

Article

Functional Morphology and Early Growth of Seedlings of Tropical Species

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Abstract

This study was undertaken to evaluate tropical species: *Calophyllum brasiliense*, *Bravaisia integerrima*, *Roseodendron donnell-smithii*, *Piscidia piscipula*, *Enterolobium cyclocarpum*, and *Dialium guianense*. The seeds were arranged in a completely randomized design under conditions of 50% shading and analyzed using the repeated measures method. In the experiment, growth was evaluated for six months after germination, and seedling morphology and phyllotaxis were described. The parameters stem height (SH), SH relative growth rate (SRGR), stem basal diameter (BD), BD relative growth rate (DRGR), number of juvenile leaves, and survivorship were recorded. Regression curves were generated with the SH and BD data. Seeds with greater length values produced seedlings with improved morphological traits, *E. cyclocarpum* and *C. brasiliense*, regardless of their functional morphology. Germination began 7 to 10 days after sowing. The average survivorship was 70.1% at six months. The highest values in seedling SH at six months were obtained in *E. cyclocarpum* and *C. brasiliense*. The number of leaves was greatest in *C. brasiliense* and *D. guianense*. Considering the features desirable for a nursery plant, production of the following species is considered feasible: *B. integerrima*, *C. brasiliense*, *Piscidia piscipula*, and *Enterolobium cyclocarpum*. The regression curves showed the tendency of the plants to present more rapid growth in the first months after germination.

Keywords: heterophylly; relative growth rate; stem height; stem basal diameter; tropical species



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1. Introduction

Deforestation has a serious impact in terms of biodiversity loss and has been one of the triggers of climate change. The main cause of deforestation is land use change, whether through agricultural or livestock production practices, or the extraction of timber [1]. In addition, there is a scarcity of appropriate strategies for the use of territories that are consistent with their ecological potential, and this often leads to rapid habitat fragmentation [2]. Particularly in the state of Tabasco, Mexico, the decline of forests has been alarming. In

1976, forests represented 15.15% of the state territory [2], but this vegetation type now accounts for less than 2.0% of the total territory of the state [3]. The United Nations warns of the urgent need to prevent, detain, and reverse the degradation of ecosystems [4]. The strategies required to rescue or rehabilitate disturbed areas include reforestation with native tree species, which have the advantage of already being adapted to environmental conditions. They can thrive in degraded sites and improve soil fertility through their association with symbionts [5]. One of the strategies selected in restoration programs is direct sowing, although seeds can be exposed to herbivores and adverse environmental conditions that could diminish both germination and recruitment percentage rates [6]. Another strategy is replanting and reforestation using nursery-produced plants, the advantage of which is the use of developed individuals that could lead to improved survivorship [7].

Nurseries play, then, an important role as custodians and providers of these plants, particularly given the recognition of their importance for the conservation of biodiversity, and can also serve as experimental sites for characterization, selection, and management. Their use allows us to design, determine, and adopt appropriate techniques for the mass production of native species.

Despite the increasing pressure to prevent their extinction, few studies have been conducted on native species [4]. González-Izquierdo et al. [8] evaluated nine species in Cuba, where techniques utilized to obtain quality nursery plants were assessed. Adegoke et al. [9] reviewed growth variables in *Terminalia ivorensis* A. Chev, Pérez-Hernández et al. [10] recorded the performance of *Aspidosperma megalocarpon* Müll. Arg., *Eugenia* sp., *Lonchocarpus castilloi* Standl., *Manilkara zapota* (L.) P. Royen, *Ormosia macrocalyx* Ducke, and *Rollinia mucosa* (Jacq.) Baill under different levels of shading. The morphology of seedling functional types is considered a useful attribute to evaluate in ecological restoration programs. It has its base in the function (reserve storage cotyledons or foliaceous cotyledons) and position of the cotyledons (hypogeal or epigeal) when germinating, which could be related to different tree species recruitment and succession strategies, as well as survivorship success [6]. It has been found that the morphological characteristics of large seeds and reserve cotyledons could facilitate rapid germination; however, environmental factors are determinant in plant survivorship in the field.

Knowledge of the morphological features of seedlings is also necessary, as their structure is often unknown at the juvenile stage but is a useful tool for field identification, especially in studies of the recovery of damaged areas [11], in particular, the morphology of the leaves and number of leaflets, as the descriptions of the species found in the literature are generally for adult plants; height and diameter are also relevant variables indicative of the timing of transplantation. To elucidate production requirements by furthering our knowledge of the initial development phases, morphology, and descriptive characteristics of seedlings, this study selected six native tree species for study: *Calophyllum brasiliense* Cambess. (Calophyllaceae), *Bravaisia integerrima* (Spreng.) Standl. (Acanthaceae), *Piscidia piscipula* L. (Sarg.) (Fabaceae), *Enterolobium cyclocarpum* Jacq. Griseb. (Fabaceae: Mimosoideae), *Roseodendron donnell-smithii* (Rose) Miranda (Bignoniaceae), and *Dialium guianense* (Aubl.) Sandwith. (Fabaceae). These species have their native distribution in the tropics of Mexico, from the state of Tamaulipas to the southeast and southwest of Mexico and Central America. They are found in high and low evergreen, medium semi-evergreen, and semi-deciduous forests and grow in secondary vegetation [12]. Some native species, such as *D. guianense*, *C. brasiliense*, *E. cyclocarpum*, and *R. donnell-smithii*, extend their distribution to Venezuela and Brazil, as well as certain zones of Africa and Asia [13]. Each of these trees can have a variety of uses, but they are mainly utilized for timber or as live fencing [14]. In particular, *D. guianense*, *C. brasiliense*, *P. piscipula*, *R. donnell-smithii*, and *E. cyclocarpum* are considered extremely useful for reforestation and restoration [15].

