

Production of seedlings of three forest tree species from the Colombian tropical rainforest

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ABSTRACT

Lecythis tuyrana, *Eschweilera coriacea*, and *Clathrotropis brunnea* are three species with high forestry and nutraceutical potential for restoring the Magdalena River Basin in the Anden region of Colombia. The study aimed to establish a baseline for producing high-quality seedlings with these species under nursery conditions. In independent experiments for each species, the treatments consisted of a factorial combination of three substrates formulated with local soil (LS), vermicompost (VC), pine bark (PB) or rice husk (RH), and three doses of a 6-month controlled-release fertilizer (CRF). In general, substrates, the addition of CRF up to 8 g L⁻¹ or its interaction had little influence on the growth, biomass, and quality of *L. tuyrana* and *C. brunnea* seedlings. The combination of S2 substrate (40% LS + 20% VC + 40% RH) + 5 g L⁻¹ produced *E. coriacea* seedlings with 1.6 times more total biomass than unfertilized seedlings. Despite being 23% more expensive than the local substrate, this substrate has a more acceptable pH, bulk density, and nutrient contents. The importance of selecting the appropriate dose of CRF among tree species of the same biome is highlighted.

Keywords: *Lecythis tuyrana*, *Eschweilera coriacea*, *Clathrotropis brunnea*, restauration, silviculture.

INTRODUCTION

Of the five natural regions that divide Colombia, the Magdalena River basin, inserted in the Andean region, comprises 23% of the national land area or 265.500 km² and is characterized by the presence of abundant endemic flora and fauna.⁽¹⁾ However, intense deforestation has led to only 13% of the original forest cover remaining.⁽¹⁾ Several forest species native to the Magdalena River Basin could be used in ecological restoration or commercial reforestation. For example, oil extracted from *Lecythis tuiyana* Pittier could be used for conventional or vacuum frying and baking.⁽²⁾

Eschweilera coriacea (DC.) S.A. Mori can be an important source of timber.⁽³⁾ Sawdust of *Clathrotropis brunnea* Amschoff has pharmacological potential against cutaneous leishmaniasis and trypanosomiasis and high-quality wood.^(4,5) The first step is establishing a baseline to produce high-quality seedlings of these species.

Among the factors that influence seedling growth and quality, the substrate plays a fundamental role, as it provides water and nutrients.^(6,7) In recent years, soil replacement has become increasingly important in substrate formulation due to environmental concerns, the presence of pests or diseases, and inadequate physicochemical properties.⁽⁸⁾ Organic residues from forestry or other industries have proven to be a viable alternative for partially or totally replacing soil, as it improves nutrient availability, porosity, moisture retention, C:N ratio, and pH.^(9,10)

Controlled-release fertilizers (CRFs) represent an alternative to conventional fertilizers in seedling production, as they improve nutrient absorption efficiency,⁽⁷⁾ while reducing losses due to leaching or volatilization and minimizing root damage caused by excessive fertilization.^(11,12) In addition, fewer applications reduce production costs, especially for species with long growth periods.^(13,14) However, the high cost of CRFs makes it necessary to adjust the optimal dose for each species.^(14,15) In Colombia, CRF doses for native forest seedlings are still based on empirical data or on rates used for exotic species in subtropical or temperate conditions.

Our hypotheses were: 1) tree species in the Tropical rainforest are affected differently by CRF use, and 2) partial substitution of soil by organic components in the substrate composition with the correct dose of CRF is commercially justified. The objective was to select the appropriate substrate with alternative components and CRF doses to produce seedlings of three tropical tree species of

the Magdalena River basin. This baseline would be a more sustainable environmental alternative in the long term than the predominant use of local soil as the sole component for substrate composition.

MATERIALS AND METHODS

Seedling production

The study was conducted at Centro de Investigación La Suiza of Corporación Colombiana de Investigación Agropecuaria (Agrosavia), located in the municipality of Rionegro, Santander, Colombia (7°22'10"N, 73°10'39"W; 550 m). According to the Köppen classification, the region shows a humid tropical climate (Af) with historical (1993 to 2022) averages of 27.5 °C and rainfall of 2,868 mm year⁻¹ (Figure S1). From January to April 2022, mature fruits were collected from tree crowns in the municipalities of Puerto Parra, Landazuri, Bolívar, and Cimitarra, Magdalena River basin, Andean region, department of Santander, Colombia (Figure 1; Figure S2).

The intense loss of native forest cover in the collection areas caused few individuals to be found, reducing the number of available seeds, especially for *Eschweilera coriacea* (Table S1). As these three species are rare or have some degree of local threat,⁽¹⁶⁾ up to 30% of the mature fruits observed in the tree canopy were collected, limiting the number of seedlings per experimental unit or replicates. Seedlings were produced in a nursery with a black monofilament screen and mesh to offer 50% shading and micro-sprinkler irrigation (water blade= 10 mm day⁻¹) (Figure S2). Seed seedlings were transplanted 65 days after sowing (das) to 1,250 cm³ polyethylene plastic bags (10 diameter x 27 cm height), the most common in local forest nurseries.

