Research article

Influence of light availability and seed mass on germinability and initial growth of two congeneric species of Fabaceae

Influencia de la disponibilidad de luz y la masa de semillas sobre la germinabilidad y el crecimiento inicial de dos especies congénitas de Fabaceae

Marcilio Fagundes[1], Pablo Cuevas-Reyes[2,3], Walter S. Araújo[1], Maurício L. Faria[1], Henrique M. Valerio[1], Marcio A. Pimenta[1], Luiz A. D. Falcão[1], Ronaldo Reis-Junior[1], Joan Sebastian Aguilar-Peralta[2], Henrique Tadeu dos Santos[1]

Abstract:

Background and Aims: Environmental factors can interact with plant life history to determinate the reproductive strategies of adult plants and seedling recruitment. We expect that widely distributed tree species produce heavier seed and with greater variation in seed size than shrubs of restricted geographic distribution. We also predict that widely distributed tree species should be capable to germinate and develop under variable range of light conditions, while sun-adapted shrubs should germinate and develop better under high light intensity. We used as models two congeneric species of Fabaceae. *Copaifera langsdorffii* is a widely distributed arboreal species and *C oblongifolia* is a shrub with restricted distribution.

Methods: Seeds were collected from two plant species in a Cerrado vegetation area, northern Minas Gerais, Brazil. The effects of light available on seed germination and seedling development was conducted in a germination chamber with controlled photoperiod, temperature and light intensity.

Key results: The widely distributed tree (*C. langsdorffii*) had greater seed mass than the shrub (*C. oblongifolia*). Seeds of *C. langsdorffii* germinated faster under available high light, while the shrub *C. oblongifolia* seeds required less time to germinate under available low light and darkness. Under high light intensity, germination percentage of *C. langsdorffii* and *C. oblongifolia* seeds did not vary. However, seeds of the shrub showed a higher germination percentage under low light intensity and darkness. In general, seed mass showed a negative relationship with germination percentage, but this relationship varied in function of species and the availability of light. *Copaifera langsdorffii* seedlings had larger shoots and roots than *C. oblongifolia*. In contrast, the root:shoot ratio was higher in shrub than in tree species.

Conclusions: Our results have important implications for understanding the patterns of distribution of two *Copaifera* species and explain the ability of *C. oblongifolia* to colonize disturbed areas.

Key words: *Copaifera*, habitat invasion, plant recruitment, plant species distribution, regeneration niche hypothesis.

Resumen:

Antecedentes y Objetivos: Los factores ambientales pueden interactuar con la historia de vida de las plantas determinando las estrategias reproductivas de individuos adultos y el reclutamiento de plántulas. Predecimos que las especies de árboles ampliamente distribuidas producen semillas más pesadas y con una mayor variación en el tamaño de las semillas que los arbustos de distribución geográfica restringida. Esperamos que las especies arbóreas ampliamente distribuidas deberían ser capaces de germinar y desarrollarse bajo un rango variable de condiciones de luz, mientras que los arbustos adaptados a condiciones de sol deberían germinar y desarrollarse mejor con una alta intensidad de luz. Usamos como modelo dos especies congenéricas de Fabaceae. *Copaifera langsdorffii* es una especie arbórea ampliamente distribuida y *C. oblongifolia* es un arbusto de distribución geográfica restringida.

Métodos: Se colectaron semillas de estas especies de plantas en un área de vegetación de Cerrado, al norte del estado de Minas Gerais, Brasil. Los efectos de la disponibilidad de luz sobre la germinación de semillas y el desarrollo de plántulas se realizaron en una cámara de germinación con fotoperíodo controlado, temperatura e intensidad de luz.

Resultados clave: El árbol de amplia distribución (*C. langsdorffii*) tuvo mayor masa de semillas que el arbusto (*C. oblongifolia*). Las semillas de *C. langsdorffii* germinaron más rápido bajo alta disponibilidad de luz, mientras que las semillas de *C. oblongifolia* requirieron menos tiempo para germinar bajo poca luz disponible y oscuridad. En alta intensidad de luz, las semillas de *C. langsdorffii* y de *C. oblongifolia* tuvieron similares porcentajes, mientras que las semillas de *C. oblongifolia* mostraron una mayor germinación en baja intensidad de luz y oscuridad. La masa de semillas mostró una relación negativa con el porcentaje de germinación, pero esta relación varió en función de las especies y de la disponibilidad de luz. Las plántulas de *C. langsdorffii* tuvieron mayor desarrollo de brotes y raíces que *C. oblongifolia*. En contraste, la relación raíz:brote fue mayor en arbustos que en árboles.

