280-cm³ tubes and transplanted after 120 days to 3.0-L citropots, followed by transplantation to 15-L pots where they remained until completing 510 days; and C, in which seedlings were established directly in 3.0-L citropots and subsequently transferred to 15-L pots, until completing 510 days. Seedling height and root collar diameter were measured monthly, and relative growth between 90 and 510 days after transplanting was calculated. The combination of biosolids with the C management system promoted the greatest height and root collar diameter growth. These results indicate that biosolids associated with direct cultivation in larger containers represent an effective strategy for producing *H. impetiginosus* seedlings for urban forestry applications.

Keywords: Sewage sludge biosolids; Initial growth; Tubes; Ipê-roxo

RESUMO

O tamanho do recipiente e o tipo de substrato estão entre os fatores que podem influenciar diretamente a qualidade das mudas, aumentando as chances de estabelecimento destas no plantio para a arborização urbana. Objetivou-se neste trabalho avaliar o biossólido como substrato alternativo e o manejo de recipientes, na produção de mudas de *Handroanthus impetiginosus* (ipê-roxo), para fins de arborização urbana. Utilizou-se dois tipos de substratos: o biossólido de lodo de esgoto e uma formulação composta por terra de subsolo, esterco e areia na proporção 5:4:1. O experimento foi constituído por um fatorial 2 x 2, totalizando 4 tratamentos, com cinco repetições de quatro mudas cada. O manejo de recipientes foi realizado de duas formas: sendo o primeiro (TC), iniciado em tubete de 280 cm³, e após 120 dias, as mudas foram transferidas para o citropote de 3,0 L. O segundo manejo (C) iniciou no citropote de 3,0 L, onde as mudas permaneceram por 240 dias, sendo posteriormente transplantadas para vasos de 15 L, onde permaneceram até os 510 dias. Dados de altura e diâmetro de coleto foram coletados mensalmente para a avaliação do experimento. Foi calculado o crescimento relativo em percentagem das variáveis H e DC, entre 90 e 510 dias após a repicagem. O manejo BIO (biossólido) + C (citropote 3,0 L + vaso 15,0 L), proporcionou maior crescimento para as variáveis altura e diâmetro do coleto, sendo indicado seu emprego na produção de mudas de ipê-roxo, destinadas à arborização urbana.

Palavras-chave: Biossólido; Crescimento inicial; Tubetes; Ipê-roxo

1 INTRODUCTION

The use of high-quality seedlings is a key factor for the success of urban afforestation programs (Oliveira et al., 2013). Seedling quality depends largely on the selection of suitable containers and substrates with adequate physical and chemical properties, as well as easy availability and low cost (Kratz; Wendling, 2016; Silva et al., 2020). Moreover, seedlings intended for urban planting require longer nursery periods, which generally increases production costs (Ribeiro et al., 2018).

Seedling production for afforestation commonly begins in small-volume containers. However, growth eventually becomes restricted, and seedlings need to be transplantated to larger containers or to a holding nursery (Oliveira et al., 2013), where plants are grown directly in the soil. These changes demand additional space and larger amounts of substrate. In order to attain the height standards required for urban afforestation, seedlings must be cultivated in containers with greater volumetric capacity to ensure adequate root development relative to shoot growth (Ribeiro et al., 2018).

Container selection should consider root system morphology, economic aspects, and the intended use of the seedlings, given the influence of container size on water and nutrient availability and consequent plant growth (Cabreira et al., 2017). Likewise, substrate composition plays a critical role in seedling development. Besides providing physical support, the substrate creates favorable conditions for root growth and supplies the nutrients required for plant development (Kratz; Wendling, 2016; Silva et al., 2021).

Recent studies have explored alternative substrates derived from residues, demonstrating their potential for forest seedling production. Compared with commercial substrates, these materials may reduce production costs while improving seedling quality (Kratz; Wendling, 2016; Abreu et al., 2017; Silva et al., 2020). Nevertheless, studies on this theme remain scarce, particularly regarding seedlings intended for urban afforestation.

