ORIGINAL ARTICLE - Silviculture



Tube Volumes and Fertilization Management in Acrocarpus fraxinifolius Seedlings

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Abstract

Acrocarpus fraxinifolius, known as Indian cedar, has little forestry development in Brazil. Thus, the objective of this study was to find the best relation between nutrition and container (tube) volume in seedlings of this species. The experiment was carried out in a 2 × 8 factorial scheme (2 containers and 8 fertilizers), with polypropylene tubes of 50 and 110 cm³ being tested, and the following fertilization treatments: one control, 4 increasing doses of osmocotee plus: 3, 5, 7, 9 kg per m³ of substrate, and fractionated mineral fertilizations with cover manure. It was possible to note that the tube with the highest volume (110 cm³) presented better results for the morphological characteristics. However, when analyzing the physiological variables, it is clear that the 50 cm³ tubes presented similar results to the largest volume tube when well fertilized. It is concluded that the indicated to form seedlings of the species is the combination of tubes of 50 cm³ with the fertilization or double of this recommendation.

Keywords: Indian cedar, fractional fertilization, osmocotee.

1. INTRODUCTION AND OBJECTIVES

The demand for forest products has increased significantly in recent decades, leading forestry to seek alternatives to high productivity (Thebaldi et al., 2015). In this context, there has been a successful experiment in Brazil to introduce species from other countries, such as Eucalyptus and Pinus (Venturin et al., 2014), and the implantation of commercial forests is important to reduce the forest deficit and to protect native remnants for carbon sequestration (Klippel et al., 2013).

Acrocarpus fraxinifolius Wight & Arn belongs to the Fabaceae family, Caesalpinioideae subfamily, is popularly known as Indian cedar and produces reddish-brown hardwood used in construction, furniture and pulp production (Firmino et al., 2015). It is native to high-rainfall regions in Asia, with rapid growth, being very promising for reforestation, and used for producing short-rotation timber (Trianoski et al., 2011).

Regarding productivity, the seedling production phase is important for establishing forest plantations, and knowledge about nutritional requirements and usage of the appropriate substrate are essential factors (Caione et al., 2012). The establishment of forest plantations, whether for commercial or restoration purposes, depends on several factors, and seedling quality is fundamental to the initial success (Gasparin et al., 2013; Simões et al., 2015).

There are several containers used to produce seedlings, but according to Dias et al. (2016), tubes have the advantage of less substrate per container, occupy less production area, reduce transportation and distribution costs in planting, and are reusable. However, smaller containers have a smaller volume, requiring fertilization to be distributed on time either as cover manure fertilization or slow release fertilizers (Dinalli et al., 2012).

The importance of nutrients to plant productivity comes from its participation in the structures and vital processes, reflecting in cellular division and elongation, and in helping the roots grow. In addition to the substrate, the application of nutrients, mainly nitrogen, phosphorus and potassium, in seedling growth has aroused the interest of several forest researchers (Caione et al., 2012; Simões et al., 2015).

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Thus, the objective of this study was to find the best relationship between nutrition and container volume in *Acrocarpus fraxinifolius* seedlings.

2. MATERIALS AND METHODS

The seedlings were produced in the forest nursery of the Tissue Culture Laboratory of the Goiano Federal Institute, Rio Verde Campus (17°48'19.56" S and 50°54'15.13" W). The climate of the region is Aw (Köppen), with mild summers and dry winters. The annual precipitation and temperature averages are 1529.5 mm and 22.4 °C, respectively (Brasil, 1992).

Acrocarpus fraxinifolius seeds were collected in a seed orchard, in which the 10 best matrices were selected, located at the Federal University of Lavras (21°13'21" S and 44°58'26" W). The seeds were collected and benefited following the recommendations of Davide & Silva (2008). The dormancy breakdown was performed in concentrated sulfuric acid for 10 minutes (Rai, 1976). The experiment was implemented using a completely randomized design in a 2×8 factorial scheme (2 container volumes and 8 fertilization treatments), 3 replicates and 20 seedlings per plot. They were arranged alternately in trays.