Piscidia piscipula is distributed across several Mexican states and is not exclusive to the state of Tabasco [13]. Two of the species selected, *C. brasiliense* and *B. integerrima*, are listed in the threatened category, according to NOM ECOL-059 [16], which increases the importance of this study. We aim to contribute to a better understanding of how tropical tree species grow and develop during their initial stages under nursery conditions, with a particular focus on those species that are useful in afforestation or reforestation programs.

These species are intended to be supplied to producers for use in the restoration of original vegetation. This study is based on the hypothesis that large-seeded forest species with cryptocotyledonous hypogeal germination and reserve cotyledons will present higher growth rates than those with phanerocotyledonous epigeal germination and leafy cotyledons. The results of this research will help foresters in tropical areas of the world to restore deforested sites or to establish forest plantations. Furthermore, the objective of this paper is to study the functional morphology and early growth of the seedlings of the six tropical species mentioned above.

2. Materials and Methods

2.1. Survey Site

The seeds were obtained from different locations in the states of Tabasco and Chiapas, Mexico (Figure 1), from April to June 2015, except for those of *D. guianense*, which were collected in June 2014 and stored at room temperature (25.2 ± 1.8 °C) until sowing. Seeds were provided by the seed bank “El guayacán”, held by the Delegation of the Comisión Nacional Forestal in Villahermosa, Tabasco, Mexico ($17^{\circ}96'$ N; $92^{\circ}95'$ W), and located at an elevation of 21.2 masl, in a warm, humid climate with summer rain. In this same area, the experiment was conducted under a black mesh umbraculum nursery shade (50%). During the experiment, a maximum temperature of 33.4 ± 0.6 °C, a minimum temperature of 24.5 ± 0.4 °C, and a relative humidity of $70.6 \pm 5.1\%$ were recorded.

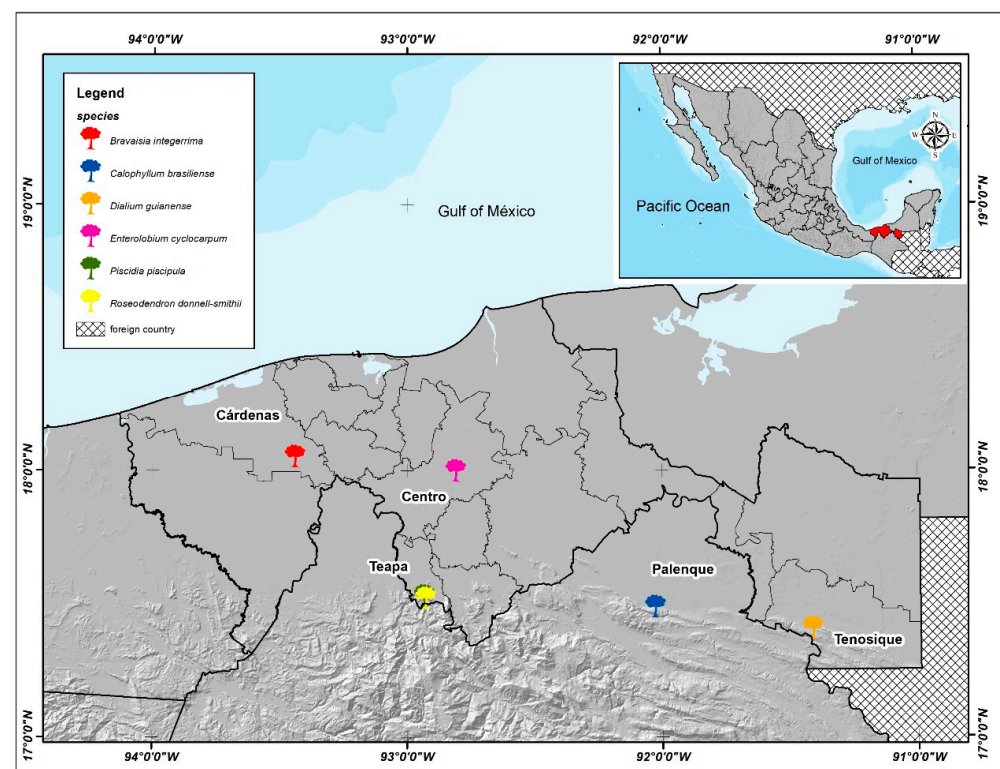


Figure 1. Location of the collection sites for germplasms from six tropical species in the states of Tabasco and Chiapas, Mexico. Prepared by Manuel L. Nuñez-Piedra.

2.2. Species Selection and Nursery Work

Six species were selected, based on seed availability during the season in which the experiment was established, to test the proposed hypothesis, as well as to assess their development and preliminary growth under nursery conditions. The seeds were donated by CONAFOR, as this is the governmental agency responsible for providing native nursery plants to producers interested in “Forest restoration, Payment for Environmental Services, and Agroforestry Plantations”, among other programs [17]. The selected species were *C. brasiliense*, *B. integerrima*, *D. guianense*, *P. piscipula*, *R. donnell-smithii*, and *E. cyclocarpum*. Seeds of *D. guianense* and *E. cyclocarpum* were subjected to a pre-germinative mechanical sanding treatment in the region opposite to the hilum, for 5 s in the former species and 10 s in the latter, using medium-grain (100–120) and coarse-grain (80) wood sandpaper, respectively. The seed coat was removed from the *C. brasiliense* seeds. All seeds were disinfected with 10% sodium hypochlorite before sowing: the small seeds of *P. piscipula*, *B. integerrima*, and *R. donnell-smithii* were soaked for 1 min; *D. guianense* and *E. cyclocarpum* for 3 min; and *C. brasiliense* for 5 min. The seeds were then sown directly into polypropylene nursery trays with a capacity of 220 cm³ per cell, with two to three seeds sown per cell to ensure the ultimate occupation of each. The substrate consisted of a mixture of peat moss (60%), vermiculite G2 8.5 (20%), agrolite hydro (20%), and 3 kg of Multicote 8 (18-06-12 of N-P-K), as recommended by the Mexican Ministry of Environment and Natural Resources [18]. Seedlings were redistributed to complete the full coverage of the tray. The respective transplantations were performed at the time when the first eophylls were well developed. Irrigation was applied ad libitum, and the cumulative rainfall for the period was 1806 mm. A fungal infection arose in July, so two types of fungicides were applied: Previcure Energy[®] (Propamocarb 53.0% + Fosetil 31%, equivalent to 840 g AI L⁻¹) (Bayer, Ciudad de Mexico, Mexico) and Derosal[®] (Carbendazim 500.76 g AI L⁻¹) (Bayer, Ciudad de Mexico, Mexico). In each case, 1 mL L⁻¹ of solution was applied, sprinkling 0.5 L of solution in each tray. Likewise, to control a hemipteran insect that affected *E. cyclocarpum* in particular, Decis Forte[®] (Deltametrina 100.0 g AI L⁻¹) (Bayer, Ciudad de Mexico, Mexico) pesticide was applied at the same doses and spray volumes as described above. Eight applications of fungicides were performed, the first four with a four-day interval and then once a month for four months. Insecticide was applied three times per month for three months, in the same periods in an intercalated manner.

