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Seed Size as Key Factor in Germination and Seedling Development of *Copaifera langsdorffii* (Fabaceae)

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Abstract

Seed size is a plastic trait of the plants that directly affect seed germination and seedling recruitment. This study aimed to investigate the relationship between seed size, seed germinability and seedling performance of *Copaifera langsdorffii* by testing four hypotheses: 1) larger seeds have higher germination percentage; 2) smaller seeds require less time to germinate and for initial development of the seedlings; 3) larger seeds produce more vigorous seedlings and 4) seed size negatively affects seedling root/shoot ratio. In 2011, we selected 30 individuals of *C. langsdorffii* from which 300 seeds were randomly collected in the plant canopy. All these seeds were weighted and placed in germination tray using vermiculite as substrate. Seed germinability and initial development of seedlings were monitored daily until cotyledons fell. Small seeds have higher germination percentage and germinate faster when compared to large seeds. Nonetheless, seedlings originated from larger seeds have longer development times, resulting in more vigorous seedlings. In addition, seedlings originating from small seeds allocate proportionally greater amount of resources to roots when compared to larger seeds. The fact that small seeds have higher germination percentage and faster germination favors the colonization of transient habitats. However, larger seeds produce more vigorous seedlings, favoring the seedling establishment in more stable habitats. Thus, we argue that high variability in seed size of *C. langsdorffii* favors its widespread geographic distribution.

Keywords

Community Organization, Plant Distribution, Seed Biometry, Seed Reserves, Seedling Vigor

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1. Introduction

Seed germination and seedling establishment are critical stages of the plant life cycles, influencing both distribution and abundance of plant species in communities [1] [2]. In fact, geographic distribution and abundance of some plant species may be associated with variations in seed germinability and seedling recruitment between different habitats due to environmental limitations, such as altitude, topography, soil quality and climate [3]-[5].

Several ecological and evolutionary factors can affect the process of seed germination and further plant establishment [6]. Seed size has been considered an important evolutionary trait that affects the reproductive outcome of many plant species [7]. Seed size directly influences the germination time [8], germination percentage [9] and seedling vigor [10], which can indirectly determine plant distribution and abundance across different habitats [11]. However, seed size produced by plants varies between and within plant species, sometimes by several orders of magnitude [12]-[14].

Smaller seeds generally germinate faster providing greater competitive advantage especially in early successional stages [15]. Nevertheless, larger seeds, although germinating slowly, often have higher percentage of germination than small seeds, being favored in predictable habitats [16]-[18]. Moreover, asynchronous and heterogeneous seed germination observed in several plants species can be associated with intra-specific variation in seed size and allow the species to colonize different habitats and expand their geographic distribution limit [5] [6] [19].

The weight and size of the seeds are directly related to the amount of nutritional reserves that will be allocated for the initial seedling growth [20]. Large seeds tend to produce more vigorous seedlings when compared to small seeds [10]. Greater amount of stored reserves allow a higher probability of seedling establishment at sites with lower resource availability [17]. However, plants respond to their environmental in such way as to optimize their resource use. Thus, according the resource optimization hypothesis, plants allocate relatively less resource to their root system when nutrient availability increases [21] [22]. In this scenario, it is expected that seedlings originated from larger seeds would present smaller root: shoot ratio, as large seeds have more nutritional reserves.

Variation in seed size is an important area of plant ecology because seed size can directly affect the processes of germination and seedling recruitment, influencing the plant performance under different environmental [23] [24]. In Brazil, *Copaifera langsdorffii* (Fabaceae) presents wide geographic distribution, occurring at different biomes (Brazilian Savannah, Dry Forest, Amazon Rainforest and Atlantic Forest) [25] [26]. Hence, the objective of this study was to evaluate the relationship between seed size, germination and seedling performance of *C. langsdorffii* by testing three hypothesis: 1) smaller seeds require less time to germinate and for initial development of the seedlings; 2) larger seeds have higher germination percentage and produce more vigorous seedlings and 3) seedling root/shoot ratio is negatively affected by seed size.