Among the alternative substrate components, biosolids derived from wastewater treatment plants (WWTPs) have received increasing attention. After treatment and stabilization, and provided that microbiological and chemical standards established by CONAMA Resolution No. 498/2020 are met, biosolids can be used in forestry activities (Brasil, 2020). Improper disposal of these residues in landfills represents a major environmental concern (Abreu et al., 2017), potentially causing groundwater contamination and soil degradation, among other adverse effects (Sharma et al., 2017).

Biosolids offer several advantages as substrate components, particularly their low cost and high concentrations of nutrients, especially N, P, and Ca, as well as organic matter. These characteristics promote seedling growth and reduce the need for supplemental fertilization (Lozada et al., 2015; Cabreira et al., 2017; Abreu et al., 2017). Thus, the use of biosolids in forest nurseries represents not only an environmentally sound but also economically attractive option for both waste generators and nursery producers (Guerrini et al., 2017; Abreu et al., 2018).

Handroanthus impetiginosus (Mart. ex DC.) Mattos (ipê roxo) is a native species commonly used in urban landscaping and street tree planting in Brazil. This species is highly valued for its ornamental characteristics, including abundant flowering between June and August and deciduous foliage, which increases light penetration during the coldest months of the year. In addition, its non-aggressive root system minimizes damage to sidewalks and urban infrastructure.

In view the suitability of *H. impetiginosus* for urban afforestation, research addressing the production seedlings for this purpose is important. In order to correlate the high nutrient content of biosolids to the optimization of container management, initiating seedling production in larger containers may be useful to reduce the time required to obtain plantable seedlings and, consequently, lower production costs.

The present study evaluated the effects of two substrates (formulated substrate and biosolids) and two container management systems (tube + citropot + pot; and citropot + pot) on the production of *H. impetiginosus* seedlings intended for urban afforestation.

2 MATERIAL AND METHODS

The experiment was conducted from 12 October 2018 to 13 March 2020 at the Luiz Fernando de Oliveira Capellão Forest Nursery, Department of Forestry, Forest Institute, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil. According to the Köppen classification, the regional climate is Aw, with a mean annual temperature of 23.5 ºC and average annual precipitation of 1,354 mm (Alvares et al., 2013).

The experiment followed a 2 × 2 factorial design. Factor 1 consisted of two substrate types: formulated substrate (FS) and biosolids (BIO). Factor 2 comprised two container management systems: tube + citropot + pot (TC) and citropot + pot (C). There were therefore four treatments, each consisting of five plots with four seedlings per replicate, totaling 80 seedlings. Plots were arranged in a completely randomized design.

The formulated substrate (FS), commonly used for urban tree seedling production, served as reference and consisted of 50% subsoil, 40% cattle manure, and 10% sand. Base fertilization was applied according to Gonçalves and Poggiani (2000), using 150 g N (through the use of sulfate), 300 g P_2O_5 (single superphosphate), 100 g K_2O (potassium chloride), and 150 g FTE micronutrients per cubic meter of substrate. The second substrate consisted exclusively of biosolids (BIO), supplied by the State Water and Sewage Company of Rio de Janeiro (CEDAE).

The subsoil used in the experiment was collected at UFRRJ from a depth of 30–40 cm to avoid contamination by pathogens and weed seeds. Cattle manure was obtained from the dairy facilities of the Agricultural Research Station of Rio de Janeiro State (PESAGRO), Seropédica unit, where the animals' diet is based on elephant grass and feed, supplemented with grazing in areas with a predominance of colonião grass. After collection, the manure was left exposed to the air under ambient conditions for approximately 30 days and subsequently sieved to remove impurities. Washed sand was purchased from a local construction materials supplier in Seropédica-RJ.

Substrate components and fertilizers were mixed manually. Moistening was performed with the aid of a watering can to improve homogenization and ensure proper compaction during container filling. After that, the 280 cm^3 tubes and the 3.0 L citropots were manually filled and subsequently taken to the seedbed. Samples of unfertilized formulated substrate and biosolids were analyzed for chemical properties at the Soil Analysis Laboratory of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (Table 1).

Three container sizes were used: 280-cm^3 tubes, 3.0-L citropots, and 15-L pots. In the tube + citropot + pot system (TC), seedlings were initially grown in 280-cm^3 tubes for 120 days, transferred to 3.0-L citropots for another 120 days, and finally transplanted into 15-L pots, where they remained for 270 days. The same substrate treatment was maintained throughout all stages. In the citropot + pot system (C), seedlings were transplanted directly into 3.0-L citropots, where they remained for 240 days, followed by transplantation into 15-L pots for 270 days.