The two polypropylene tube sizes tested were 50 and 110 cm³. The tubes were sanitized with 1% sodium hypochlorite before use to avoid fungal diseases.

The eight fertilizations consisted of one control without fertilization, 4 increasing doses of osmocote plus 3-4 months (15-09-12 + micro): 3, 5, 7, 9 kg of osmocotee per m³ of substrate respectively, and 3 mineral fertilizations composed of one, half and double the recommended fractionated fertilization recommendation proposed by Gonçalves & Benedetti (2005) which consisted of 150 mg dm⁻³ N, 300 mg dm⁻³ P₂O₅, 150 mg dm⁻³ K₂O and 150 mg dm⁻³ FTE Br 12 (micronutrient cocktail: B = 1.8%; Cu = 0.8%; Fe = 3.0%; Mn = 2.0%; Mo = 0.1%; Zn = 9.0%) and cover manure fertilization with 20 mg N and 15 mg K₂O per tube, applied weekly (K₂O applied every 2 weeks until the end of the experiment), as shown in Table 1.

Treatments*	Base fertilizer	Cover manure fertilizer
T1	0	No
Τ2	3 kg osmocotee/m ³ of manure	No
Τ3	5 kg osmocotee/m ³ of manure	No
Τ4	7 kg osmocotee/m ³ of manure	No
Τ5	9 kg osmocotee/m ³ of manure	No
T6	Half of T7	Half of T7
Τ7	150 mg dm 3 N, 300 mg dm 3 P $_2O_5$, 150 mg dm 3K_2O_5 and 150 mg dm 3 of FTE Br 12	20 mg N and 15 mg K ₂ O per tube**
Т8	Double T7	Double T7

Table 1. Fertilization applied to A. fraxinifolius seedlings in nursery.

*All fertilization treatments are in factorial with 2 container volumes (50 and 110 cm³).

** N applied weekly and K2O every 2 weeks, started 30 days after sowing.

The substrate used for the seedlings formation was a mixture of carbonized rice husks, expanded vermiculite, chicken litter and swine sludge in a ratio of 1:1:1:1. The chemical characteristics of this substrate are pH 7.2, 1.58 g/dm³ K, 6.22 g/dm³ P, 4.1 cmol/dm³ Ca, 12.6 cmol/dm³ Mg, 63.9 mg/dm³ S, 63.9% M, 3.8 dag/kg OM, 59.57 mg/LP-Rem.

After the substrate was prepared, the base fertilizer was added to it. The tubes were filled with the substrate soon after, and the seed sowing continued; three seeds were then placed in each tube with the dormancy breakage, and thinning was carried out one week after germination, leaving only one seedling per tube.

Seedling growth occurred in full sun with an automatic irrigation system (4.3 L m⁻², three times a day). Cover manure fertilization was started for treatments 6, 7 and 8 after 30 days.

Cover manure fertilization was not carried out in treatments with controlled release fertilizer, since by theory it provides nutrients throughout the plant cycle.

At 120 days after sowing, the following morphological characteristics were analyzed: shoot height (H) and stem diameter (D). A millimeter ruler was used for height measurements (H), and a digital caliper was used for diameter (D) at 0.5 cm height of the substrate.

The physiological analyzes conducted in the *A. fraxinifolius* seedlings were: gas exchanges of the expanded plants to record the stomatal conductance [gs, mol (H_2O) m⁻²s⁻¹], photosynthetic [*A*, µmol (CO_2) m⁻¹] and transpiration rate [*E*, mmol (H_2O) m⁻²s⁻¹], the internal and external CO₂ concentration (*Ci/Ca*). These evaluations were performed using a LI6400xt (Li-Cor, Nebraska,

USA) portable infrared gas analyzer (IRGA), with photon flux density at 1000 μ mol m⁻²s⁻¹, between 8 a.m. and 12 p.m.

The chlorophyll content was evaluated with a ClorofiLOG1030[°] portable meter, in which chlorophyll a, b and totals were measured in the leaves along with gas exchanges.