2.3. Morphological Description of Seedlings

To obtain the morphological descriptions of each species, the following were determined: days to the beginning of germination after sowing and seedling functional morphology, according to Pérez-Harguindeguy et al. [11]; general limb form (eophylls and metaphylls) and phyllotaxis, according to Vozzo [19]; and length of metaphyll blade at 6 months of age. The term eophyll was applied to the first true green leaf of the expanded blade, while the metaphylls were juvenile leaves that are morphologically defined as mature leaves. In *B. integerrima* in particular, a species that originates from adventitious roots, the diameter of the set of adventitious roots was measured once they were inserted into the substrate.

2.4. Growth Variables

In periods of 2, 4, and 6 months, stem height was measured from the base to the apical meristem, and stem basal diameter was recorded at the substrate level. In addition, the number of eophylls plus metaphylls in the juvenile leaves was quantified, along with the number of leaflets per leaf in the compound leaves. Relative growth rate (RGR) was

determined and analyzed for the same periods as for stem height relative growth rate (Equation (1)) and stem basal diameter relative growth rate (Equation (2)), as follows:

$$SRGR = \frac{\ln(h_2) - \ln(h_1)}{(t_2 - t_1)} \quad (1)$$

where h_2 is the stem height at the end of the period (t_2), and h_1 is the stem height at the start of the period (t_1).

$$DRGR = \frac{\ln(d_2) - \ln(d_1)}{(t_2 - t_1)} \quad (2)$$

where d_2 is the stem basal diameter at the end of the period (t_2), and d_1 is the stem basal diameter at the start of the period (t_1).

2.5. Survivorship

The survivorship probability of the species was calculated with the non-parametric Kaplan–Meier estimator [20], using the statistical software IBM SPSS v29.0 (Armonk, NY, USA). This is a very commonly used resource for calculating survivorship rates, especially in ecological restoration projects in which the prediction of plant vulnerability to a new environment is important.

2.6. Data Analysis

The experiment was established following a completely randomized design, originally quantifying 28 seedlings in each tray, representing replicates; the number of trays and seedlings quantified for each species was different in each measurement since this depended on the availability of seeds and seedling survivorship over time. The data pertaining to stem height and stem basal diameter, total number of leaves, and relative growth rate were statistically analyzed in three periods after sowing (0–2, 2–4, and 4–6 months) via repeated measures analysis following the three evaluation times, with mean separation using a Tukey test in the software Statistix 10.0 (Tallahassee, FL, USA). The curve of best fit was drawn for each species based on its highest R^2 value using the software XLStat 2024 from Lumivero (Denver, CO, USA).

3. Results

3.1. Plant Germination Documentation

Initiation of germination varied among species, with *Bravaisia integririma*, *Piscidia piscipula*, and *Enterolobium cyclocarpum* being the species that germinated soonest (7 days after sowing), while *Calophyllum brasiliense*, *Dialium guianense*, and *Roseodendron donnell-smithii* germinated between 10 and 12 days after sowing.

3.1.1. *Calophyllum brasiliense*

Germination in this species is cryptocotylar hypogeal with reserve cotyledons. The first event is the emergence of the plumule, which occurs 10 days after sowing. The young plant initially consists of a small hypocotyl and an elongated epicotyl; then, at 9 days after germination, a pair of eophylls is observed, lanceolate, with an attenuated base, an acute apex, and smooth margins (Figure 2a). The blade is bright green on the adaxial side; pale green on the abaxial side, which is also typical of the metaphylls that are subsequently formed; leathery; and always in a decussated position. Mature metaphylls have a height of 11.7 ± 0.4 cm at six months of age. At the end of the experiment, the juvenile leaves formed in the basal area of the epicotyls, which were smaller (Figure 2b).

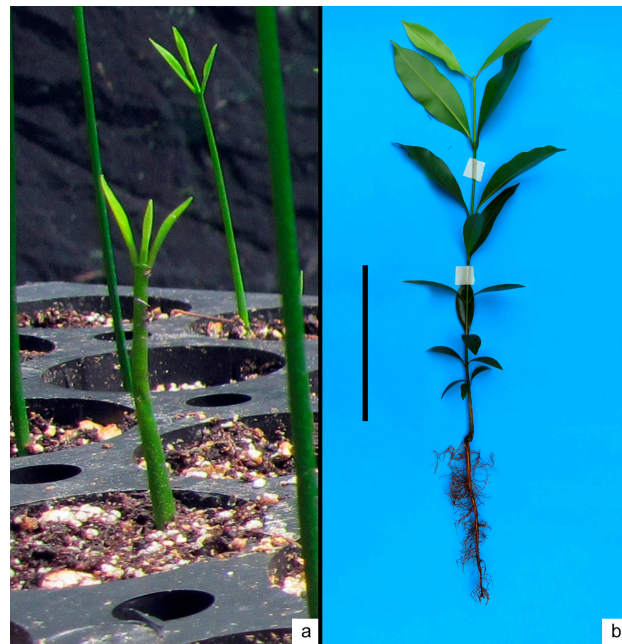


Figure 2. Seedling of *Calophyllum brasiliense* 19 days after sowing (a) and *Calophyllum brasiliense* at six months of age (b), developed in a nursery. The line corresponds to 15 cm.