Table 1 – Chemical analysis of macro- and micronutrients of the formulated substrate (FS) and biosolids (BIO) used for the production of *Handroanthus impetiginosus* seedlings in a nursery

Substrate	pH	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	B	C/N
	-				%					mg kg^{-1}			
FS	7.2	0.45	0.15	0.56	0.47	0.34	0.16	164	15245	283	17	6.7	11.4
BIO	5.4	1.96	0.68	0.16	1.84	0.29	1.12	1009	25294	230	209	8.9	4.9

Source: Authors (2022)

In where: Total levels, determined in the acid extract (nitric acid with perchloric acid); N - Kjeldahl method; CO - Walkley-Black method.

Handroanthus impetiginosus, a species commonly used in urban afforestation programs, was used in the experiment. Seeds were collected from selected mother trees located on the UFRRJ campus.

After processing and drying, seeds were sown indirectly in sand-filled seedbeds. Seedlings were transplanted to the experimental containers when they had developed two pairs of leaves. All seedlings were maintained in the nursery under automatic irrigation twice daily, in the morning and in the late afternoon, supplying approximately 15 mm of water per container per day.

In the TC management, the 280-cm³ tubes were allocated in plots of four seedlings in a randomized manner in polypropylene box-type trays with 54 cells and maintained on the nursery floor. Seedling density was 206 plants m^{-2} during the first 30 days. Between 30 and 120 days, the seedlings were spaced in a way to produce a density of 103 seedlings m^2. In the C management system, 3.0-L citropots were arranged in double rows and secured with wire and bamboo stakes. The citropot plots were also arranged in a randomized manner, resulting in a density of 40 plants m^{-2} during the first 120 days. In the C management, from 0 to 120 days, the density was 40 seedlings m^2.

After 120 days, in the TC management, seedlings grown in 280-cm³ tubes were transferred to 3.0-L citropots. At this point, since 3.0-L citropots were now used in all treatments, they were randomized again and container spacing was adjusted by alternating occupied and empty positions. In the period from 120 to 240 days, the density was 20 seedlings m^2 for all seedlings in all treatments.

After 240 days, to promote the proper growth, all seedlings were transplanted into 15-L pots and maintained until completing 510 days, when they reached the standards required for urban planting. Pots were spaced at 1 × 1 m, corresponding to a density of one plant m^{-2}. Bamboo stakes were used to guide stem growth and promote crown formation with a single stem and well-distributed scaffold branches.

Routine maintenance included weed control and pruning. Beginning at 150 days, branches corresponding to approximately 50% of total height were removed to reduce light competition and minimize phytosanitary problems. Formative pruning was also performed to eliminate natural bifurcation. Once stems reached 2.0 m without lateral branches, pruning was discontinued, as seedlings had achieved the quality standards required for urban afforestation.

Topdressing fertilization was applied only to seedlings grown in the formulated substrate, following Gonçalves and Poggiani (2000). Nutrient solution consisted of 200 g N (ammonium sulfate) and 180 g K_2O (potassium chloride) per 100 L of water. A volume of 5 mL per seedling was applied using a syringe. The first topdressing fertilization occurred 30 days after transplanting, then nitrogen applications were performed every 15 days and potassium applications every 30 days until completing 240 days. Thereafter, the volume applied was increased to 50 mL per seedling, maintaining the same application intervals.

Growth assessments began 90 days after transplanting and were performed every 30 days until 510 days. The following morphological variables were measured: shoot height (H), root collar diameter (RCD), and diameter at breast height (DBH, at 1.30 m), using a graduated ruler, digital caliper, and measuring tape, respectively.

Relative growth (%) for H and RCD between 90 and 510 days was calculated to compare container management systems and substrates. The TC system served as a reference to calculate the growth percentage of seedlings in the C system, Equation (1). For the comparison of substrates, the FS was taken as a reference to estimate the percentage growth of seedlings produced in the BIO substrate, Equation (2):

$$\text{Relative growth (\%)} = (C*100)/TC \tag{1}$$

$$\text{Relative growth (\%)} = (BIO \times 100)/FS \tag{2}$$

In where: C – height (cm) or root collar diameter (mm) of seedlings in the C system (citropot); TC – height (cm) or root collar diameter (mm) of seedlings in the TC system (tube + citropot); FS – height (cm) or root collar diameter (mm) of seedlings in the FS substrate (formulated substrate); BIO – height (cm) or root collar diameter (mm) of seedlings in the BIO substrate (biosolids).