At 120 days after the end of the nursery phase, the seedlings were removed from the tubes, washed in running water to remove all residues from the substrate and separated into stems, leaves and roots, and dried in a forced circulation air oven at 65 °C until reaching constant weight. Leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (RDM) were obtained by the sum of plant dry matter expressed in g plot⁻¹, according to Hunt (1990). The ratio of height/diameter (H/D), root/shoot (R/S), and Dickson quality index (DQI) were also calculated.

The evaluated macronutrients were nitrogen (N), phosphorus (P) and potassium (K). The samples were ground in a Wiley mill, and 0.3 g of the dry leaf biomass plant material were collected from the muffle oven and extracted by dry digestion for quantification of the phosphorus contents. P determination was performed by spectrophotometer, and N by distillation according to the methodology of Empresa Brasileira de Pesquisa Agropecuária (Embrapa, 2009).

The data were processed by statistical assumptions, ascertaining the normality and homogeneity of the variances. A variance analysis (at 5% significance) was performed, and the means were separated by the Scott-Knott test. The SISVAR 5.3 software was used in the analyses (Ferreira, 2011).

3. RESULTS AND DISCUSSION

The results found in the shoot height measurement at 120 days indicated that both treatments with osmocote and those with fractionated fertilization influenced the seedling growth, but the best values were found in the treatments 6, 7 and 8, with those being the weekly fertilizations, showing that the slow release effect was lower than weekly fertilization. It can be observed that all the 110 cm³ tubes obtained higher averages in height, highlighting the treatments that were fertilized weekly (Figure 1a and 1b).

Shoot height is an excellent parameter to evaluate the quality of forest seedlings: the larger seedlings have greater vigor, and N increased the mean height in *Schizolobium amazonicum* (Huber ex Ducke) seedlings (Caione et al., 2012). Wendling & Dutra (2010) place 2 mm as the minimum D threshold for well-formed eucalyptus seedlings, and all treatments were above this minimum limit at 120 days after sowing.

Lisboa et al. (2012) found similar results for *Toona ciliata* (Australian cedar) seedlings, in which they observed that larger volume containers tend to provide greater amounts of nutrients and retained water, thus helping in the shoot height growth. In the same way in eucalyptus seedlings, Bamberg et al. (2012) found that the higher tube volume provided a greater number of leaves, because the restriction on the root system imposed by small tubes limits the growth and development of several species.



Figure 1. Mean (a) height, (b) diameter, (c) stem mass, (d) leaf mass, and (e) root mass in grams (g) at 120 days in *Acrocarpus fraxinifolius* seedlings under different fertilizations and tube volumes. Means with different letters differ significantly by the Scott Knott test at 5% significance; upper case letters differ in size from lower case letters and different treatments.

Analyzing the stem dry mass, it was possible to observe a difference for the 110 cm³ tubes in the treatments 6, 7 and 8 for fractionated fertilization with manure mix in relation to the slow release (Figure 1c), while treatment 8 had better results inside the 50 cm³ tube.

A greater variation was noticed in treatments 7 and 8 in the leaf dry mass means for the 110 cm³ tubes, while the 50 cm³ tube did not present statistical differences (Figure 1d).

The root dry mass results show that the treatments did not have significant differences (Figure 1e), but it is possible to notice a trend in osmocote treatments to invest more in the root system. In the analysis of all these parameters, we found that the osmocote mineral fertilizer influenced the seedling development, but the fertilization by cover manure was more efficient.

When we analyzed the height-to-diameter ratio (H/D), we noticed that only treatments 5 and 6 presented lower averages for the 50 cm³ treatment, and the fractional fertilization with cover manure provided higher height/diameter, showing that well-nourished plants tend to grow in height (Figure 2a).

No significant differences were found in the Dickson Quality Index (DQI) analysis for either the treatments or the tubes (Figure 2b).

