



Intraspecific variation in seed size and light intensity affect seed germination and initial seedling growth of a tropical shrub

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ABSTRACT

Seed germination and seedling performance are affected by environmental factors and seed traits. In this study we investigated the effects of seed size and light intensity on germinability and seedling development of *Copaifera oblongifolia*. A total of 225 seeds were individually weighed and sown in three germination trays composed of 75 cells each. Each tray was placed in a different germination chamber with controlled photoperiod, temperature and light intensity. Seed size showed a positive relationship with time required for seed germination, and seeds exposed to high light intensity required more time to germinate. Seed size did not affect germination percentage, but seeds sown under high light intensity had a lower germination percentage than seeds sown under low light intensity and darkness. Seedling shoot mass showed a positive relationship with seeds mass, and seedlings grown in high light intensity had greater shoot mass than seedling growth in low light intensity and darkness. Thus, seed germinability of *C. oblongifolia* was higher in darkness while seedlings exhibited greater development under light. Looking to explain the ability of *C. oblongifolia* to colonize open/disturbed sites, it seems possible that plowing soil can bury seeds, thereby stimulating the germination of seeds present in the seed bank.

Keywords: Cerrado, *Copaifera oblongifolia*, diversity conservation, dominancy, habitat invasion, seed-seedling conflict

Understanding why some plants become dominant and even form homogeneous stands inside specific habitats would help in the development of strategies for species management and conservation of species diversity (Herrera & Laterra 2009; Gallagher *et al.* 2015). Several morphological (e.g. size, form, stiffness) and physiological (e.g. age, photoblastism, dormancy) attributes of seeds can interact with ecological variables (e.g. light, temperature, moisture, soil quality, seed predation) to determine the

local abundance of plant species (Rojas-Aréchiga *et al.* 2013; Ribeiro & Borghetti 2014; Souza & Fagundes 2017). Thus, finding a predictable relationship between plant life history and environmental variables will help determine the ability of plants to become established and elucidate traits that allow species to become dominant in specific habitats (Murray *et al.* 2005; Ferreras *et al.* 2015; Jelbert *et al.* 2015).

Seed mass is an important evolutionary trait that affects seed germination, seedling performance and plant

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community organization (Yanlong *et al.* 2007; Herrera & Laterra 2009; Souza & Fagundes 2014). While smaller seeds generally germinate faster, larger seeds often have a higher germination percentage than small seeds (Cordazzo 2002). Seed size is also directly related to the amount of nutritional reserves that can be allocated to initial seedling development (Yanlong *et al.* 2007; Souza & Fagundes 2014). In general, when compared to small seeds, larger seeds produce more vigorous seedlings (Gharoobi 2011; Fagundes *et al.* 2011). Plant species that produce larger-sized seeds are favored in predictable and more competitive habitats (Baskin & Baskin 1998; Ferreira & Borghetti 2004), while plant species that produce a greater number of smaller seeds have greater competitive advantage, especially in early successional habitats (Baskin & Baskin 1998; Moles & Westoby 2006; Rojas-Aréchiga *et al.* 2013).

Seed size can interact with other environmental factors (e.g. light, moisture, temperature, soil quality) in determining the success of seed germination and plant recruitment among habitats (Pearson *et al.* 2003; Khan & Gulzar 2003; Yanlong *et al.* 2007; Fagundes *et al.* 2011). Small seeds generally germinate better in light than in darkness, while large seeds do not exhibit a difference in germination between these two conditions (Milberg *et al.* 2000; Fenner & Thompson 2005). However, some studies have shown the effect of light intensity on seed germination is variable and may be species dependent (Moles & Westoby 2006; Onyekwelu *et al.* 2012). In addition, light is perhaps the main environmental factor involved in seedling survival and growth among habitats (Onyekwelu *et al.* 2012). The biomass of roots, stems, leaves and whole plant was found to decrease under low light intensity (Zervoudakis *et al.* 2012). However, because light intensity varies temporally and spatially, plants species acclimate and develop plasticity to cope with variation in light regimes. Plants characterized as sun species must have a positive response to increased light intensity while light excess can inhibit the growth of plants shade adapted (Gonçalves *et al.* 2005). Thus, environmental traits that are suitable for seed germination may not be ideal for seedling or adult plant development, generating a seed-seedling conflict (Moles & Westoby 2006; Herrera & Laterra 2009).