Conclusiones: Nuestros resultados tienen implicaciones importantes para comprender los patrones de distribución de dos especies de *Copaifera* y poder explicar la capacidad de *C. oblongifolia* para colonizar áreas perturbadas.

Palabras clave: *Copaifera*, distribución de especies de plantas, hipótesis del nicho de regeneración, invasión de hábitat, reclutamiento de plantas.

[1]Programa de Pós-Graduação em Biodiversidade e Uso dos Recursos Naturais, Departamento de Biologia Geral, Universidade Estadual de Montes Claros, 39401-089, Montes Claros, Minas Gerais, Brazil.

[2]Laboratorio de Ecología de Interacciones Bióticas, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, Morelia, 58030 Michoacán, Mexico.

[3]Author for correspondence: pcragalla@gmail.com

Received: November 21, 2019.
Reviewed: February 18, 2020.
Accepted by Moisés Méndez Toribio: June 4, 2020.
Published Online first: June 22, 2020.
Published: Acta Botanica Mexicana 127 (2020).

To cite as: Fagundes, M., P. Cuevas-Reyes, W. S. Araújo, M. L. Faria, H. M. Valerio, M. A. Pimenta, L. A. D. Falcão, R. Reis-Junior, J. S. Aguilar-Peralta and H. T. Dos Santos. 2020. Influence of light availability and seed mass on germinability and initial growth of two congeneric species of Fabaceae. Acta Botanica Mexicana 127: e1638. DOI: 10.21829/abm127.2020.1638

e-ISSN: 2448-7589

Introduction

Abiotic conditions such as topography, soil fertility, humidity, temperature, and light availability are the main factors that affect plant distribution and abundance (Buckley et al., 2003; Longas et al., 2016). In addition, biotic factors (e.g. herbivores, pollinators, seed predators and dispersers) can also affect plant distribution, considering that these are associated with their dispersal capacity, germination, and recruitment (Ranieri et al., 2012; Jelbert et al., 2015). These facts agree with the regeneration niche hypothesis, which states that the distribution of each plant species is restricted to a particular set of biological conditions in which optimal germination and establishment occur (Grubb, 1977). Regeneration niche can be defined as biotic and abiotic requirements in earlier stages of the life cycles of a plant species (Grubb, 1977; Poorter, 2007). In this context, many studies have evaluated seed and seedlings responses to variables of the regeneration niche with the objective of understanding patterns of plant species distribution and their ability of habitat colonization (Poorter, 2007; Murray et al., 2005; Ranieri et al., 2012; Sõber and Ramula, 2013; Guerrero et al., 2016).

Light is an environmental factor known to exert greatest effect on seed germination and shape the regeneration niche of plant species (Benvenuti et al., 2001; Boyd and Van Acker, 2004; Gonçalves et al., 2015). Because light intensity varies temporally and spatially, several plant species have been acclimated showing different levels of plasticity as a mechanism of response to variations in these light regimes (Davidson et al., 2011; Gonçalves et al., 2015). For example, plants that occur in open areas generally show a positive relationship between seed germination and light incidence (e.g. Milberg et al., 1996; Knüsel et al., 2017). In contrast, shade tolerant tree species developed the ability to germinate their seeds under different conditions of light incidence (Simão and Takaki, 2008; Souza and Fagundes, 2014).

Morphological (i.e. size, form, hardness) and physiological (i.e. age, dormancy) seed traits can interact with their regeneration niche variables. Specifically, light can interact with seed size which, in turn, affects both the success of seed germination and seedling establishment (Milberg et al., 2000; Aud and Ferraz, 2012). Generally, smaller seeds germinate faster, but often have lower percentage of germination in comparison with large seeds (Herrera and Laterra, 2008; Souza and Fagundes, 2014). However, small seeds have a higher percentage of germination under high light availability than in darkness conditions (Moles and Westoby, 2006). Large seeds exhibit low sensitivity of light variation, suggesting more plasticity levels to light regimens (Milberg et al., 2000; Fenner and Thompson, 2005).