A randomized complete block experimental design with three replications in a 3 x 3 factorial arrangement was adopted for each species (independent experiment). The experimental unit for *Clathrotropis brunnea* and *Lecythis tuiyana* was nine seedlings and six for *Eschweilera coriacea*. Factor A referenced three substrates formulated with different volumetric proportions of the following alternative components: pine bark (PB), local soil (LS), collected at a depth of 0-30 cm in a forest area; rice husk (RH); and commercial vermicompost comprising bovine manure and plant residues (VC). PB and LS were sieved through a 0.5 cm mesh before mixing with the other components. Substrate treatments were denominated as follows:

S1= 60% LS + 20% RH + 20% VC or the substrate used by local nurseries without CRF to serve as our control and two proposed alternative substrates, S2= 40% LS + 40% RH + 20% VC and S3= 20% LS + 40% VC + 40% PB.

A sample of the soil used was analyzed, indicating high acidity ($\text{pH} = 4.1$) and exchangeable aluminum ($3.37 \text{ cmol}_c \text{ kg}^{-1}$), low extractable P (7.46 mg kg^{-1}), and cation exchange capacity (CEC) with values of 0.11, 0.19 and $0.59 \text{ cmol}_c \text{ kg}^{-1}$ of K^+ , Mg^{+2} and Ca^{+2} , respectively. Data provided by the manufacturers indicates that vermicompost contains: $\text{pH} = 7$, bulk density= 0.83 cm g^{-3} , $\text{CEC} = 25 \text{ cmol}_c \text{ kg}^{-1}$, C:N ratio= 13, and, in g kg^{-1} , N= 2.0, P= 0.66, and K=1.0; pine bark contains: $\text{pH} = 6.2$, bulk density= 0.21 cm g^{-3} , and water holding capacity= 28%; and rice husk contains: $\text{pH} = 6.8$, water holding capacity= 26% and, in g kg^{-1} , P= 0.40 and K= 4.15.

Factor B comprised three doses of CRF (Basacote ® Plus 6M) with granule size of 2 to 4 mm, a formulation of 16% N, 8% P_2O_5 , 12% K_2O , 2% MgO , 5% S, 0.02% B, 0.05% Cu, 0.4% Fe, 0.06% Mn, 0.015% Mo, and 0.02% Zn. *Clathrotropis brunnea* and *Lecythis tuyaana* received 4 and 8 g L^{-1} of CRF. In contrast, the smaller seedlings of *Eschweilera coriacea* received a dose of 2.5 and 5 g L^{-1} to reduce the risk of toxicity. The control treatment for all three species did not receive CRF. For a better homogenization, for every 60L of prepared substrate the corresponding amount of fertilizer dosage was added and so on until completing the filling of the all bags.

Evaluation of seedling growth and quality

Based on previous studies on similar conditions, a time of seedling production of 180 das was determined for *Lecythis tuyaana* and 210 das for *Clathrotropis brunnea*.^(17,18) In *Eschweilera coriacea*, the criterion was based on seedlings reaching a mean height greater than 20 cm in the best treatment, which was at 275 das. Height and stem diameter were measured after 95 days and every three or four weeks. A total of 4, 5, or 7 evaluation periods were measured until the seedling production time was completed, depending on the species. Stem diameter (SD) was measured at the epicotyl scar (*Lecythis tuyaana*) or the substrate level (*Eschweilera coriacea* and *Clathrotropis brunnea*) with a digital calibrator and height (H) was measured from the substrate level to the apical bud with a flex meter. Once seedling growth was complete (at 180, 210, or 275 days), destructive sampling was performed. Shoot dry mass (SDM) and root dry mass (RDM) were determined by drying the plant material in an oven at 65°C up to constant weight. With these values, the Dickson's quality index (DQI) was determined⁽¹⁹⁾ (Formula 1):

$$\text{DQI} = (\text{TDM}) / ((\text{H} / \text{SD}) + (\text{SDM} / \text{RDM})) \quad (\text{Formula 1})$$

Where height (H [cm]), stem diameter (SD [mm]), total dry mass (TDM) = shoot dry mass (SDM [g seedling⁻¹]) and root dry mass (RDM [g seedling⁻¹]).