Copaifera oblongifolia Mart. (Fabaceae) is a heliophyte shrub species that reaches up to 2.5 m in height in the Brazilian Cerrado. The plants of the studied population are semideciduous with the production of new leaves from July to September. Flowering occurs from February to May and fruits ripen from August to October. Upon opening, each fruit exposes one ellipsoid seed (rarely two), which is black and shiny and partially covered by a yellow-orange aril. The species occurs in open areas such as abandoned pastures, borders of Cerrado fragments and highways. Moreover, this shrub often invades pastures and agricultural systems, becoming dominant and inhibiting the development of cultivated plants in northern Minas Gerais State (ME,

personal observations). In this study we hypothesized that light intensity and seed size affect germination success and seedling performance of *C. oblongifolia*. Specifically, the following predictions of this hypothesis were tested: (i) seeds will experience greater germinability and seedlings will have greater initial growth under high light intensity, and (ii) larger seeds will have a greater germination percentage and will produce more vigorous seedlings than smaller seeds.

In order to perform germination tests, mature seeds were collected from 21 individuals of *C. oblongifolia* in an area of Cerrado vegetation (16°17'20''S and 44°09'02''W) in the municipality of Mirabela in northern Minas Gerais, Brazil, during October 2015. The region has a semi-arid climate with well-defined dry and wet seasons. The average annual temperature is 23 °C and rainfall is about 1000 mm/year (Souza & Fagundes 2017). A total of 20 well-formed seeds were haphazardly collected from each individual plant, packed in individual paper bags and weighed with an analytical scale to determine the biomass of each seed. Thus, seed mass was used as a proxy for seed size (see Souza & Fagundes 2014). All 420 seeds collected were manually examined, with malformed seeds and those with visual signals of attack by predators or pathogens being eliminated. Finally, a set of 225 homogenous seeds (about nine to 11 seeds for each individual plant) was selected to perform the germination experiment.

The selected seeds were individually sown in three germination trays composed of 75 individual cells (2 cm long, 2 cm wide and 3 cm tall) with vermiculite used as a substrate. Each tray with 75 seeds represented a specific light-intensity treatment, and was placed in its own germination chamber with the photoperiod, temperature and light intensity as follows: t_1 = high light intensity, 12 h/light at 28 °C and 12 h/dark at 28 °C, 4 fluorescent-lamp equivalent to 47.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ irradiation; t_2 = low light intensity, 12 h/light at 28 °C and 12 h/dark at 28 °C, 2 fluorescent-lamp equivalent to 23.8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ irradiation; and t_3 = 24 h/darkness at 28 °C. This experimental design assumed each seed with their specific mass to be a statistically independent experimental unit, thus permitting comparisons of seed germinability among treatments (see Warton & Hui 2011; Souza & Fagundes 2014). Before sowing, all seeds were subjected to disinfection by immersion in a 1 % sodium hypochlorite solution for two minutes (Souza *et al.* 2015). The humidity of the germination substrate was maintained daily by adding three milliliters of distilled water to each germination cell. Seeds were monitored daily to determine the germination percentage and time to germination. Seeds were considered germinated when they exhibited primary root protrusion.

Seedlings from the germination test were used to evaluate the effects of light intensity and seed mass on biomass accumulation by seedlings. Time for seedling development was defined as the time elapsed between seed germination and the fall of the seedling cotyledons. All



seedlings were removed from the substrate after the fall of the cotyledons to determinate their dry biomass. Seedlings were individually placed in properly identified paper bags and transferred to an air circulation oven at 60 °C for 72 hours. After this time, the dry weight of aerial portion and the root system of each seedling were determined using an analytical scale. Finally, the ratio of root:shoot biomass of all seedlings was calculated using the ratio between dry mass of the root and dry mass of the shoot.

The effects of light intensity and seed mass on time required for seed germination or germination percentage were tested using General Linear Models (GLM) followed by ANOVA. Thus, time required for seed germination or germination percentage was used as a response variable while the treatments of light intensity, seed mass and the interaction between them were the explanatory variables. Because the seed germination data is binary (germinated or not-germinated), a binomial error distribution was used in model construction (Warton & Hui 2011). To test the effect of light intensity and seed mass on seedling vigor, different Generalized Linear Models (GLM) were constructed with total biomass, aerial biomass, root biomass or root:shoot ratio being used as response variables and treatments, seeds mass and the interaction term as the explanatory variables. All significant models that presented an explanatory variable with more than two levels were submitted to a contrast

analysis to combine levels that were not significantly different, and separation of significantly different levels.