2. Material and Methods

2.1. Studied Plant

Copaifera langsdorffii (Fabaceae) is a heliophytic, deciduous tropical tree, growing up to 10 to 15 m height. The species have a wide distribution, occurring from northern Argentina to southern Bolivia, throughout the Brazilian Cerrado [25]. *C. langsdorffii* presents supra-annual fruiting, alternating years of high and low or no fruit production. The flowering occurs from November to January and fruits are mature by July to September, coinciding with the time of greatest leaves' deciduousness [26]. Upon opening, each fruit exposes a single ellipsoid seed, which is black and shiny and is partially covered by a yellow-orange aril. The seeds of *C. langsdorffii* have orthodox behavior and slow germination, which extends up to 70 days after sown [27].

2.2. Seed Collection

Seeds used in this study were collected from 30 individuals of *Copaifera langsdorffii* located in an area of Cerrado *stricto sensu* (16˚40'26"S, 43˚48'44"W) in Montes Claros municipality, north of Minas Gerais State, Brazil. The region have semi-arid climate with well-defined dry and wet seasons. The average annual temperature is about 23˚C and average rainfall is about 1000 mm/year. The study area presents dystrophic soils and developed herbaceous-subshrub strata often affected by fire [4] [28].

During August 2011, 30 reproductive individuals of *C. langsdorffii* were selected at study area. Those trees were from five to seven meters high, with a well-formed crown and in a good phytosanitary state (e.g. without presence of lianas or parasitic plants). A total of 100 fruits were haphazardly collected throughout the canopy from each selected tree and taken to Laboratory of Conservation Biology of State University of Montes Claros (UNIMONTES). All collected fruits were manually benefited, eliminating malformed seeds and those with visual signals of attacks by predators or pathogens. Thus, a set of 300 seeds (ten seeds per plant) were selected and weighed with an analytical scale. Finally those seeds were categorized in two size classes: smaller seeds (seeds < 0.394 g, n = 150) or larger seeds (seeds > 0.397 g, n = 150).

2.3. Germination Experiment

After seeds were weighed, all 300 seeds were individually sown in germination tray formed by 300 cells (2 cm length, 2 cm width and 3 cm height) and vermiculite was used as substrate for seed germination. All seeds were subjected to disinfection in a 1% sodium hypochlorite solution for two minutes before sown. The germination experiment was conducted in a germination chamber with controlled photoperiod and temperature (12 h/light at 30˚C e 12 h/dark at 25˚C). The humidity of the substrate was maintained daily by adding three ml of distilled water in each germination cell. The seeds were monitored daily to determine the percentage and time for germination. Seed was considered germinated when they presented primary root protrusion [18].

At the same time, a soaking test was conducted under same germination conditions using others 80 seeds (40 seed for each size class). At this case, dry mass of all seeds was determined and so, all those 80 seeds were immersed in distilled water and weighed after 2, 4, 8, 18, 24, 30 and 48 hours of water absorption. Relative increase in fresh weight (Wr) of seeds was calculated as $\mathrm{Wr} = \left[\left(\mathrm{Wf} - \mathrm{Wi}\right)/\mathrm{Wi}\right] \times 100$, where Wi is the initial seed weight and Wf the weight after each time of water absorption [29]. Thus, imbibitions curves for smaller and larger seeds were based on increase seed mass at different times of seed immersion in distilled water.

2.4. Seedling Development

The seedlings originated from seed germination experiment were used to evaluate the effects of seed size on time for seedling development and seedling vigor. The time for seedling development was defined as the time from seed germination (root protrusion) until seedling cotyledon fell. After cotyledon falling, seedlings were removed from substrate to determinate their length and dry mass of shoot and root system. The root and shoot lengths were determined with a digital caliper. In order to get dry biomass of root and shoot all seedlings were individually placed in paper bags, identified and transferred to an oven with air circulation at 60˚C for 48 hours. After this time the dry weight of shoot and root system was determined on an analytical scale. Finally, the ratio of root: shoot biomass (RSR) of all seedlings was calculated as $\mathrm{RSR} = \left(\mathrm{W}_{\mathrm{root}} / \mathrm{W}_{\mathrm{shoot}}\right)$, where $\mathrm{W}_{\mathrm{root}}$ is dry mass of the root and $\mathrm{W}_{\mathrm{shoot}}$ is dry mass of shoots.