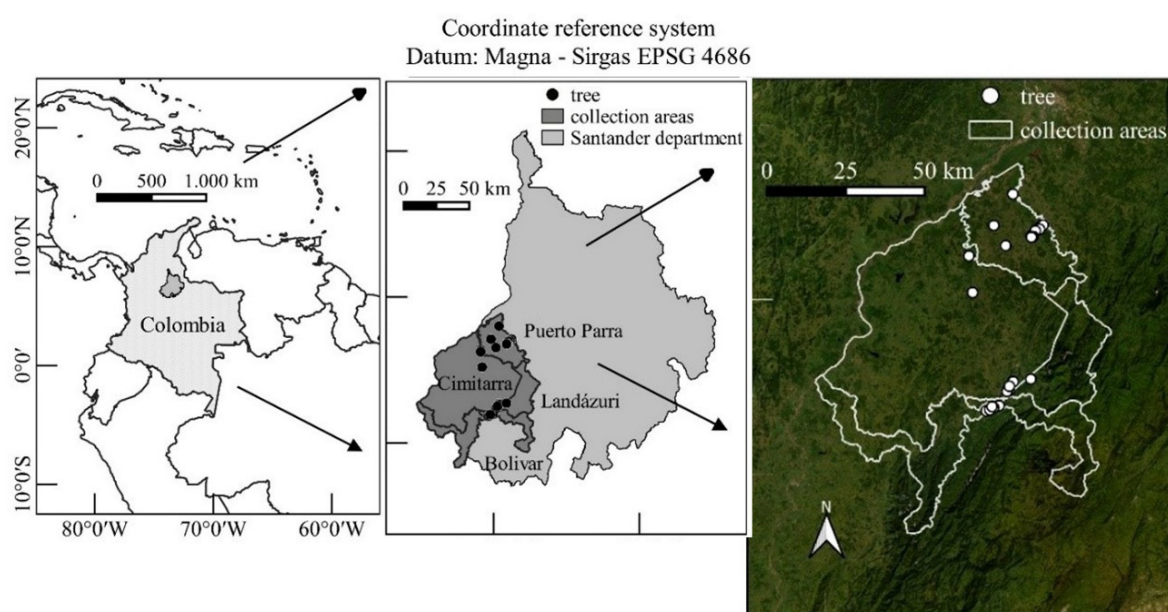


Figure 1. Location of the area for seed collection of three tree species in the municipalities of Cimitarra, Puerto Parra, Landázuri and Bolívar, Magdalena River basin, Santander, Colombia.

The mean \pm standard deviation temperature and relative humidity recorded inside the nursery during the experimental period (May 2022 to January 2023) were 24.6 ± 4.5 °C and $82.3 \pm 16.1\%$, respectively.

Substrates characterization and costs

A sample of saturated substrate paste (S1, S2, and S3 substrates), with water added until the point of saturation, was sent to characterize its chemical and physical properties per Colombian Technical Standard NTC 5167.⁽²⁰⁾ The substrate's bulk density (Bd) was determined following the methodology standard.⁽²¹⁾ The cost of each substrate (USD\$ m⁻³) included transportation from the acquisition site to the municipality of Rionegro, plus the CRF added. Transportation cost was standardized for all components at 46.0 USD m⁻³ and one bag (25 kg) of CRF for 172.5 USD. Prices of the components were 32.2, 202.4, 128.8, and 358.8 USD m⁻³ for local soil, vermicompost, rice husk, and pine bark, respectively.

Statistical analysis

Normality and homoscedasticity of variance of the attributes biometric and quality indices of the seedlings were verified in each experiment, according to the Shapiro-Wilk and Bartlett tests, respectively. A two-way analysis of variance was conducted (factors: substrates x doses of CRF) to verify the effect of the factors and the interaction on the response variables. When a significant effect was identified, the treatment mean was adjusted to the regression models (linear and quadratic) for the dose of CRF (quantitative factor). The Tukey test was applied for the substrates (qualitative factor). The best model selection was based on the significance of the parameter estimates and R² values. The same regression analysis was performed on height and stem diameter to analyze growth in different evaluation periods. However, substrate, CRF, and days after planting (time) were considered sources of variation. All analyses were performed in the statistical program R version 4.2.2) using the ExpDes package.⁽²²⁾

RESULTS

Evaluation of seedling growth and quality

Depending on the species, the growth of seedlings responded in different ways to the substrates formulated, dose of CRF, days after sowing (das), or its interaction. The increase over evaluation periods (95, 120, 145, and 180) in

stem diameter of *Lecythis tuiyana* seedlings were affected ($p < 0.05$) by the interaction of the factors CRF x substrate x das and height by the interaction's substrate x das and CRF x das (Table S2). At the end of seedling production time (six months), there was an isolated effect ($p < 0.05$) of the CRF factor for SDM and TDM (Table S2). These four variables showed a linear response with increasing doses of CRF, but the equation fit was lower for SDM and RDM (Figure 2). The RDM, SDM: RDM, and DQI ($p > 0.05$) had a mean of 2.49 g seedling⁻¹, 5.85, and 1.40, respectively (data not shown). Seedlings fertilized with 8 g L⁻¹ compared to unfertilized seedlings were taller (53.3 cm vs. 44.6 cm; +20%) and accumulated more SDM (15.5 g seedling⁻¹ vs. 13.1 g seedling⁻¹; +19%) and TDM (18.0 g seedling⁻¹ vs. 15.5 g seedling⁻¹; +16%) (Figure 2A and 2C). However, the increase in stem diameter (7.8 mm vs. 7.6 mm; +3%) was almost invariable (Figure 2B).

The analysis of variance showed an effect ($p < 0.05$) for the interaction of the factors CRF x substrate x das on height growth over evaluation periods (100, 130, 160, 190, and 210 das) and the factor das on stem diameter of *Clathrotropis brunnea* seedlings (Table S3). Considering the last evaluation period at seven months, SDM depended ($p < 0.05$) on the isolated effect for the CRF and substrate factors; RDM, SDM: RDM and DQI on the effect of the CRF factor and TDM on the effect of the substrate factor (Table S3). In general, the height of seedlings had a strongly linear growth with increasing doses in three substrates, as well as stem diameter (Figure 3). The coefficient of determination was low for the linear behavior of RDM, SDM, SDM: RDM, and DQI (Figure 4).