3.1.2. *Bravaisia integerrima*

Germination in this species is epigeal phanerocotylar; its foliaceous cotyledons are orbicular, with an attenuated base and rounded apex. The metaphylls are ovate, the bases are attenuated, and the apex is acute, with slightly sinuate margins in the first months (Figure 3a), accentuating in the mature leaves by the end of the experiment. 7.7 young leaves form on average with opposed phyllotaxis, and the stem is articulate with lenticels. At the end of the experiment, 17% of the plants assessed presented the formation of prominent adventitious roots (2–4 per plant), reaching an average stem basal diameter of 3.5 ± 1.3 cm (Figure 3b).



Figure 3. Seedling of *Bravaisia integerrima* 23 days after sowing (a) and *Bravaisia integerrima* at six months of age (b), developed in a nursery. The line corresponds to 15 cm.

3.1.3. *Dialium guianense*

Germination in this species is epigeal phanerocotylar with thin reserve cotyledons, initially forming a hypocotylar hook nine days after sowing; then, at 16 days, a pair of ovate paracotyledons emerge, petiolate with a caudate apex and smooth margins, of light green color and membranous consistency (Figure 4a). Simple eophylls are formed in a third event, and then, imparipinnate metaphylls form of three and five leaflets in number, occasionally seven, as with the first eophylls. A slight light brown incipient lignification was noted on the stem during the last period of the experiment (Figure 4b).



Figure 4. Seedling of *Dialium guianense* 14 days after sowing (a) and *Dialium guianense* at six months of age (b), developed in a nursery. The line corresponds to 15 cm.

3.1.4. *Piscidia piscipula*

The emission of foliaceous cotyledons is promoted seven days after sowing; germination is epigeal phanerocotylar with foliaceous cotyledons (oblong), with both the base and apex rounded. The development process continues with the production of the first pair of eophylls, which are petiolate and very pubescent in the margins, and on stem (simple trichomes); the blades are oval with an acute apex and smooth margins (Figure 5a). Between 30 and 60 days after sowing, up to 3.4 simple eophylls appear on average, with the same form as those described above. At 4 months, three and five imparipinnate metaphylls are produced, with pulvinated petioles. The stem is slightly woody with lenticels and presents spiral phyllotaxis (Figure 5b).

3.1.5. *Roseodendron donnell-smithii*

Germination in this species is epigeal phanerocotylar with foliaceous cotyledons, which are oblong, with markedly slit apices observed 10 days after sowing. At 49 days, the first pair of eophylls emerges. These are petiolate, ovate in shape, have an acuminate apex, and develop a serrated margin. Four months after sowing, the first eophylls are unifoliate, then bifoliate, and finally trifoliate (Figure 6a). At six months, some metaphylls are observed to be largely petiolate and pentafofiate, with the same blade features as the initial examples. The stem presents light-colored lignification, and phyllotaxis is alternate. The leaves show reddish pigmentations (Figure 6b).



Figure 5. Seedling of *Piscidia piscipula* 12 days after sowing (a) and *Piscidia piscipula* at six months of age (b), developed in a nursery. The line corresponds to 15 cm.



Figure 6. Seedling of *Roseodendron donnell-smithii* 18 days after sowing (a) and *Roseodendron donnell-smithii* at six months of age (b), developed in a nursery. The line corresponds to 15 cm.

3.1.6. *Enterolobium cyclocarpum*

Germination in this species is epigeal phanerocotylar with reserve cotyledons. Elevation of the cotyledons is considered to denote the time of germination, which was five days in the case of this experiment. During the following three days, a simple eophyll emerges with nine pairs of leaflets (paripinnate) and, one day after that, a paripinnate bipinnate eophyll emerges with nine pairs of leaflets—opposite and linear—with a sessile base and mucronate apex; three days after that, an eophyll with three pairs of leaflets is formed, containing 10, 11, and 11 pairs of secondary leaflets (Figure 7a). At a later stage, bipinnate paripinnate metaphylls develop with up to five pairs each and, after 4 months,

up to six pairs. From 5.7 ± 0.4 to 16.1 ± 2.6 pairs of secondary leaflets appear at six months old. In all the leaves, the last pair of secondary leaflets is unguled. Phyllotaxis of the plantlet is in a spiral, and lenticels can be observed in the stem (Figure 7b).



Figure 7. Seedling of *Enterolobium cyclocarpum* seven days after sowing (a) and *Enterolobium cyclocarpum* at six months of age (b), developed in a nursery. The line corresponds to 15 cm.

3.2. Growth Variables

Based on the repeated measures analysis over time, the results obtained for the different evaluated variables are shown (Tables S1 and S2).

3.2.1. Stem Height and Stem Basal Diameter

The species that grew the most over the course of the experiment was *E. cyclocarpum*, with a mean height of 38.0 cm, followed by *C. brasiliense*, at 22.0 cm, at 6 months after germination, showing highly significant differences in this regard ($F_{5,27} = 345.61$ ($p < 0.0001$)). In the case of stem basal diameter, the plants with greater stem thickness were *B. integerrima* and *R. donnell-smithii*, each of which presented values between 5.0 and 4.2 mm after six months of assessment; highly significant differences were also found in this parameter ($F_{5,27} = 203.43$ ($p < 0.0001$)). The analysis of variance for these variables is shown in Table S1. The Tukey test is presented in Table 1.

Table 1. Stem height, stem basal diameter, and eophyll number for different juvenile tropical plant species established in nursery conditions for six months (repeated measures analysis in time).