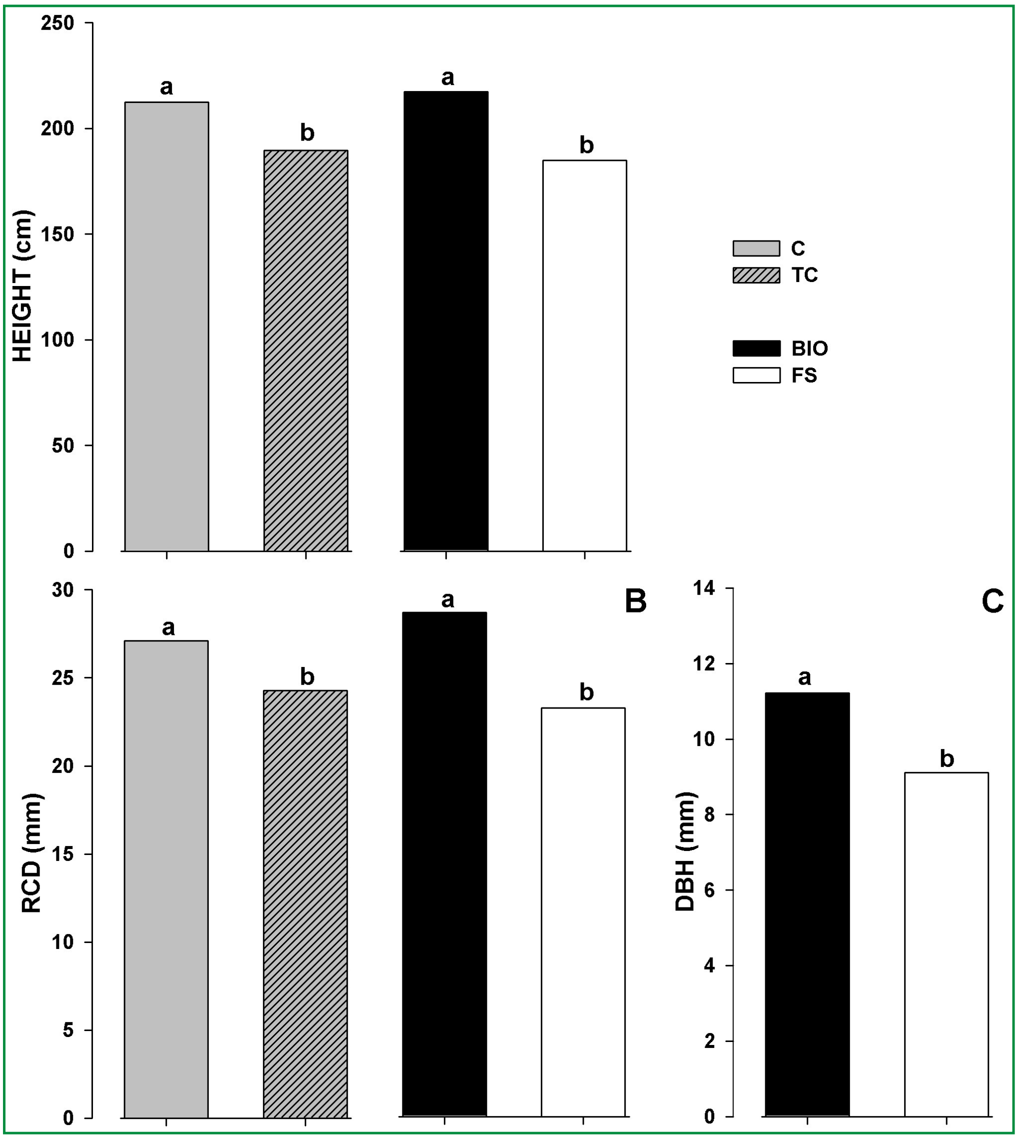
After obtaining the data, they were tested for normality and homogeneity of variances. Subsequently, two-way analysis of variance (ANOVA) was performed, and treatment means were compared using Tukey's test at the 5% significance level. Statistical analyses were conducted using Sisvar 5.6 software (Ferreira, 2019). Equation adjustments were also performed. The equations were adjusted and tested for model identity (Leite; Oliveira, 2002).