The maximum dose of 8 g L⁻¹ implied that the seedlings were taller than unfertilized seedlings in the S2 substrate (40.5 cm vs. 33.7 cm; +20%) but practically constant in the S1 (35.1 cm vs. 35.0 cm) and S3 substrates (41.4 cm vs. 39.5 cm) (Figure 3A, 3B and 3C). Regardless of the substrates, there was a decrease in RDM (2.98 g seedling⁻¹ vs. 2.48 g seedling⁻¹; -17%) and DQI (1.30 versus 1.15; -11%) but an increment in SDM: RDM (2.84 vs. 2.10; +35%) when increased to 8 g L⁻¹ (Figure 4A, 4B and 4C). The SDM did not fit any mathematical model and showed a mean of 6.26 g seedling⁻¹ (Figure 4A). The SDM (7.06 g seedling⁻¹) and TDM (9.59 g seedling⁻¹) in S3 substrate were 12 e 3% higher than S1 substrate, respectively (Table S4).

During the 9.2 months in which the *Eschweilera coriacea* seedlings were evaluated (95, 120, 150, 180, 215, 235,

and 275 das), the analysis of variance showed influence ($p < 0.05$) in height for the interactions of the factors CRF x das and substrate x das and the stem diameter for the interaction of the factors CRF x substrate x das (Table S5). In the last seedling evaluation at 275 das, the interaction of CRF and substrate factors influenced ($p < 0.05$) the SDM, TDM, and DQI; the isolated effect of CRF and substrate factors the SDM: RDM (Table S5). The seedlings' growth

in the S3 substrate (17.8 cm) reached a height higher than the S1 (12.6 cm; +42%) and S2 (16.2 cm; +10%) substrates, respectively (Figure 5A). The maximum doses of 5 g L^{-1} (17.5 cm) increased the height of unfertilized seedlings (14.3 cm) and fertilized with 2.5 g L^{-1} (14.2 cm) (Figure 5B). It was typical that all the treatments fitted a linear model.

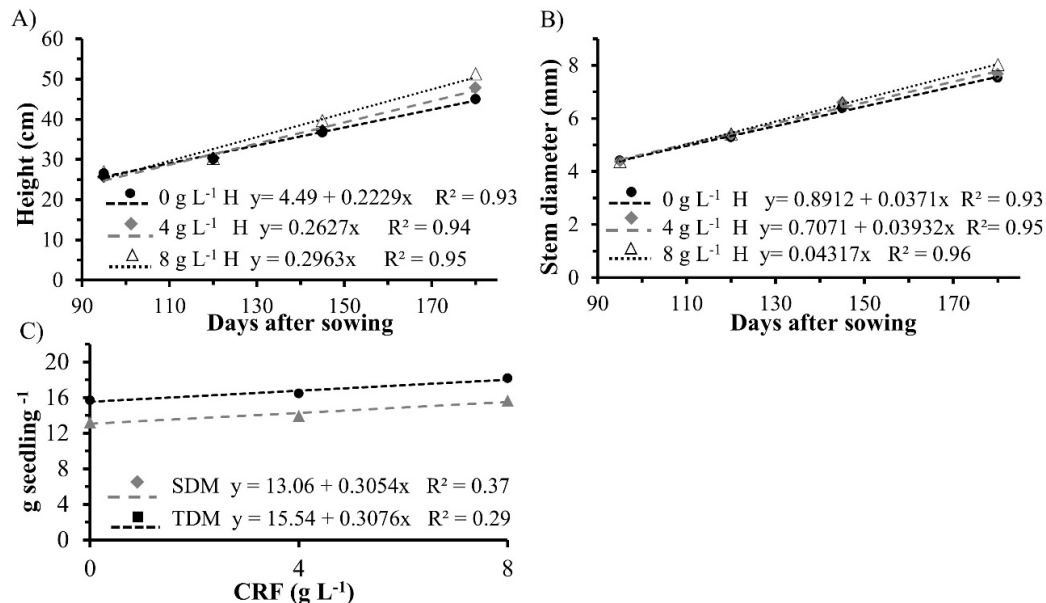


Figure 2. Regression equations for height (A), stem diameter (B), shoot dry mass SDM, and total dry mass TDM (C) in *Lecythis turyrana* seedlings depending on the interaction of CRF x das factors (height and stem diameter) or CRF factor (SDM and TDM). das= days after sowing.

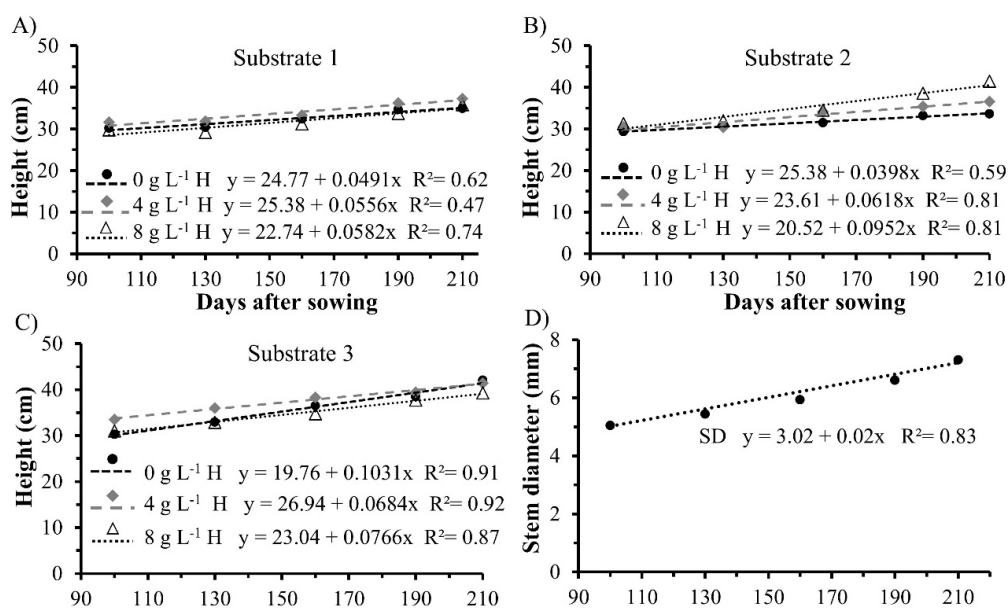


Figure 3. Regression equations for height (A, B, and C) and stem diameter (D) in *Clathrotropis brunnea* seedlings depending on the interaction of CRF x substrate x das (height) factors or interaction of CRF x das (stem diameter). das= days after sowing.