Species	Stem Height (cm)	Stem Basal Diameter (mm)	Number of Eophylls
<i>Calophyllum brasiliensis</i>	22.0 b	2.6 d	8.8 a
<i>Bravaisia integerrima</i>	14.5 cd	5.0 a	7.1 b
<i>Dialium guianense</i>	13.1 de	1.9 e	7.2 b
<i>Piscidia piscipula</i>	16.3 c	3.0 cd	3.9 d
<i>Roseodendron donnell-smithii</i>	11.6 e	4.2 b	7.7 ab
<i>Enterolobium cyclocarpum</i>	38.0 a	3.1 c	6.0 c

Means with different lowercase letters differ statistically according to the Tukey test ($p \leq 0.01$).

3.2.2. Number of Juvenile Leaves

The total number of leaves recorded in the experiment was highest for *C. brasiliensis*, followed by *R. donnell-smithii*, *B. integerrima*, and *D. guianense*, with average values from 7.2 to 8.8. *Calophyllum brasiliense* and *B. integerrima* produced only simple leaves, while the other species comprised juvenile compound leaves. Highly significant differences were found in this parameter ($F_{5,27} = 52.28$ ($p < 0.0001$)). The Tukey test is presented in Table 1.

Table S3 shows the means of the numbers of young leaves (eophylls and metaphylls), as well as the number of leaflets developed, in which the species with compound leaves are prominent, *D. guianense*, *R. donnell-smithii*, and *E. cyclocarpum*, which had formed 5–7 leaflets per leaf by the end of the experiment.

3.2.3. Relative Growth Rates

For all species, the relative growth rate (RGR) for stem height was higher in the first assessment period (0–2 months) than in the later periods (2–4 and 4–6 months) and decreased at the end of the experiment. Highly significant differences were identified in this parameter ($F_{5,27} = 85.30$ ($p < 0.0001$)); the analysis of variance for these variables is shown in Table S2. The highest rates obtained during the study period were for *E. cyclocarpum* ($0.0210 \text{ cm cm}^{-1} \text{ day}^{-1}$), followed by *C. brasiliense* ($0.0203 \text{ cm cm}^{-1} \text{ day}^{-1}$). The species with the lowest RGR was *Piscidia piscipula* ($0.0091 \text{ cm cm}^{-1} \text{ day}^{-1}$); The Tukey test can be observed in Table 2.

Table 2. Relative growth rate for stem height (SRGR) and stem basal diameter (DRGR) for different juvenile tropical plant species established in nursery conditions for six months (repeated measures analysis).

Species	Stem Height SRGR ($\text{cm cm}^{-1} \text{ day}^{-1}$)	Stem Basal Diameter DRGR ($\text{cm cm}^{-1} \text{ day}^{-1}$)
<i>Calophyllum brasiliensis</i>	0.0203 ab	0.0076 a
<i>Bravaisia integerrima</i>	0.0179 b	0.0108 a
<i>Dialium guianense</i>	0.0154 c	0.0057 a
<i>Piscidia piscipula</i>	0.0091 d	0.0080 a
<i>Roseodendron donnell-smithii</i>	0.0147 c	0.0098 a
<i>Enterolobium cyclocarpum</i>	0.0210 a	0.0101 a

Means with different lowercase letters are statistically different according to the Tukey test ($p \leq 0.01$).

Based on the Tukey test, no significant differences were found in the case of RGR for basal stem thickness (Table 2). Values ranged from 0.0076 to $0.0108 \text{ cm cm}^{-1} \text{ day}^{-1}$.

3.2.4. Regression Curves

Regarding stem height and stem basal diameter growth curves during the six-month period, all species fit adequately to second-degree polynomial equations (Figure 7). The species Bi, Cb, and Pp maintained fairly constant growth during the six months of evaluation, while Rd, Dg, and Ec showed a good growth rate in terms of height at the beginning and then tended to decrease with time. Rd was the only species whose stem basal diameter maintained a constant growth rate. On the other hand, Ec stood out for its absolute growth and reached a total stem length of $45.35 \pm 1.17 \text{ cm}$, well above Cb, which occupied the second position with $29.3 \pm 2.11 \text{ cm}$.

For stem height, a high level of fit was observed for *C. brasiliensis* ($R^2 = 0.993$), but moderately low fits were observed for *R. donnell-smithii* ($R^2 = 0.758$) and *D. guianense* ($R^2 = 0.862$). For stem basal diameter, fits were high for all species, with coefficients of determination ranging from 0.998 for *E. cyclocarpum* to 0.889 for *C. brasiliensis* (Figure 8).