In relation to the root/shoot ratio (R/S), there is a tendency for the 50 cm³ tubes to have values higher than the 110 cm³ tubes; in addition, lower values were found for those fertilized weekly, as well as treatments 6, 7 and 8 when comparing the treatments (Figure 2c). Moreover, the seedlings in smaller containers tend to have a higher R/S ratio due to the space limitation, and we noticed the fractionated fertilization treatments by cover manure are better nourished by showing greater investment in the shoots. There is a direct relationship between increased R/S ratio and lower supply of nutrients to plants (Glass, 1989), meaning that slow release fertilization.

A similar result was found by Muniz et al. (2013) with NPK fertilization in eucalyptus seedlings, observing a higher root/shoot ratio in low fertility environments, meaning a higher volume of roots, which may be a strategy for the plant to obtain the maximum nutrients in this situation.



Figure 2. Mean (a) height per diameter (H/D), (b) Dickson Quality Index (DQI), and (c) root/shoot ratio (R/S) at 120 days in *A. fraxinifolius* seedlings according to volume and fertilization management. Means with different letters differ significantly by the Scott Knott test at 5% significance; upper case letters differ in size from lower case letters and different treatments.

When analyzing the nitrogen content in *A. fraxinifolius* leaves, we found no variation for the tube size within the treatments, as the contents of N in the leaves were higher for treatments 7 and 8 regardless of the tube volume, which is a reflection of better nutrition generating higher growth, as seen in previous data, and consequently higher nutrient contents in leaves (Figure 3a).

The phosphorus content in the leaves indicated that only treatment 1 (the one without fertilization) presented higher P levels in the leaves. This probably occurred due to a P concentration effect, since it was the treatment with less biomass accumulation (Malavolta, 2006) (Figure 3b).

The potassium content in the leaves showed a linear increase with increasing doses, and the fractionated fertilization with treatments 6, 7 and 8 had higher K content for the 100 cm³ tube, and treatments 7 and 8 for the 50 cm³ tube (Figure 3c).

The optimal N, P and K contents for *A. fraxinifolius* are 23.28, 1.98 and 5.15 g Kg⁻¹, respectively, and the contents of plants in deficiency are 11.3, 1.3 and 2.17 g Kg⁻¹, respectively (Munguambe et al., 2017). The study showed that the main limiting factor for growth was nitrogen, since only treatments 7 and 8 reached adequate levels, while all the treatments with slow release fertilization and the treatment with half of the fractional fertilization (T6) obtained intermediate values between deficient and adequate. The leaf contents for P and K were above those found for the species in well-nourished conditions, meaning the fertilizations as well as the nutrients in the substrate itself were probably able to supply the plants' needs.

In their research with *Eucalyptus camaldulensis*, Lôbo et al. (2014) observed that fertilized tubes obtained greater availability of essential nutrients in the substrate such as nitrogen, phosphorus and potassium, and could be absorbed by the seedlings, thereby justifying greater growth.



Figure 3. Mean (a) nitrogen, (b) phosphorus, and (c) potassium content at 120 days in *A. fraxinifolius* leaves according to volume and fertilization management. Means with different letters differ significantly by the Scott Knott test at 5% significance; upper case letters differ in size from lower case letters and different treatments.

When analyzing the photosynthetic rate (A) (Figure 4a), the slower doses of slow release fertilizer for the 110 cm³ tubes showed higher averages in relation to the same treatments in the 50 cm³ tubes, with the exception of treatment 1. There was no significant difference for the variable among the treatments for the 110 cm³ tube, while the best results among the treatments for the 50 cm³ tube occurred with fractional fertilization tubes 7 and 8. This shows that with a higher volume of the cover manure, the plants are photosynthesizing adequately, being indicative of good physiological response, and that good fertilization is necessary for the smaller tube (50 cm³) to obtain physiologically similar seedlings with the highest volume, showing that there is a gain due to all the advantages of producing in a smaller container.