2.5. Data Analysis

Survival analysis was performed in order to test the effect of seed size on percentage and time for germination. Thus, germination percentage within each size class was used as response variables while the germination time was the explanatory variable. Survival analysis evaluates the likelihood of germination at a certain time avoiding the temporal pseudo-replication inherent to the data. The effect of seed size on seed water absorption was tested by constructing Generalized Linear Mixed Models (GLMM), since these data also showed temporal pseudo-replication. Thus, increased seed mass (Wr) at different times (2, 4, 8, 18, 24, 30 and 48 hours of water absorption) was used as the response variable and seed size classes as explanatory variable.

The effect of seed size on seedling development time, seedling vigor and resource allocation between root: shoot (RSR) was tested by constructing GLMs. Thus, to evaluate the effect of seed size on time of development of the seedlings, time required for the cotyledon to fall was the response variable and seed weight was the explanatory variable. To evaluate the effect of seed size on seedling vigor, length and dry weight of shoot and root system were the response variables and seed weight was the explanatory variable. To evaluate the differences in resource allocation, the root: shoot ratio (RSR) was used as the response variable and seed weight as explanatory variable. The hypotheses proposed in this study were tested on R software [30] and all of the models were constructed using an appropriate error distribution for each response variable [31].

3. Results

Seed germination percentage of *Copaifera langsdorffii* reached 72%. Seed germination started in the eighth day after sawn and continued until the 55th day. The percentage of seed germination varied between seed size classes ($\chi^2 = 8.9$, $P < 0.01$) and small seeds have higher germination percentage (80%) compared with large seeds (64.4%). Time required for seed germination also varied between seed size classes ($\chi^2 = 11.02$, $P < 0.001$) with small seeds germinating faster than large seeds. The average time taken to 50% of small seeds germinate was approximately 29 days while the time for the same proportion of larger seeds germinate was about 42 days (**Figure 1**). The soaking test also showed that water absorption varied between seed size class ($F_{x,y} = 22.56$, $P = 0.003$). In fact, small seeds were approximately two times more permeable to water than large seeds (**Figure 2**).

Seed weight positively affected time for cotyledon fall and ratio of root: shoot biomass of the seedlings (**Table 1**). Thus, seedlings originated from larger seeds retained their cotyledons for longer time compared with seedlings originated from smaller seeds (**Figure 3(a)**) and small seeds allocate proportionally more resources to root system compared to large seeds (**Figure 3(b)**). Furthermore, seed size positively affected dry weight of root and shoot, and shoot length of *C. langsdorffii* seedlings (**Table 1**, **Figures 4(a)-(d)**).

4. Discussion

Seed germination percentage of *Copaifera langsdorffii* was affected by seed size. However, the result was different than expected because the percentage of germination was 15.6% higher in small seeds. In addition, small seeds require less time for germination than large seeds, corroborating the general patterns (see [8]). At this point it is important to emphasize that increase in time for germination can cause seed viability loss due to deterioration and microorganisms attack [32]-[34]. Thus, this fact can be used to explain the variation in percentage of seed germination observed in this study.

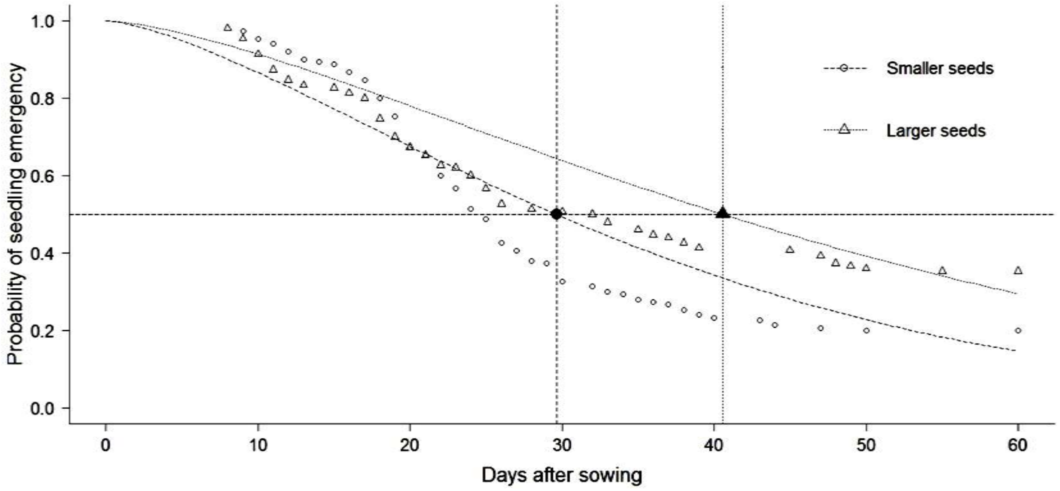


Figure 1. Germinability of *Copaifera langsdorffii* seeds between different size classes. Vertical lines indicate the time required for germination of 50% of smaller and larger seeds.