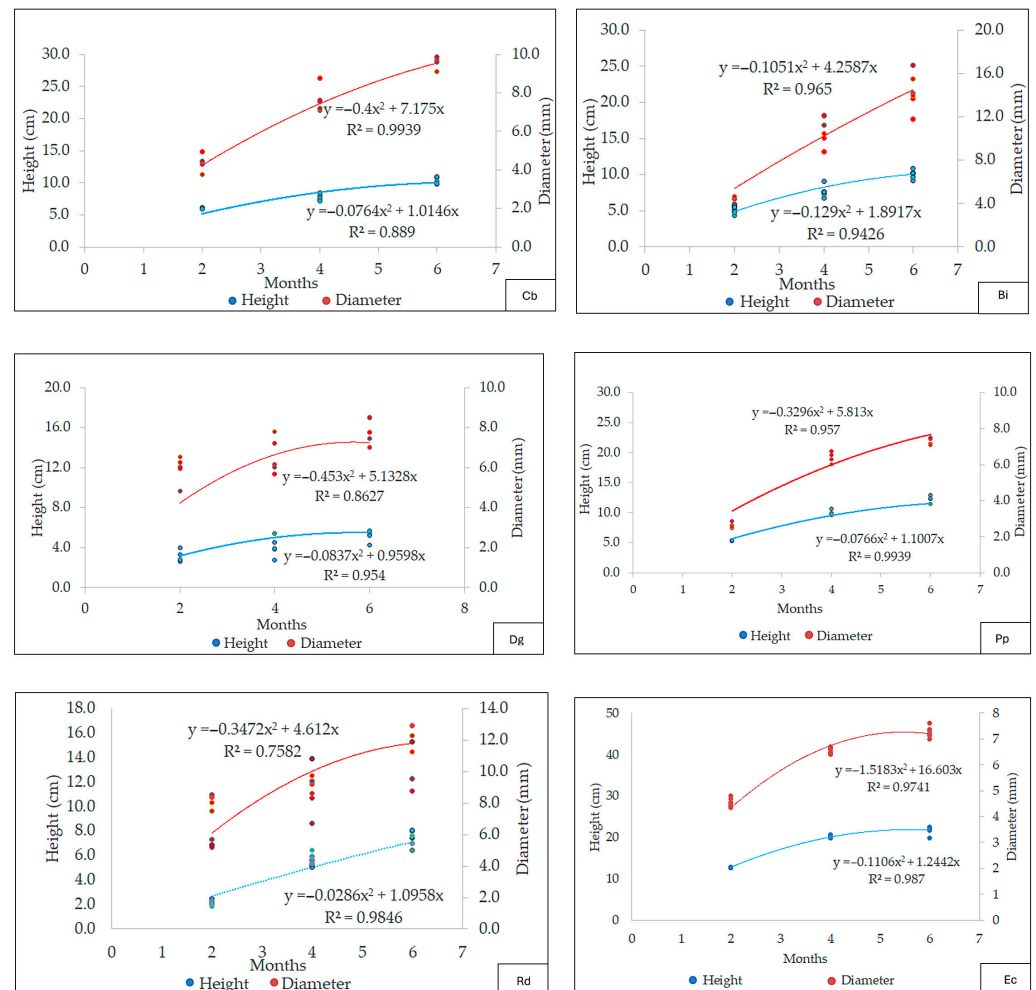


Figure 8. Regression curves of initial growth in height and stem basal diameter in seedlings of six native forest species: *Calophyllum brasiliense* (Cb), *Bravaisia integerrima* (Bi), *Dialium guianense* (Dg), *Piscidia piscipula* (Pp), *Roseodendron donnell-smithii* (Rd), and *Enterolobium cyclocarpum* (Ec). The data points and curves in blue refer to height, while the data points and curves in red refer to diameter.

3.2.5. Seedling Survivorship

The species that presented the highest percentages of survivorship after six months of experimentation were *C. brasiliense*, which obtained 92.14%, and *P. piscipula*, with 90.17%. The species with the lowest survivorship was *R. donnell-smithii*, which decreased from 96.87% at two months of age to 43.75% at the end of the study due to a severe fungal infection that caused the death of some plants and defoliation during the final assessment period. The other species, *B. integerrima*, and *E. cyclocarpum*, presented survivorship values of 61.42%, and 73.21%, respectively.

According to the Kaplan–Meier estimator analysis, the probability of survivorship was high (100%) during the first 2 months of evaluation, decreasing six months after germination, reaching values of 61.3–85.7% for *R. donnell-smithii*, *D. guianense*, *B. integerrima*, and *E. cyclocarpum*. A survivorship probability between 92.9 and 95.1% was reported for *P. piscipula* and *C. brasiliense* (Figure 9). Highly significant differences were identified in this parameter ($\text{Chi}^2 = 48.155$, $p = 0.001$). This indicates a variation between the observed and expected frequencies in the Kaplan–Meier model.

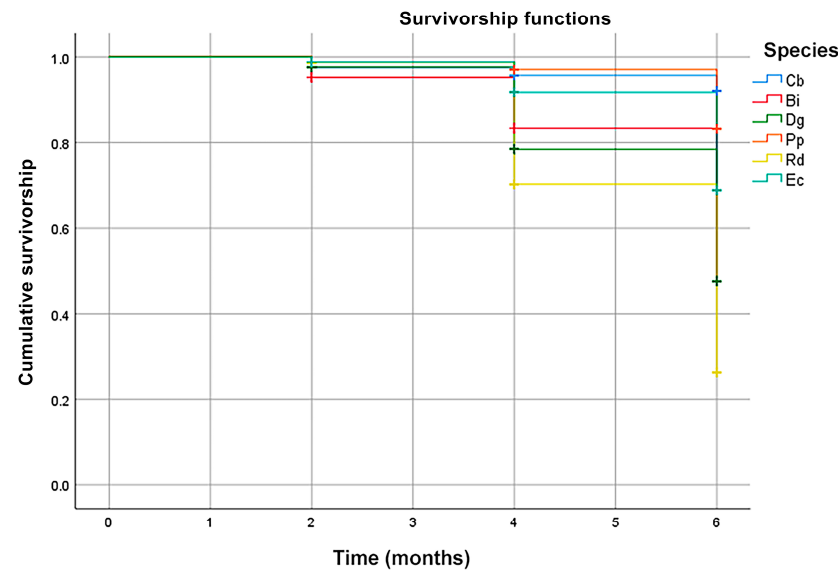


Figure 9. Survivorship curve estimation with the Kaplan–Meier model for six tropical tree species over six months of growth. *Calophyllum brasiliense* (Cb), *Bravaisia integerrima* (Bi), *Dialium guianense* (Dg), *Piscidia piscipula* (Pp), *Roseodendron donnell-smithii* (Rd), and *Enterolobium cyclocarpum* (Ec).

4. Discussion

4.1. Germination and Morphological Description of Seedlings

The success of germination depends on several factors, mainly seed quality, genetic traits, and the properties of the germination medium [21,22]. *C. brasiliense*, a hydrochoric species with high moisture content [23], exhibits short germination times, which in this study were accelerated by coat removal. For *B. integerrima*, scarce references exist; its small seed size (3.5 mm), absence of reserve tissues, thin coat, and preference for floodable soils [24] favor rapid germination. This work represents the first detailed description of *B. integerrima* seedlings, providing background for related taxa. Similarly, *R. donnell-smithii* produces exalbuminous seeds with only an embryonal axis and foliaceous cotyledons, which are 1.5 cm in length, including the wing [25].