The analyses were carried out in R software (R Core Team 2014). All models were submitted to residual analysis to check for model fit and suitability of error distribution for each response variable (Crawley 2007). The Akaike Information Criterion for small samples (AICc) was used to order explanatory variables and select the most parsimonious model. These procedures were carried out using the MuMIn package on R software (Bartoń 2015).

The seeds of *Copaifera oblongifolia* began germinating on the 12th day and continued until the 81st day after sowing. Light intensity, seed mass and the interaction between light intensity and seed mass affected the time required for seed germination (Tab. 1). Seed size showed a positive relationship with time required to seed germination, and seeds exposed a high light intensity required more time to germinate. However, a significant interaction term suggests that the relationship between seed size and time for germination changes according to light intensity. In fact, results of contrast analysis showed that the positive relationship between seed mass and time required to seed germination was evident only under the low light intensity treatment (Fig. 1A). Only light intensity affected seed germination percentage (Tab. 1), and seeds sown under high light intensity had a lower germination percentage than seeds subjected to low light intensity and darkness (Fig. 1B).

Table 1. Summary of minimal adequate models showing the effects of explanatory variables (light intensity and seed mass) on the variables responses (time required to seeds germination, percentage of seed germination, total mass, shoot mass, root mass and root:shoot ratio) of *Copaifera oblongifolia* shrubs.

Response variables	Explicative variables	Deviance	Df from residue	Residual deviance	F-values	P-values
Time for seed germination	Light intensity	10113.6	184	22066	45.059	<0.01
	Seed mass	793.9	183	21272	7.067	<0.01
	Light intensity x seed mass	939.9	181	20332	4.183	0.016
Seed germination	Light intensity	5.721	184	25.026	2.860	0.047
Total mass	Seed mass	0.003	111	0.065	112.6	<0.01
	Light intensity x seed mass	0.004	109	0.060	0.041	0.021
Aerial mass	Light intensity	0.003	112	0.093	4.072	0.019
	Seed mass	0.041	111	0.052	91.312	<0.01
	Light intensity x seed mass	0.004	109	0.048	4.679	0.011
Root mass	Seed mass	0.002	113	0.005	50.925	<0.01
Root: Shoot ratio	Light intensity x seed mass	0.082	109	0.896	4.993	<0.01

Total mass of seedlings showed a positive relationship with seed mass and the significant interaction between light intensity and seed mass suggests that seedlings grown in low light intensity are more dependent on seed mass (Tab. 1, Fig. 2A). In fact, the results of contrast analysis showed the slope of curves to vary among light treatments indicating seed mass is a more important trait in low light intensities.

Seedling shoot mass was affected by seed mass, light intensity and the interaction between seed mass and light intensity (Tab. 1, Fig. 2B). In general, seed mass had a positive effect on seedling shoot mass, and contrast analysis revealed slope curves varied among treatments, indicating shoot mass of seedlings grown on low light intensity was

more affected by seed mass. Moreover, contrast analysis also showed that seedlings grown in high light intensity exhibited greater shoot mass than seedlings grown in low light intensity and darkness.

Among all the explanatory variables tested, only seeds mass affected root mass, and seedlings from heavier seeds exhibited higher root mass (Tab. 1, Fig. 2C). Finally, the interaction between light intensity and seed mass was observed to affect the root:shoot ratio, with the root:shoot ratio of seedlings grown in darkness having a positive relationship with seed mass, while the root:shoot ratios of seedlings grown in low and high light intensity had a negative relationship with seed mass (Tab. 1, Fig. 2D). Thus,



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light intensity appears to affect resource allocation from seeds between root system and shoot.

Seeds can be classified into three groups according to their sensitivity to white light: (1) positive photoblastic, in which seeds germinate only under white light (produced principally by heliophyte plants); (2) negative photoblastic, in which seeds germinate only in the dark, with white light inhibiting germination; and (3) light insensitive, in which seeds germinate both in the dark and under white light (produced by understory plants) (Takaki 2001). Based on this classification, perhaps the most unexpected result found in this study was that seeds subjected to high light intensity had lower germinability; this is surprising because *C. oblongifolia* is a heliophyte species, growing only in open areas. However, other studies have also shown that seeds of some weed species have high germinability in the absence of light or after being buried in the soil for a period of time (Ohadi *et al.* 2010; Wang *et al.* 2016; Hu *et al.* 2017).