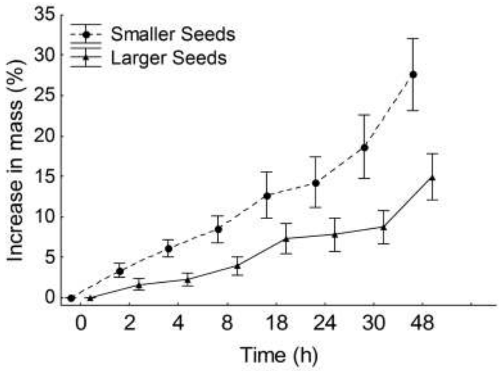
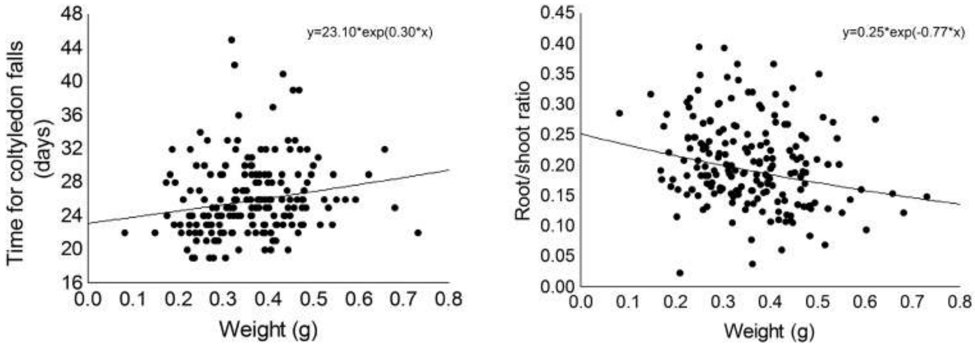
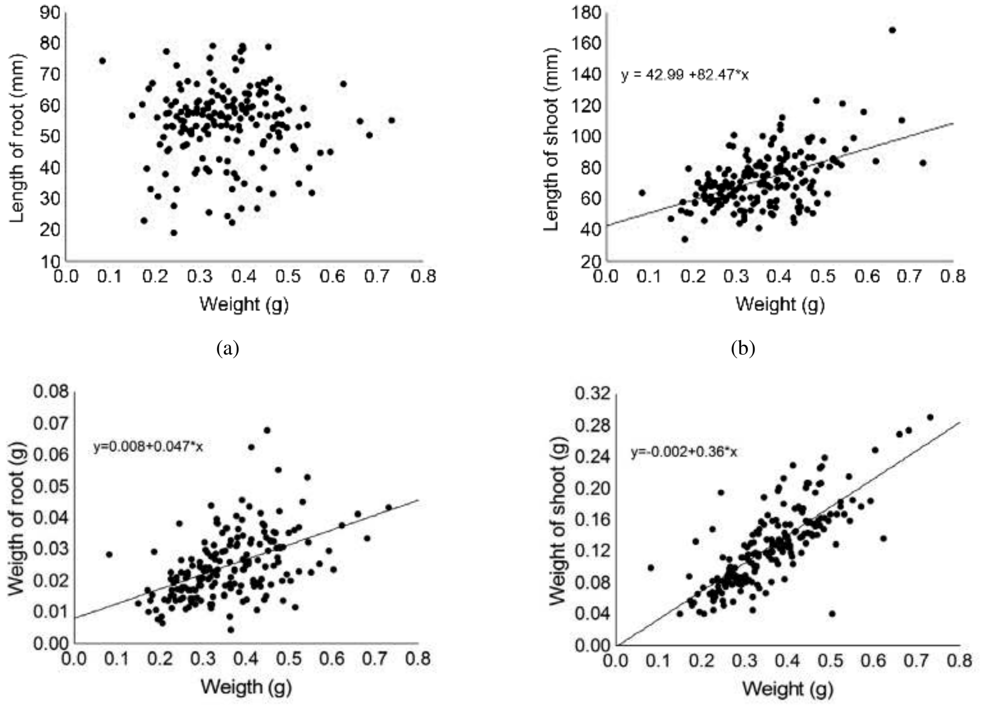


Figure 2. Imbibition curves for smaller and larger seeds of *Copaifera langsdorffii*.