Scarification is commonly applied to legumes such as *D. guianense*, *P. piscipula*, and *E. cyclocarpum*, whose hard coats hinder germination [26–28]. Without scarification, *D. guianense* shows only 5% germination under field conditions [29]. For *E. cyclocarpum*, Semarnat [30] recommends soaking seeds in hot water (75 °C, 3–5 min). *Piscidia piscipula*, with small seeds (0.5 cm), germinates in 7 days without treatment, consistent with Dzib-Castillo [31], who reported 95% germination at 20 days. Arceo-Gómez et al. [28] obtained 76% with boiling water for 10 s.

Seedling functional morphology is crucial for physiological, taxonomic, and ecological studies, aiding accurate field identification [11]. The type of germination and the number and morphology of the leaves are specific characteristics, especially in tropical taxa [32]. In this study, most revised seedlings were phanerocotylar with foliaceous cotyledons (PER), except for *C. brasiliense*, which is classified as cryptocotylar hypogeal with reserve cotyledons (CHR). These function as nutrient reservoirs, and this characteristic could apparently delay germination and seedling development since the photosynthetic process begins when the epicotyl emerges [21,33].

In this experiment, species with seed lengths between 0.3 and 0.5 cm (*B. integerrima*) or 1.5 and 2.3 cm (*P. piscipula*, *E. cyclocarpum*) and PER-type seedlings germinated earliest (5–7 days). In contrast, *D. guianense* and *R. donnell-smithii* germinated later (10–12 days), similar to *C. brasiliense* (CHR, 0.5 cm). No strict relationship was observed between seedling

type, size, and germination speed, although CHR species may show greater survivorship due to higher reserve content [6].

It was observed that the leguminous *D. guianense* and *P. piscipula* develop paracotyledons (embryonic leaves with photosynthetic functions), similar to other Fabaceae species [32]. The shape of the protophylls in *B. integerrima*, *R. donnell-smithii*, and *C. brasiliense* is also maintained in the metaphylls and even in the adult leaves, like that reported by Pennington and Sarukhán and de Jesus et al. [34], as well as in the leaflets of *D. guianense*, *P. piscipula*, and *E. cyclocarpum*, which also correspond to that reported in adult trees [12].

4.2. Growth Variables

Morphological traits such as stem height and basal diameter are widely used as indicators of seedling quality. Broadleaf nursery species should ideally reach at least 15 cm in height and exceed 5 mm in basal diameter to ensure field survivorship [35]. In this study, *E. cyclocarpum*, *C. brasiliense*, *P. piscipula*, and *B. integerrima* showed the greatest stem growth, with values equal to or higher than those reported in the literature [26,28,36,37]. For *B. integerrima*, no comparative references exist.

Roseodendron donnell-smithii and *D. guianense* exhibited shorter stems, although the former was affected by fungal infection. Records indicate that *R. donnell-smithii* can reach 42.3 cm in height and 4.6 mm in diameter in 3.5 months in a peat–perlite–vermiculite substrate (3:1:1) ratio with 10 gL^{−1} Multicote fertilization [35]. The stem height recorded in *D. guianense* (13.1 cm) is similar to values reported for *Dialium guineense* Wild. in Africa, grown under similar conditions [38,39].

The growth in height of species depends on the ecological strategies [35]. *Calophyllum brasiliense*, *D. guianense*, *B. integerrima*, *R. donnell-smithii*, and *P. piscipula* are late-successional, shade-tolerant species with slower growth, in contrast to *E. cyclocarpum*, a shade-intolerant pioneer [31,40–42]. Plants with low growth rates are determined by their adaptive response at low resource availability [40]. Regarding basal diameter, the highest values corresponded to *B. integerrima* and *R. donnell-smithii*, surpassing previous reports [35,43]. Intermediate diameters (0.3–0.5 cm) were recorded in *P. piscipula*, *C. brasiliense*, and *E. cyclocarpum*. Tamayo-Chim et al. [44] reported higher values (0.94 cm at five months) in *P. piscipula* due to larger containers with a mixture of luvisol soil and sand (2:1) that promoted root expansion. For *Calophyllum inophyllum*, Maulidya et al. [37] recorded 0.3 cm as the average stem basal diameter at three months in peat soil.

Overall, the evaluated species equaled or exceeded the literature values, except *R. donnell-smithii* and *D. guianense* [26,38,45]; this indicates that the light conditions (50% shade), substrate, and fertilization regime in which the plants developed were adequate. It has been observed that seedlings growing under lower light, such as in the understory, produce higher chlorophyll a and b contents, which allows them to better absorb the available light energy to photosynthesize and have a higher quantity of carotenoids, which protect the chlorophyll from photo-oxidation by dissipating excess energy [46].

All species developed typical dicotyledonous taproots with secondary roots, except *B. integerrima*, which showed fasciculate roots and early adventitious roots that later form stilt roots, conferring flood tolerance [12]. No nitrogen-fixing nodules were detected in legumes, consistent with observations in other Caesalpinoideae [47,48]. Although *P. piscipula* is reported as a nodule-forming species [49], this feature was absent here.

4.3. Number of Juvenile Leaves

Calophyllum brasiliense formed, on average, 8.8 leaves, fewer than the 18.4 reported by Jardim et al. [50] in 120 days with high fertilization using Osmocote (8.0 g L^{−1}). *Bravaisia*

integerrima produced 7.1 leaves, slightly lower than its homolog *A. marina*, which reached 10 leaves in five months in loamy sand [38]. For *D. guianense*, the number of leaves was similar to that reported in *D. guineense* by Osaigbovo and Nwaoguala [34]. The number of leaflets per metaphyll (7.2) corresponded to that of adult compound leaves. In *P. piscipula*, each compound leaf bore five leaflets, fewer than the seven to nine typically observed in mature trees [12]. *R. donnell-smithii* produced only 7.7 leaves, contrasting with the 22.5 recorded at five months [41], likely due to fungal infections that limited development. Adult leaves of this species usually present seven leaflets [12], but in this study, only metaphylls of five were formed.