3 RESULTS AND DISCUSSIONS

At 510 days, significant effects of both substrate and container management were detected for seedling H and RCD, whereas DBH was affected only by substrate type. No significant interaction between factors was observed for any morphological variable. In all cases where differences were detected, seedlings grown under management C outperformed those produced under management TC, and seedlings grown in BIO showed superior performance compared with those grown in FS (Figure 1).

The superior values of H and RCD observed under the C system (Figure 1) are likely attributable to the larger substrate volume available since the beginning of seedling production, which provided greater rooting space and enhanced water and nutrient availability. Similar responses have been reported for *Enterolobium contortisiliquum* (Abreu et al., 2015), *Ceiba speciosa* (Lima Filho et al., 2019), and *Cedrela fissils* (Antoniazzi et al., 2013), all species intended for restoration programs.

Figure 1 – Height (A), root collar diameter (B), and diameter at breast height (C) of *Handroanthus impetiginosus* seedlings grown under different container management systems and substrates after 510 days in the nursery



Source: Authors (2022)

In where: Height (H), root collar diameter (RCD), and diameter at breast height (DBH) of seedlings produced in the citropot + pot (C) and the tube + citropot + pot (TC) management systems and using biosolids (BIO) and formulated substrate (FS). Means followed by different letters differ significantly according to Tukey's test ($p \leq 0.05$).

Antoniazzi et al. (2013) demonstrated that larger-volume plastic bags represented the most suitable alternative for producing large seedlings of *C. fissilis*, owing to improved root development and, consequently, enhanced shoot growth. A similar pattern was observed in the present study.

Substrate type significantly affected all three morphological variables, with seedlings grown in the BIO substrate exhibiting superior H, RCD, and DBH (Figure 1). This response was most likely associated with the higher nutrient concentrations found in BIO (Table 1).

Likewise, in a evaluation of *Schinus terebinthifolius* seedlings produced in biosolids and commercial substrate under different rates of monoammonium phosphate (MAP) in topdressing fertilization, Abreu et al. (2018) reported greater H and RCD throughout the production cycle when biosolids were used. These findings are consistent with the results obtained here and suggest that, if growth trends are maintained, seedlings produced in biosolids from the beginning of cultivation would ultimately attain greater H and RCD than those grown in formulated substrates receiving both basal and topdressing fertilization.

Height growth from 90 to 510 days exhibited a linear trend in both BIO and FS substrates, regardless of container management (Figure 2), indicating that seedlings remained in an active growth phase throughout the experimental period. In contrast, RCD showed a quadratic pattern, suggesting a tendency toward growth stabilization under the prevailing cultivation conditions.

At 90 days, all treatments exhibited similar growth patterns. However, differences became evident after 120 days, when seedlings grown in BIO under management C began to exhibit superior performance (Figure 2). As growth progressed, treatments increasingly diverged, with biosolid-based treatments displaying steeper growth trajectories and the FS + TC treatment showing the lowest growth rates.