(a) (b)

Figure 3. Relationship between seed weight and time for cotyledons fall (a) and ratio of root: shoot biomass (b) of *Copaifera langsdorffii* seedling.



(a) (b)

(c) (d)

Figure 4. Relationship between seed weight and root length (a), shoot length (b), root dry weigh (c) and shoot dry weigh (d) of *Copaifera langsdorffii* seedling.

Small seeds of *C. langsdorffii* are more water permeable and germinate faster than large seeds. In general, seed size is directly related with seed coat thickness and inversely related with water absorption [35]. The increase in seed size also implies decrease in the surface: volume ratio, resulting in lower relative ability to absorb water and initiate the process of germination [36]. Thereby, smaller seeds have thinner coats and higher relative surface. This seed trait ensures greater permeability in small seeds and, consequently, less time for germination [37]. The fact that smaller seeds have thinner coat and high surface: volume ratio can justify effectively imbibition and variation in germination time between seed size classes of *C. langsdorffii*.

After germination, *C. langsdorffii* seedlings originated from larger seeds retained their cotyledons for longer time. Large seeds have higher amount of reserves in theirs cotyledons and need longer time to incorporate theses nutrients in seedling tissues [20]. Thus, seedlings originated from larger seeds have both more time for development and nutrients available for growth. Thus, the result observed in this study agrees with the general predic-

Table 1. Deviance analysis of the complete models evaluating the effect of seed weight on initial development and resource allocation of *Copaifera langsdorffii* seedlings.

Explanatory variable	Response variable	Deviance	Residual Df.	Residual deviance	X^2	P
Seed weight	Time for cotyledons fall	5.0181	180	116.32	5.0181	<0.01
	Length of shoot (mm)	13659	180	43381	56.676	<0.001
	Length of root (mm)	5.6645	180	26246	0.0386	0.8444
	Weight of shoot (g)	0.0048	180	0.015	58.52	<0.001
	Weight of root (g)	0.2773	180	0.153	325.79	<0.001
	root: shoot ratio	0.0184	180	0.768	0.0184	<0.05

tion that larger seeds produce seedlings with larger initial size as reported by others authors [4] [10] [23] [38] [39]. In this scenario it would be reasonable to think that larger seeds/seedlings have higher competitive ability relative to small seeds. However, several other forces act synergistically to shape seed size. For example, nutritionally stressed habitat acts in favor of larger seeds/seedlings because larger seedlings (originated from larger seeds) have more nutritional reserves [2] [19] [40] [41]. On the other hand, larger seeds are preferentially attacked by predators, favoring smaller seeds survivorship [42].

Another important aspect of the relation between seed and seedling size is related with resource allocation for root or shoot. In general, seedlings originated from smaller seeds must allocate a greater proportional amount of resources to roots development, resulting in higher root: shoot ratio [22] as observed in this study. The high investment in root tissues promotes greater development of root systems that reach deeper levels of substrate with more water and nutrients [43]. Thus, this resource reallocation between root and shoot could increase the survivorship chance of seedlings with poor cotyledons reserves.

Within a given population, plants should develop seeds of similar size because there is often a single best range of seed size that optimizes the local fitness [44]. However, seed size within species is one of the more plastic components of plant life history [45]. It is plausible that the variation in seed size within species is associated with differential seed performance among habitats [46]. For example, smaller seeds are favored in transient habitats, because they present greater percentage of germination and germinate more quickly. In contrast, larger seeds have more reserves, producing vigorous seedlings that present greater competitive ability in predictable habitats. The *C. langsdorffii* seeds showed broad variation in biomass. This variation in seed size allows the species to colonize different habitats, helping to justify *C. langsdorffii* widespread geographic distribution.

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