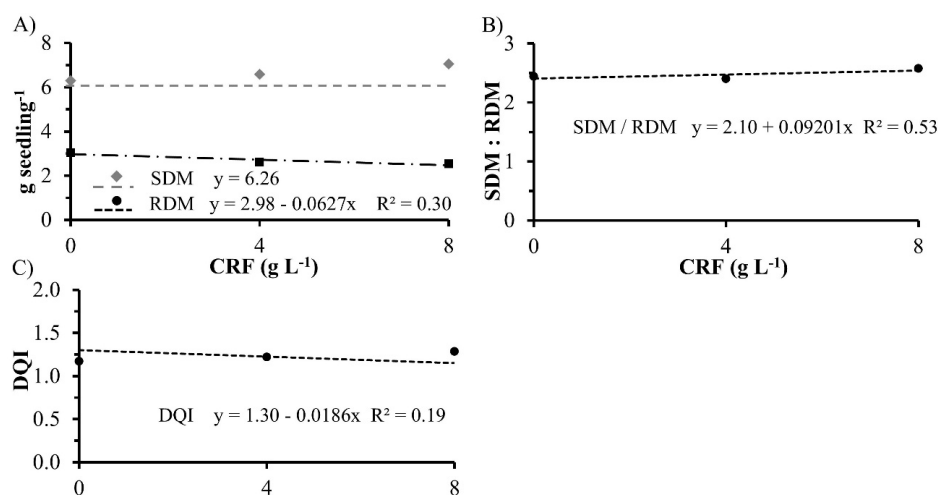


Figure 4. Regression equations for shoot dry mass (SDM), root dry mass (RDM), and SDM: RDM and Dickson quality index (DQI) in *Clathrotropis brunnea* seedlings depend on the CRF factor.

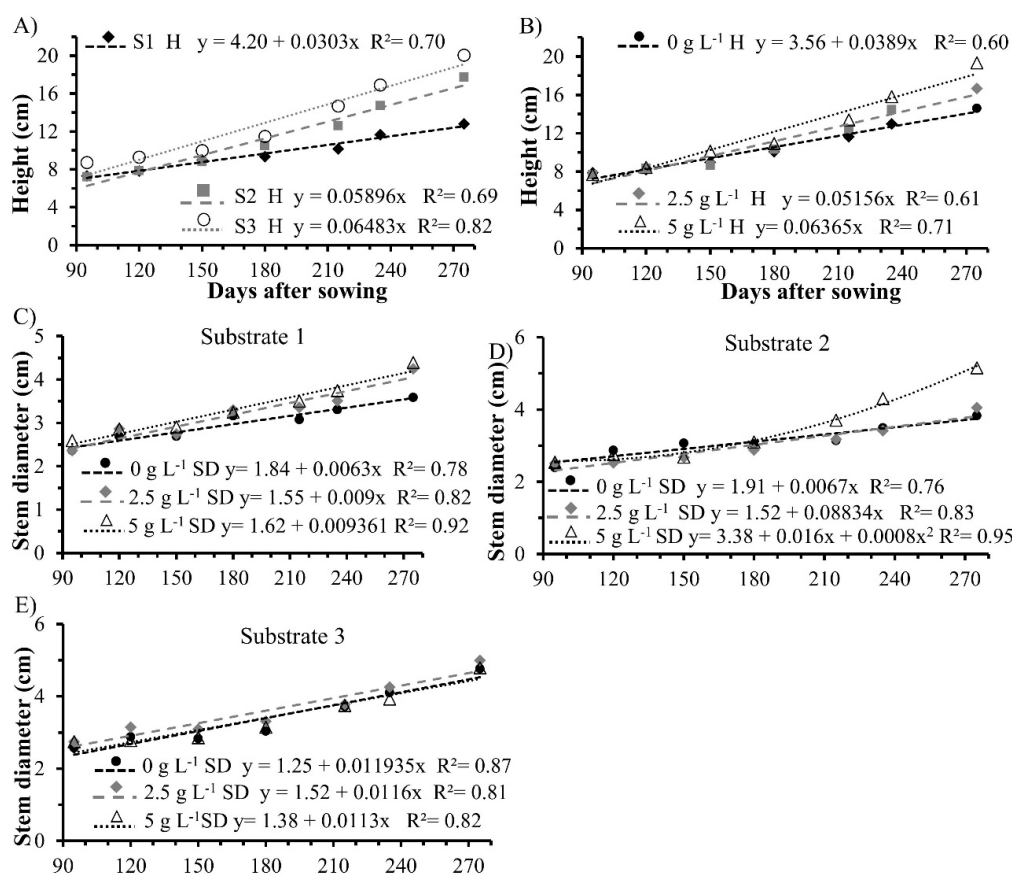


Figure 5. Regression equations for height (A, B, and C) and stem diameter (D and E) in *Eschweilera coraceae* seedlings depending on interaction CRF x das (height) or interaction of CRF x substrate x das (stem diameter). das= days after sowing.