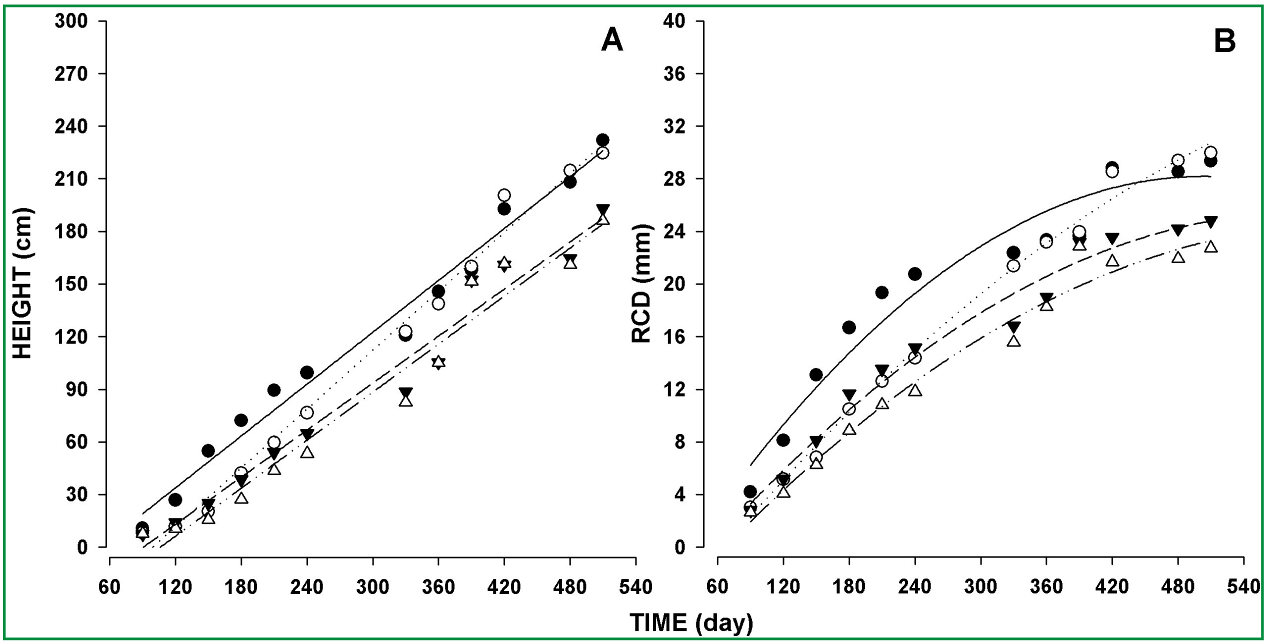
These results suggest that, during the initial stages, all containers provided sufficient substrate volume and nutrient availability to sustain seedling growth. In addition, basal and topdressing fertilization in FS compensated, to some extent, for the higher macronutrient concentrations (except potassium and manganese) found in BIO. As seedlings developed, however, the smaller containers became restrictive, whereas BIO proved more efficient in meeting plant nutritional requirements, despite the additional fertilization supplied to the formulated substrate.

The superior performance observed under management C can therefore be attributed to the larger substrate volume available from the outset, which promoted root development and increased water availability. Lima Filho *et al.* (2019) evaluated the performance of biosolids as a substrate and different tube volumes for production of *C. speciosa* seedlings and they reported greater growth in 280-cm³ tubes than in 55-cm³ tubes. The authors believed that the reduced volume of smaller containers limited seedling growth, resulting in lower height and root collar diameter values. These observations are consistent with the present findings for *H. impetiginosus*, in which seedlings established directly in 3.0-L containers exhibited the greatest growth in height and diameter.

In a study of the effects of biosolids from two wastewater treatment plants on the production of *Luehea divaricate* sedlings compared to a commercial substrate, Sousa *et al*. (2019) reported linear height increase over time in all substrates tested. Their findings corroborate the pattern observed in the present study, particularly the steeper growth trajectories obtained with BIO (Figure 2).

At 510 days, seedlings grown in BIO, irrespective of container management, and those produced under FS + C reached the recommended height range for planting in sidewalks (1.5–2.5 m). However, seedlings produced under the BIO + C treatment attained the greatest H and RCD, indicating the possibility of shortening the nursery production time. Since urban tree seedlings commonly require more than two years to reach planting standards (BIONDI *et al*., 2009), reducing production time would lower nursery management costs. Conversely, seedlings produced under FS + TC had not reached the minimum recommended height after 510 days, suggesting the need for prolonged nursery maintenance and, consequently, higher production costs.

Figure 2 - Height (A) and root collar diameter (B) growth of *Handroanthus impetiginosus* seedlings under different substrate and container management systems from 90 to 510 days in the nursery



Source: Authors (2022)

In where: Height (H) and root collar diameter (RCD) of *Handroanthus impetiginosus* seedlings from 90 to 510 days after transplanting and grown in the citropot + pot (C) and tube + citropot + pot (TC) management systems using biosolids (BIO) and formulated substrate (FS).

Comparisons between regressions showed that seedlings produced in BIO consistently exhibited greater relative growth in terms of both H and RCD than those grown in FS, either in the C and TC managements (Figure 3).