In *E. cyclocarpum*, the number of eophylls and secondary leaflets matched values reported by Gurgel et al. [32]. Adult leaves in this species may reach 5–10 pairs of compound leaflets and 15–35 pairs of secondary leaflets [12], but in this study, the number of young leaves was lower (13.2). Compound leaves, typical of legumes and Bignoniaceae such as *R. donnell-smithii*, confer physiological advantages by increasing leaf area and enabling effective stomatal control, which reduces transpiration while allowing convective cooling. Additionally, legumes exhibit paraheliotropic leaflet movements through pulvini, reducing radiation load during high solar exposure [44].

Other adaptive traits include pubescence observed in *P. piscipula* and *B. integerrima*. This feature increases the boundary layer, thereby reducing excessive transpiration under stressful conditions. Such morphological and physiological attributes directly influence water balance and resilience, and should, therefore, be considered when evaluating the potential field performance of these species [51].

4.4. Relative Growth Rate

Growth rates in tropical trees vary among species and depend on both biotic and abiotic factors, particularly light availability, which determines whether species are pioneers or late-successional [52]. In this experiment, all six species grew under identical conditions, so differences reflected genetic traits and adaptive strategies [40,53]. *Calophyllum brasiliense*, despite its cryptocotylar hypogeal germination, surpassed phanerocotylar species in growth, consistent with its tolerance to variable shade [42]. *Enterolobium cyclocarpum*, typically showing rapid growth of ~ 100 cm year⁻¹ in height and 1 cm year⁻¹ in diameter [54], grew more slowly here, likely constrained by shade, as it thrives in disturbed, high-light environments similar to *Enterolobium contortisiliquum* (Vell.) Morong [55].

Conversely, *D. guianense* exhibited low relative growth, characteristic of late-successional species adapted to resource scarcity [40]. However, in the referenced experiment, no significant growth differences were detected under contrasting environments (flooded soils vs. hills), suggesting a genetic basis for its responses. *Bravaisia integerrima* demonstrated favorable growth, aligning with its ecological dominance in canacoitales, highlighting its high importance in natural stands [24]. *Roseodendron donnell-smithii*, although heliophilous, is generally reported as slow-growing in natural conditions [41].

Growth rates among tropical forest species cover a broad spectrum depending on genotype and environment. For instance, saplings in humid tropics show basal diameter increments of 0.007–0.017 cm year⁻¹ [56], contrasting with fast-growing taxa that may reach 0.3 cm year⁻¹ [57]. In this experiment, and over the period of evaluation, the proposed hypothesis was not proven since the functional morphology did not relate positively to the greater rates of growth. We believe that the evaluation period was too short to reach a definitive conclusion, as the cryptocotyledonous hypogeal germination species with reserve cotyledons (*Calophyllum brasiliense*) did not have the longest stems, although it did have a greater number of leaves, which would subsequently give it a photosynthetic advantage over the phanerocotyledonous epigeal germination species (*Enterolobium cyclocarpum*).

4.5. Seedling Survivorship

The overall survivorship rate at six months in this experiment was 70.1%, like that recorded by Viani and Rodrigues [58] when working with native species in nursery conditions. It should be noted that, during the experiment, there was little rainfall and high temperatures, which facilitated the dispersal of fungal spores. When seeds from wild trees are harvested in tropical conditions, there is a risk that they may be internally contaminated by fungi [59]. One limitation of this study was that all the seedlings were grown under similar conditions (protected by a shade net throughout their growth), and therefore, the recommendations on their cultivation are only directly applicable in this particular environment. The values recorded in this study are positive, but the performance of the plants could be improved with strict phytosanitary control of their seed provenance. However, this was not possible because this region lacks germplasm production units for these kinds of species.

Species-specific requirements were observed: Broadleaf species (*B. integerrima*, *P. piscipula*, *R. donnell-smithii*) require wider spacing to reduce light competition and ensure robust aerial and root system development for higher field survivorship [45]. *C. brasiliense*, *B. integerrima*, and *P. piscipula* demonstrated potential for reforestation programs, with optimal production times of approximately six months. *E. cyclocarpum* requires open-field cultivation to increase stem thickness suitable for transplantation, while *D. guianense* needs about seven months to develop adult-like leaf structures, primarily requiring stem height growth.

Recalcitrant species such as *R. donnell-smithii* have small reserves, and up to five seeds can be sown per hole to guarantee coverage of the tray, with subsequent transplantation on the emergence of the first eophylls. Ideally, if there is a sufficient budget to plant these species in larger containers of at least 2 L capacity, this will help growth since the roots have more room to grow, and this will also be reflected in the dimensions of the stem. Forest polypropylene trays are suitable for tree species, but it is recommended that seedlings with wide leaves, such as *P. piscipula* and *B. integerrima*, be planted alternately to avoid competition for light. This study can be used to generate research to obtain the technological packages necessary for these native species and thus contribute to their conservation.

4.6. Regression Curves

In general, the increase in stem height and stem basal diameter, represented by quadratic equations, reflects the tendency of the plants to present a higher growth rate in the first months after germination, following a parabolic curve. However, it was observed that Dg and Ec had a notable decrease in stem growth rate during the last month. On the other hand, the high total stem growth of Ec, which greatly surpassed the rest of the species, reflects important differences among them.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ecologies6040069/s1>: Table S1: Analysis of variance of the studied variables in six forest species maintained under nursery conditions. Repeated measures analysis over time. Table S2: Analysis of variance of the relative growth rate (RGR) of the studied variables in six forest species maintained under nursery conditions. Repeated measures analysis over time. Table S3: Means and standard deviations of the numbers of young leaves (eophylls and metaphylls) over three growth periods in six humid tropical forest species grown under nursery conditions.

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