The increase in final stem diameter when using 5 g L⁻¹ was greater in unfertilized seedlings from substrate S2 (5.03 mm vs. 3.75 mm; + 34%) than in those from substrate S1 (4.19 mm vs. 3.56 mm; +17%) (Figure 5C and 5D). In substrate S3, the seedling doses reached similar values among the CRF doses (mean 4.58 mm) (Figure 5E).

The dose of 5 g L⁻¹ in substrate S2 showed a substantial quadratic adjustment, while the other treatments followed a linear adjustment. SDM, DQI, and TDM were more favored using CRF in the S2 substrate than in the S1 and S3 substrates. In substrate S3, these three variables showed a constant response (Figure 6A, 6C and 6E).

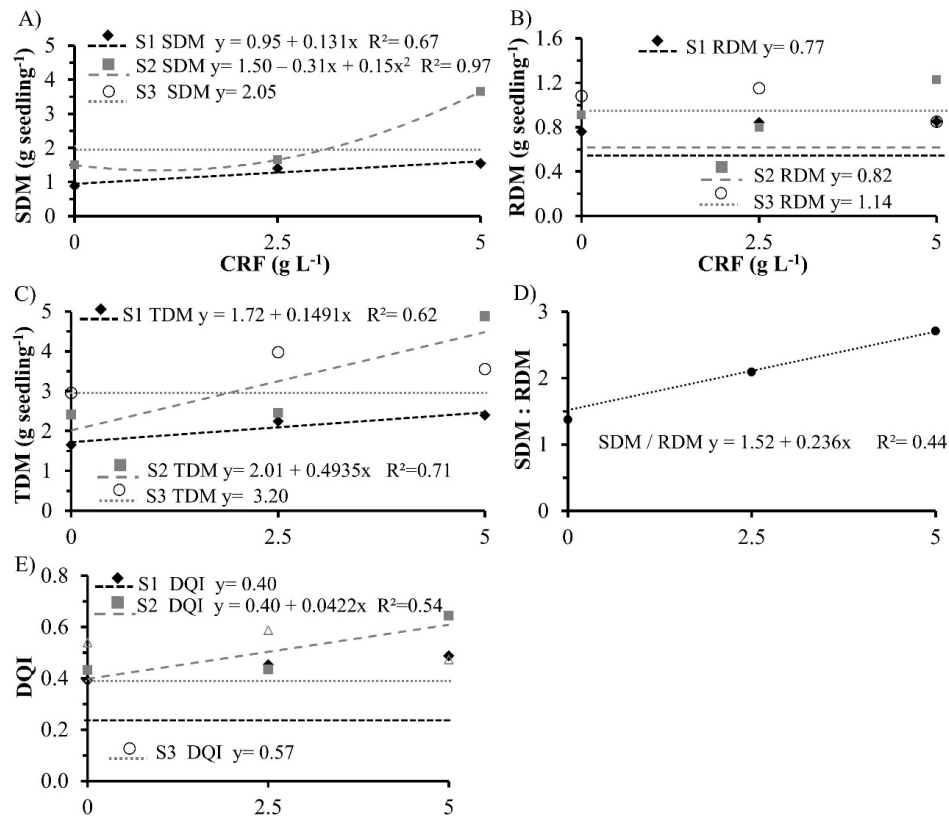


Figure 6. Regression equations for shoot dry mass SDM (A), root dry mass RDM (B) and total dry mass TDM (C), SDM: RDM (D) in *Eschweilera coraceae* seedlings depending on interaction of doses of controlled-release fertilizer x substrate (SDM, RDM, TDM and DQI) or doses of controlled-release fertilizer (SDM: RDM), after nine months in the nursery.

The maximum dose of 5 g L⁻¹ implied a positive second-degree polynomial adjustment of the SDM in substrate S2 reaching a value 1.3 times higher than unfertilized seedlings (3.45 g seedlings⁻¹ vs. 1.50 g seedlings⁻¹). In substrate S1, the response was linear with a 0.7 times higher value (1.61 g seedlings⁻¹ vs. 0.95 g seedlings⁻¹) (Figure 6A). TDM had a linear behavior in both substrates S1 and S2 (Figure 6C). The RDM had a constant value (2.05 g seedling⁻¹) among the doses evaluated in the three substrates (Figure 6B). Independent of the substrate, SDM: RDM showed a positive linear response as a function of CRF (Figure 6D). Finally, DQI in the S2 substrate responded linearly, increasing its value to 43% with the 5 g L⁻¹ relative to the unfertilized seedlings (0.40 vs. 0.57). On the contrary, in S1 and S3 substrates, the response was constant between doses (Figure 6E).