Under management C, the superiority of BIO reached approximately 120% for H and 60% for RCD at around 150 days. This response was likely associated with the larger substrate volume and the higher fertility of BIO, particularly with respect to nitrogen and phosphorus availability. Then there was a drop, which is probably explained by the reduced availability of nutrients due to seedling absorption (for growth and biomass production) and leaching caused by irrigation.

Under management TC, the higher N availability provided by biosolids resulted in smaller relative growth gains than those observed under management C, owing to the smaller initial container volume (280 cm³). At approximately 150 days, the superiority of biosolids reached about 50% and 20% for H and RCD, respectively. This response became evident after seedlings were transferred, at 120 days, from the 280-cm³ containers where nutrient availability had likely declined because of plant uptake and leaching to larger 3.0-L, containers containing fresh, more fertile substrate. Following transplantation, seedlings under management TC exhibited accelerated growth, thereby reducing the differences relative to those produced under management C. Thereafter, the same declining trend observed under management C was also evident.

Figures 3B and 3D illustrate the relative superiority of seedlings produced under management C over those grown under management TC in terms of H and RCD, for both BIO and FS throughout the experimental period.

In BIO, the superiority of management C over TC initially increased. The larger volume of the BIO substrate in management C, associated with the greater fertility and nutrient availability of this substrate, provided relative gains of approximately 170% in H and 90% in RCD. In the FS, these gains were smaller: 60% and 30%, respectively, for height and root collar diameter, at 120 days.

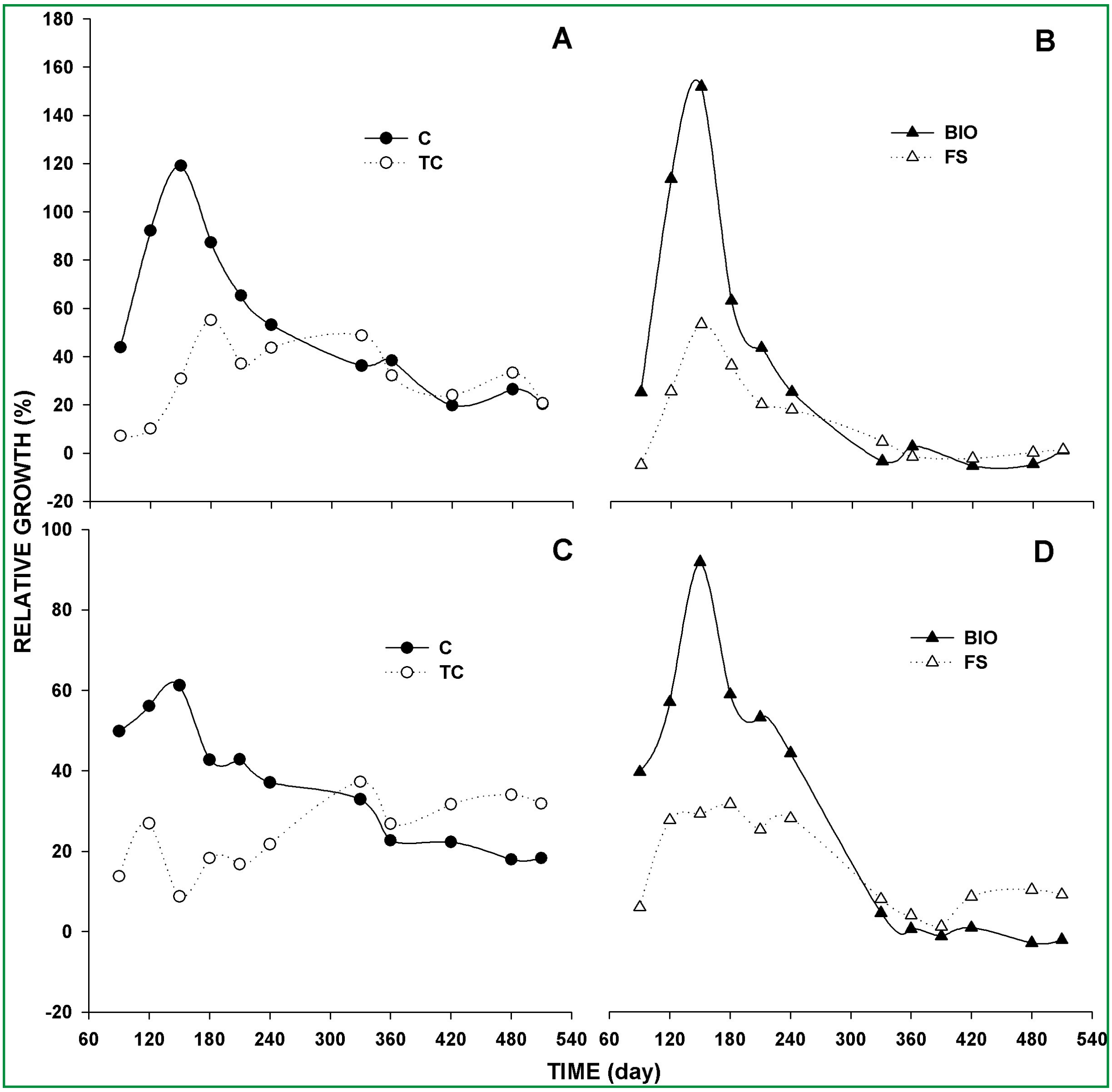
Similar responses have been reported for *S. terebinthifolius* (CABREIRA *et al*., 2017), whose seedlings exhibited superior morphological characteristics when grown in larger tubes (280 cm³) compared to those with smaller tubes (110 cm³) at 112 days after transplanting.