It is also important to highlight that seed size did not affect the germination percentage of *C. oblongifolia* seeds, but larger seeds required more time to germinate, especially under low light intensity. Seed mass is a trait that many authors have related to photoblastic response (Rojas-Aréchiga *et al.* 2013). In general, light requirement increases as seed mass decreases and shade tolerant species have larger seeds than open sites species (Pearson *et al.* 2003; Souza & Fagundes 2014). Therefore, our results agree partially with the general prediction that larger seeds have greater germination percentage and require more time to germinate.

Another important result observed in this study was total and shoot mass of seedlings were positively related to seed mass, and seedlings grown under high light intensity also possessed greater total and aerial biomass. In general, larger seeds have a greater amount of reserves in their cotyledons that can be allocated to initial growth, resulting in more vigorous seedlings (Mendes-Rodrigues *et al.* 2011; Souza & Fagundes 2014). Moreover, the greater biomass production by plants subjected to high light intensity can be easily explained on the basis of the general relationship between light radiation and photosynthesis rates (Onyekwelu *et al.* 2012; Leal *et al.* 2013). Therefore, the results observed in this study agree with our initial prediction that larger seeds would produce more vigorous seedlings and seedlings grown in darkness would have lower initial growth.

Root mass was not affected by light intensity and seedlings originating from heavier seeds produced more root mass. Thus, it is probable that photoassimilates produced during initial seedling development are drained principally for the production of aerial parts. Moreover, the significant interactive effect observed between seed mass and light intensity on root:shoot mass indicates that, under low and high light intensity, bigger seeds produce relatively more shoot mass than root mass, while smaller seeds invest more resources in the root system. In general, seedlings originating from smaller seeds must allocate a proportionally greater

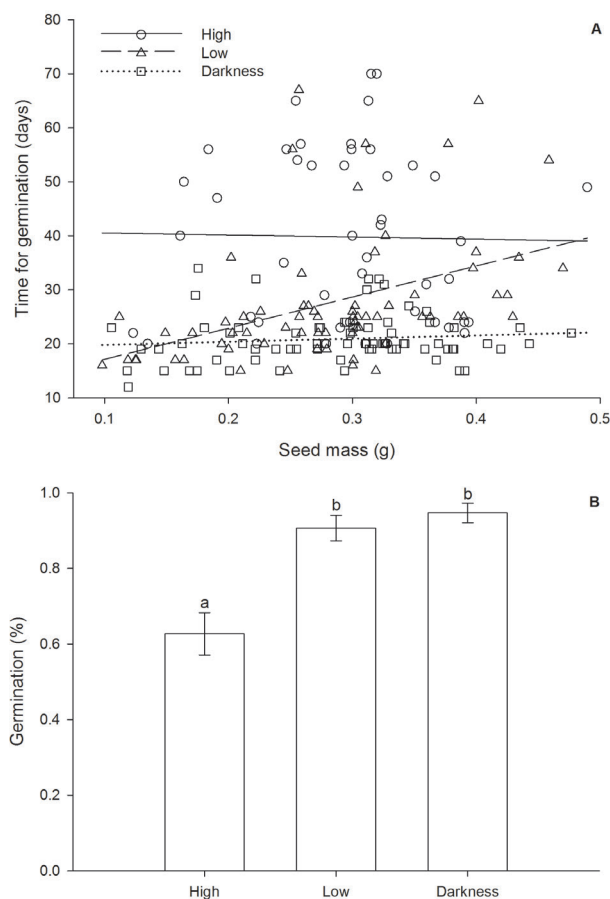


Figure 1. Seeds germinability of *Copaifera oblongifolia*: (A) the relationship between seeds mass and time required to seed germination under different light intensity and (B) effect of light intensity treatments on seeds germination percentage.

amount of resources to root development, resulting in a larger root system that reaches deeper into the substrate and acquiring more water and nutrients (Canadell & Zedler 1995; Yang & Midmore 2005).

In summary, the present study showed that while seed germinability was higher in darkness, seedlings exhibited greater development under light, and seed size positively affected seedling development. At first glance these results might seem to suggest a seed-seedling conflict. However, the apparent conflict could be solved based on the assumption that many seeds that present higher germinability in darkness should germinate better when they are buried (Pearson *et al.* 2003; Yanlong *et al.* 2007). Looking for an explanation for the relationship between the ability of *C. oblongifolia* colonize open/disturbed sites (many times invading areas that are being prepared for cultivation), and considering the results of this study, we hypothesize that human plowing of the soil can bury seeds and stimulate germination of seeds present in the seed bank. However, this hypothesis still needs to be tested under field conditions.