Light is also the main environmental factors that affects seedling survival and plant growth in different habitats. Because seed size is directly related with the amount of nutritional reserves to be allocated for initial seedling growth (Geritz, 1995; Fagundes et al., 2011), it is possible this affects the success of the recruitment of plant species across different habitats (Milberg et al., 2000; Ribeiro and Borghetti, 2014; Onyekwelu et al., 2012; Souza et al., 2015b). In general, there are positive relationships between plant size and seed biomass (Leishman, 2001; Souza et al., 2016) and larger seeds develop more vigorous seedlings (Delgado et al., 2009; Sõber and Ramula, 2013; Souza and Fagundes, 2014). Hence, plant species that produce larger seeds are favored in predictable, more competitive habitats, while those that develop greater number of small seeds have greater competitive advantage, especially in early successional habitats (Moles and Westoby, 2006; Souza et al., 2015b).

The knowledge of the regeneration niche of phylogenetically related plant species allows us to find relationships between plant species traits and the colonization probability of a particular habitat (Brown et al., 2003; Gallagher et al., 2014; Ferreras et al., 2015). In this study, we evaluated the effects of seed size and light intensity on seed germination, aerial and belowground growth of two congeneric species of Fabaceae (*Copaifera langsdorffii* Desf. and *C. oblongifolia* Mart.) to characterize traits of regeneration niche that favor plant species colonization of specific habitat. The two species share a zone of sympatry in Cerrado areas in the north of Minas Gerais State, Brazil. *Copaifera langsdorffii* is a heliophytic, arboreal species with wide geographic distribution (Fagundes, 2014). *Copaifera oblongifolia* is a shrub with restricted distribution that grows in open areas such as abandoned pastures, edges of

Cerrado fragments and highways. Additionally, this species is invasive in grasslands and agricultural systems, dominating and inhibiting the development of cultivated plants, especially in northern Minas Gerais, Brazil (Fernandes et al., 2018; Coutinho et al., 2019). Therefore, we expect that widely distributed tree species produce heavier seeds and with greater variation in seed size than shrubs that present more restricted geographic distribution. We also predict that, while widely distributed tree species should be capable to germinate and develop under a variable range of environmental conditions, sun-adapted shrubs should germinate and develop better under high light intensity.

Materials and Methods

Study species

Copaifera langsdorffii (Fabaceae) is a heliophytic tropical shrub, reaching up to 30 m height. It is widely distributed in South America, occurring from northern Argentina to southern Bolivia, and can be found in different biomes in Brazil, such as the Cerrado (Brazilian Savanna), Amazon Forest and Atlantic Forest (Souza et al., 2015a; Fagundes, 2014). *Copaifera oblongifolia* is a shrub of 1-2.5 m height that occurs in disturbed/open areas (i.e. abandoned pastures, edges of forest fragments and highways) of the Cerrado in central Brazil (Coutinho et al., 2019). Since the last decade, *C. oblongifolia* has caused economic loss for farmers of Minas Gerais State, because it has a great capacity to invade and colonize pastures and agricultural systems, and therefore, has become a dominant species capable to inhibit the development of cultivated plants (Fernandes et al., 2018; Fagundes et al., 2019).

The two *Copaifera* L. species share a sympatry zone in Cerrado areas in northern Minas Gerais State, Brazil. In this region, both flower from December to February and their fruits mature by September to October, coinciding with the time of their highest deciduousness (Costa et al., 2016; Fernandes et al., 2018). The fruits and seeds of both species have very similar morphology. 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, with slow germination rate which extends up to 70 days after sowing (Fernandes et al., 2018). The seed size is a key factor for germination and seedling vigor (Souza and Fagundes, 2014). Although other animals attack seeds of *C. langsdorffii* in the pre-dispersal phase, the seed predation by the weevil *Rhinochenus brevicollis* Chevrolat represents the main cause of mortality of these seeds (Souza and Fagundes, 2017).

Seed collection

Seeds were collected in September 2015, which represents the period of seed dispersal peak of both species. We collected mature seeds from 17 individuals of *Copaifera langsdorffii* and 21 individuals of *C. oblongifolia* in an area of Cerrado vegetation (16˚17'20''S and, 44˚09'02''W) in Mirabela municipality, northern Minas Gerais State, Brazil. This region is characterized by semi-arid climate with well-defined dry and wet seasons. The average annual temperature is 23 ˚C and rainfall is about 1000 mm/year (Fernandes et al., 2018).

We randomly collected a total of 2212 well-formed seeds (malformed seeds and seeds with visual signals of attack by predators or pathogens were eliminated from the sampling) from both two plant species (*C. langsdorffii* N = 1445; *C. oblongifolia* N = 1775). Each seed was packed in an individually labeled paper bag and weighed with an analytical scale in order to compare mean and coefficient of variation of seed biomass between both species.