Both the stomatal conductance (*gs*) (Figure 4b) as well as the transpiration (*E*) (Figure 4c) showed the same trend as was found for the 50 cm³ differences, as only treatments 1 and 8 were higher. The different fertilizations did not modify the variables in the case of larger volume fertilizers, while

the plants with the best fertilization in the case of smaller volumes (in the treatment case 8) presented a better stomatal regulation due to the higher potassium content in the leaf (Figure 3c), which added to a higher photosynthetic rate, consequently resulting in higher height growth and stem dry mass (Figures 1a and 2a).

Tonello & Teixeira Filho (2013) obtained similar results in *Eucalyptus* sp seedlings, in which they observed that stomatal conductance (gs) follows transpiration (*E*). Davila et al. (2011) found similar results in clonal Eucalyptus seedlings: treatments with potassium fertilization showed higher development in height and diameter due to the greater stomata opening.

These data mainly reveal the importance of fertilization in smaller containers, since these presented better responses in the physiological parameters of the seedlings in relation to the implemented treatments, thus showing that greater care should be taken in relation to fertilization in seedlings grown in smaller containers; it is also clear that a seedling grown in a small but well fertilized tube has a physiological apparatus that provides excellent development in the field.



Figure 4. Mean (a) photosynthesis (*A*), (b) stomatal conductance (*gs*), and (c) transpiration (*E*) rates at 120 days in *A. fraxinifolius* seedlings according to volume and fertilization management. Means with different letters differ significantly by the Scott Knott test at 5% significance; upper case letters differ in size from lower case letters and different treatments.

There was no statistical difference regarding chlorophyll a/b (Figure 5a). The photosynthesis/nitrogen (Figure 5b) and photosynthesis/phosphorus (Figure 5c) ratios verified that the fractionated fertilization for the 50 cm³ tube presented

statistical differences, further showing that higher fertilization increased the deficiency in the use of both nitrogen and phosphorus; an increase that improves physiological conditions and may influence survival in the field. When analyzing the different container volumes, we noticed that when there is lower fertilization the 110 cm³ tubes are more efficient, meaning they have good efficiency even with low doses of nutrients. Due to the larger diameter in these tubes, fertilization is already sufficient to meet the

nutritional and physiological demands of photosynthesis, even in small quantities; in contrast, the volume restriction in the small tube accentuates the effect of the smaller fertilizations, interfering in the parameters, including the photosynthesis/nutrition ratio.



Figure 5. Means of the (a) chlorophyll a/b ratio, (b) photosynthesis/nitrogen ratio, and (c) photosynthesis/phosphorus ratio at 120 days in *A. fraxinifolius* seedlings according to volume and fertilization management. Means with different letters differ significantly by the Scott Knott test at 5% significance; upper case letters differ in size from lower case letters and different treatments.

There is a trend in the lower volume fertilizer for both A/N and A/P when fertilized with the higher doses of fractionated fertilization (treatments 7 and 8), which showed a tendency towards better values in these relationships, indicating a better use of nutrients in photosynthesis, and that despite a growth restriction due to the size of the tube, the physiological parameters show the seedling is physiologically suitable to go to the field with great chances of survival.

4. CONCLUSION

Fractional fertilization had better results compared to slow release fertilization, while 110 cm³ tubes presented seedlings with better morphological characteristics and are less sensitive to fertilization restriction. The 50 cm³ tubes are more sensitive to fertilization due to the lower volume.

The better fertilization treatments were 7 (150 mg dm⁻³ N, 300 mg dm⁻³ P₂O₅ and 150 mg dm⁻³ K₂O) and 8 (300 mg dm⁻³ N, 600 mg dm⁻³ P₂O₅ and 300 mg dm⁻³ K₂O in the substrate, plus 40 mg N and 30 mg K₂O per tube in weekly manure cover).

Due to the better physiological quality/container volume ratio, the best treatment combination is the 50 cm³ tube and fertilizations 7 and 8.

ACKNOWLEDGEMENTS

We would like to thank God, Instituto Federal Goiano, campus Rio Verde, our supervisor and families for the support.

SUBMISSION STATUS

Received: 24 Oct. 2017 Accepted: 31 July 2018 Associate Editor: João Latorraca

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