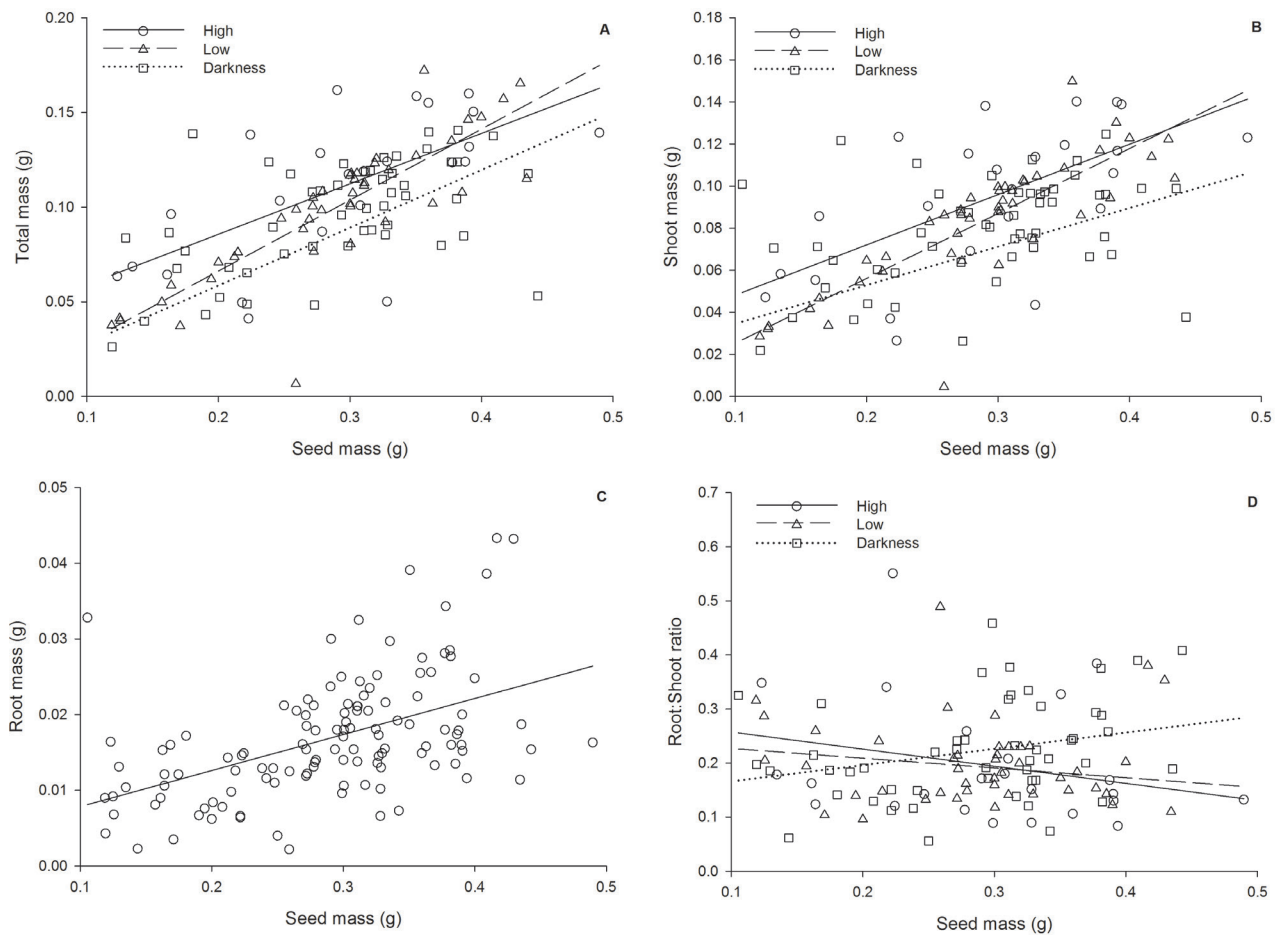


Figure 2. Relationship between seed mass and total mass (A), shoot mass (B), root mass (C) and root:shoot ratio (D) of *Copaifera oblongifolia* seedling growth under treatments of high and low light intensity, and darkness.

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