DISCUSSION

Biometric variables and quality indices

No reference values were found for the parameters evaluated in the seedlings of the three species. Therefore,

the comparison of their suitability for field planting must rely on other species. Reports on other forest species (e.g. *Pinus* sp. or *Eucalyptus* sp.) indicate heights from 20 to 30 cm, stem diameter > 4.00 mm, or an optimal SDM:RDM ratios fall between 2 and 3, providing better resistance to hydric stress by balancing shoot, photosynthetic capacity with root nutrient and water absorption.^(23,24) All seedlings of *Lecythis tuyaana* (height > 45 cm and SD > 7.6 mm) and *Clathrotropis brunnea* (height > 34 cm and SD = 7.22 mm) exceeded the required height and stem diameter. The DQI, which groups non-destructive and destructive variables, indicates a positive correlation with seedling quality. Stem diameter, a non-destructive method, can be considered the best practical indicator for estimating DQI.^(18,25) Judging by the constant results of the DQI and the minor variation in the other attributes, seedlings of both species can be produced on any substrate without the addition of CRF.

Lecythidaceae, the third most abundant tree family in Amazonian forests, is characterized by having many emergent species. Together with *Clathrotropis* (Fabaceae), they are common in non-flooded lowlands.^(5,26) High-density wood species tend to grow slowly, while pioneer

species show rapid growth but low wood density.^(3,27) The basic wood density of *Clathrotropis brunnea* (0.89 g cm^{-3}), *Eschweilera coriacea* (0.81 g cm^{-3}) and *Lecythis* sp (0.77 g cm^{-3}) suggests slow growth.⁽²⁷⁾ Therefore, it is unlikely that differences in response to CRF are related to succession status. Pioneer species tend to benefit more from nutrient enrichment due to reduced competition.⁽²⁸⁾ A longer-term assessment of seedling growth could better clarify the strategies of each species in its different phases. For example, *E. coriacea* may require higher initial light levels and then a more shaded environment to achieve higher growth rates.

A common characteristic between *Lecythis tuiyana* and *Clathrotropis brunnea* seeds is their dimensions, $4.5 \text{ cm} \times 7.0 \text{ cm}^2$ and $4.4 \text{ cm} \times 3.0 \text{ cm}$,⁽²⁹⁾ respectively, which are larger than the seeds of *Eschweilera coriacea*, measuring $2.5 - 3.0 \text{ cm} \times 1.5 - 2.0 \text{ cm}$.⁽³⁰⁾ Larger seeds generally produce more vigorous seedlings with higher survival probability.^(18,31) As the release time of CRF (5-6 months at 21°C) was sufficient according to the manufacturer for soils with low natural fertility, *E. coriacea* seedlings would probably increase establishment rates if fertilized. This is especially true for the initial phase, where abiotic and biotic factors play an important role.

The increase of up to 15% in some attributes (height, SDM, and TDM) with the addition of 8 g L^{-1} of CRF in *Lecythis tuiyana* seedlings was probably due to the seed and nutrient reserves in the three substrates being sufficient with the size of the bag used. The near absence of influence of substrates, CRF, or their interaction with *Clathrotropis brunnea* seedlings reinforces our hypothesis. When 3 g L^{-1} of CRF was added to a substrate like substrate S1, there was hardly a significant effect on the SDM of *Lecythis tuiyana* seedlings produced with this same bag volume.⁽¹⁷⁾ Similar results to our study were found in *Clathrotropis brunnea* seedlings without CRF.⁽¹⁸⁾

If the stem diameter is considered as the only quality parameter, fertilized and unfertilized seedlings from substrate 3 ($> 4.5 \text{ mm}$), the combination of substrate S1 + 2.5 g L^{-1} (4.05 mm) or substrate S2 + 5 g L^{-1} (4.09 mm) reached the required. The best positive response with the combination substrate S2 + 5 g L^{-1} for the height, stem diameter, SDM, TDM, and DQI without altering the ideal SDM:RDM indicates that it should be chosen over the other combinations. Unlike *L. tuiyana* and *C. brunnea*, the strong substrate \times CRF interaction observed in *E. coriacea* may be due to

a more rapid depletion of nutrient reserves in its smaller seeds. High-quality seedlings also showed a higher shoot/root ratio, which improves photoassimilate production.

It is important to interpret the results for the three species cautiously because the attributes and quality indexes vary on factors such as nursery duration, container volume, substrates, and species characteristics.^(6,13,25,32) For example, stem diameter, height, and DQI predict the field performance of seedlings; however, sometimes, the effect in the nursery does not continue in the field.⁽¹⁰⁾ We suggest that seedlings from these best treatments be evaluated in the field to propose minimum quality parameters for each species. This would establish a baseline for selecting seedlings suitable for planting in the field.

Some variables did not present a good fit to the model, and only three doses were evaluated in our study, but there was no evidence that increasing the doses negatively affected the growth of the seedlings. In various tree species, doses within or above the range evaluated in this study have been recommended because no toxicity effects were reported.^(11-13,15) Thus, higher doses can be evaluated. We are aware that the smaller number of seedlings per repetition in the *E. coriacea* experiment could cause concern in the interpretation of the results. We suggest establishing seed orchards and germplasm banks and conducting a more exhaustive identification of mature trees of this species in the region under study.