Likewise, Abreu et al. (2015) found that *E. contortisiliquum* seedlings produced in 1,248-cm³ plastic bags achieved greater H and RCD than those grown in 280- and 180-cm³ tubes. These authors attributed such responses to the larger substrate volume, which provided more favorable conditions for seedling development. The present study showed a similar trend in ipê-roxo, as seedlings established directly in 3.0-L containers achieved superior growth.

After approximately 150 days, relative differences between substrates gradually decreased. Nutrient depletion in BIO due to plant uptake and leaching, combined with the absence of supplemental fertilization in BIO, likely contributed to this pattern.

In contrast, seedlings grown in the FS received both basal and periodic topdressing fertilization, ensuring a more continuous nutrient supply. Differences in growth parameters (H and RCD) of the seedlings decreased between the two substrates.

Figure 3 – Relative growth in height (A and B) and root collar diameter (C and D) of *Handroanthus impetiginosus* seedlings under different substrate and container management systems from 90 to 510 days in the nursery



Source: Authors (2022)

In where: Height (H) and root collar diameter (RCD) of *Handroanthus impetiginosus* seedlings from 90 to 510 days after transplanting and grown in the citropot + pot (C) and tube + citropot + pot (TC) management systems using biosolids (BIO) and formulated substrate (FS).

An additional advantage of BIO is that no mineral fertilization was required throughout the production cycle, resulting in lower fertilizer costs and reduced labor requirements.

Following the initial peak, the superiority of management C progressively declined, and after approximately 330 days no additional growth gains relative to management TC were observed. From 240 days onward for H and from 300 days onward for RCD, seedlings in both managements exhibited similar performance until 510 days regardless of substrate.

Thus, although the larger initial volume provided by management C promoted faster early growth, this advantage was not sustained toward the end of the production cycle, likely because nutrient availability in BIO gradually decreased in the absence of fertilization.

The use of native species in urban forestry contributes to the conservation of local biodiversity. Although *H. impetiginosus* is widely used in urban landscaping, information regarding its nursery production remains scarce. Because seedlings intended for urban planting require specific quality standards and generally demand larger containers and greater substrate volumes, identifying alternative substrates and optimizing nursery practices are essential.

Overall, the results demonstrate that sewage sludge-derived biosolids represent a promising alternative for the production of urban tree seedlings. Their use reduces the need for mineral fertilization and enables direct cultivation in 3.0-L containers, eliminating one transplanting stage and potentially reducing labor requirements and overall production costs.

4 CONCLUSIONS

The combination of biosolids (BIO) and the C management system (3.0-L citropot followed by a 15-L pot) promoted the greatest height and root collar diameter growth of *H. impetiginosus* seedlings. Therefore, this management strategy is recommended for the production of this species for urban forestry purposes.

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