Substrate characterization and costs

All substrates exhibited a C:N ratio of less than 20, suggesting good stability and maturity of the components, which avoids the immobilization of N.^(33,36) The properties of each substrate explain the difference observed between them when CRF was not applied. Substrate S2 had a higher content of P and K and a slight decrease in bulk density compared to substrate S1 (Table 1). Even so, it was the S3 substrate that showed greater fertility, water retention capacity, pH, and bulk density that was closer to the recommended of $5.5 - 6.5$ and $< 0.85 \text{ g cm}^{-3}$, respectively.⁽⁸⁾ The electrical conductivity, however, was the highest and should be monitored. The increase in vermicompost used in the composition of substrates led to a greater CEC of up to 20 times higher than the very acidic local soil with a $\text{CEC} < 1 \text{ cmol}_c \text{ kg}^{-1}$ (Table 1). The improvement in soil fertility with vermicompost, chicken, or bovine manure, which tends to be neutral to basic, has been demonstrated by other studies on forest species.^(10,32,34)

Table 1. Physicochemical properties and cost of substrates in seedling production of three tropical forests with the use of a 6-month controlled-release fertilizer (CRF)

S	pH	E.C	C	N	P	K	CEC	C:N ratio
		dS m ⁻¹	g kg ⁻¹					
S1	7.1	5.0	2.21	0.32	< 0.01	< 0.01	9.7	7.0
S2	6.6	5.8	1.88	0.30	0.20	0.13	10.1	6.4
S3	6.0	9.1	9.74	0.56	0.17	0.26	19.4	17.5
	Bd	WHC	Dose of CRF (g L ⁻¹)					
	(g cm ⁻³)	%	0	2.5	4	5	8	
S1	1.25	49.6	85.6	92.7	97.1	99.9	108.6	
S2	1.02	48.2	USD\$ m ⁻³ *	106.4	112.1	116.4	119.3	127.9
S3	0.78	93.9	230.9	238.1	242.4	245.3	253.9	

E.C= electrical conductivity. C= organic carbon. Bd= bulk density. CEC= cation exchange capacity; K⁺ + Mg²⁺ + Ca WHC= water holding capacity.

* 1 USD\$ = COP\$ 4,344 (2025 Jan 17) ⁽³⁷⁾

S1 substrate with the lowest proportion of vermicompost promoted the lowest growth in the *Eschweilera coraceae* seedlings and, to a lesser extent, in *Clathrotropis brunnea*. This substrate's high proportion of soil reduced the aeration capacity, drainage, and fertility (Table 1). Therefore, the quality of seedlings even using the CRF. The higher proportion of rice husk (40%) in substrate S2 - a stable organic component known for its neutral pH, good aeration capacity, and low bulk density⁽³⁵⁾ - increased the pH and improved water holding capacity in this substrate (Table 1). The same positive effect with pine bark composition in the S3 substrate could occur where the quality of *Eschweilera coraceae* seedlings was similar, even without fertilization, to those obtained with the other fertilized substrates.

Pine bark is an organic material with high organic carbon content, Ca²⁺ and K⁺, low bulk density, high C:N ratio, and adequate drainage,^(15,36) as the physicochemical analysis showed (Table 1). The low Ca²⁺ available in the local soil and its absence in the CRF indicate a relevant effect of pine bark and, to a lesser extent, vermicompost on seedling growth.

In Colombia, most nurseries select soil as the main component for substrate formulation in seedling production. Disregarding the negative environmental impact and its inadequate physicochemical properties (sanitation, poor drainage, high density, and nutritional deficiencies) that predispose seedlings to sanitary and physiological problems, soil is preferred due to availability and lower cost.^(8,34,35) In unfertilized substrates, the partial substitution of the soil of substrate S1 with pine bark, vermicompost, or rice husk increased the costs of the other substrates, ranging from 23 (S2) to 170% (S3) (Table 1). Although it does

not equal the best quality of the S3 substrate, replacing pine bark with rice husk lowers costs and provides acceptable fertility and aeration capacity in the S2 substrate.

Considering the substrate S1 as a reference because it is the most common in local nurseries, the fertilization with 8 g L⁻¹ increases costs by 27% compared to unfertilized *Lecythis turyana* and *Clathrotropis brunnea* seedlings but with a gain in biomass or height of less than 20%. In *Eschweilera coraceae*, the substrate S2 + 5 g L⁻¹ would increase costs by 39% compared to the unfertilized seedlings of substrate S1 (119.3 USD\$ m⁻³ versus 85.6 USD\$ m⁻³) but with a substantial increase in the quality of the seedlings (height=+116%, stem diameter= +41, SDM= +311%, RDM= +61%, and DQI=+ 64%). Improvements in these parameters will most likely increase the survival chances of seedlings. Large-scale forest seedling production presents significant environmental drawbacks due to the dependence on the soil as a substrate principal component.^(9,10) Therefore, there is an urgent need to replace soil with alternative components in these three species.

CONCLUSIONS

The combination of S2 substrate + 5 g L⁻¹ of CRF is likely justifiable because it improves the growth parameters of *Eschweilera coriacea* seedlings and reduces production time in the nursery. Still, these seedlings should be evaluated in the field.

Although it is more expensive and does not imply a substantial improvement in growth and quality, it is suggested that *Lecythis turyana* and *Clathrotropis brunnea* use the S2 substrate as an alternative to the local substrate without fertilization.

SUPPLEMENTARY DATA

Supplementary data are available at: https://docs.google.com/document/d/11_sKQy7EBEVVuV2njDekHMLFssxwCfHZ/edit

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DATA AVAILABILITY

All datasets supporting the results of this study are available upon request to the corresponding author, Andrés Iván Prato. The datasets are not publicly available due to restrictions imposed by the funders.

AUTHOR CONTRIBUTIONS




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