Seed germination tests

Of the total of 2212 seeds collected, we selected 225 of each species (eight to 20 seeds from each individual) to establish the germination experiment. All selected seeds were individually sown in six germination trays with 75 individual cells (2 cm length, 2 cm width and 3 cm height) and vermiculite was used as substrate for seed germination. This experimental design considers that each seed is a statistically independent experimental unit and allows for the comparisons of seed germination percentage among treatments using a binomial error distribution (see Warton and Hui, 2011; Souza and Fagundes, 2014). All seeds were subjected to disinfection by immersion in a 1% sodium hypochlorite solution for two minutes before sowing (Souza et al., 2016).

Finally, each set with two trays of 75 seeds of *C. langsdorffii* and 75 seeds of *C. oblongifolia* was placed in its own germination chamber (climatic chamber MA402 Marconi, Piracicaba city, Brazil) with controlled 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, 47.5 μmol.m^{-2}s^{-1} irradiation; T_2 = low light intensity: 12 h/light at 28 °C and 12 h/dark at 28 °C, 23.8 μmol.m^{-2}s^{-1} irradiation and; and T_3 = 24 h/darkness at 28 °C). The humidity of the germination substrate was maintained constant daily by adding three ml of distilled water in each germination cell. Seeds were monitored daily to determine seed germination percentage and time required for germination. Seeds were considered germinated when they presented primary root protrusion.

Aerial and belowground plant growth experiment

All seedlings originated from this germination experiment were cultivated at the same experimental conditions of the seed germination test (T_1 = high light intensity, T_2 = low light intensity and T_3 = darkness), in order to evaluate the effects of light intensity, plant species and seed mass on aerial plant growth. We estimated a time required for seedling development and biomass accumulation by seedling. The time for seedling development was calculated as the time elapsed between seed germination until seedling cotyledons fell. All seedlings were removed from the substrate soon after cotyledons fell to determine their dry biomass, and next, all seedlings were individually placed in individually labeled paper bags and transferred to an oven (QUIMIS oven Q31 4M272, Sao Paulo, Brazil) with air circulation at 60 °C for 72 hours. After this time, the dry weight of shoot and root systems was determined on an analytical scale. Finally, the ratio of root:shoot biomass (RSR) of all seedlings was calculated as RSR = Wroot/Wshoot, where Wroot is dry mass of the root and Wshoot is dry mass of shoots.

Statistical analysis

To determine the differences in seed mass and coefficient of variation of seed mass between both species, we built two General Linear Models (GLMs). The models consider the species (*C. langsdorffii* and *C. oblongifolia*) as explanatory variable and average seed mass or coefficient of variation of seed mass per individual plant as response variables. The two models were tested with ANOVA, considering the Gaussian error distribution.

Variations in time required for seed germination and percentage of germination among treatments were tested with GLMs followed by ANOVA. For model constructions, time to germination or germination percentage was used as response variables and treatments of light intensity, plant species and seeds mass as explanatory variables. We used the Gaussian distribution when the time to germination was the response variable. Moreover, because data of seed germination are binary (germinated or not germinated), Binomial distribution error (corrected for Quasibinomial) was used in model construction when germination percentage was the response variable. This statistical approach provides a significant gain in analysis power (Warton and Hui, 2011).

We used five different GLMs to compare the effects of light intensity treatments, plant species and seed mass on seedling vigor. The models considered time to cotyledons shedding, total biomass, aerial biomass, root biomass or root:shoot ratio as response variables and light intensity treatments, plant species and seed mass as the explanatory variables. The models were tested with ANOVA, using Gaussian distribution. 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 Akaike Information Criterion for small samples (AICc) was used for ordered explanatory variables, followed by selection of the more parsimonious model. These procedures were carried out using the MuMIn package in R software (Bartoń, 2015). All models were submitted to residual analysis to check for model fit and suitability of error distribution for each response variable (Crawley, 2007). All analyses were carried out in software R version 3.6.3 (R Core Team, 2020).

Results

We found differences in seed mass between plant species (Residual DF = 36, F = 5.193, P = 0.020). Seeds of *Copaifera langsdorffii* were 14% heavier than those produced by *C. oblongifolia* (Fig. 1A). However, the coefficients of variation of seed mass did not differ statistically between both species (Residual DF = 36, *F* = 1.752, *P* = 0.194, Fig. 1B).

The time required for seed germination varied in function of light intensity and seed mass (Table 1). Contrast analysis showed that time for seed germination was greater in high light intensity treatment, followed by low light intensity, and darkness. Seed mass affected positively time required for seed germination (y = 22.93 + 19.37x).

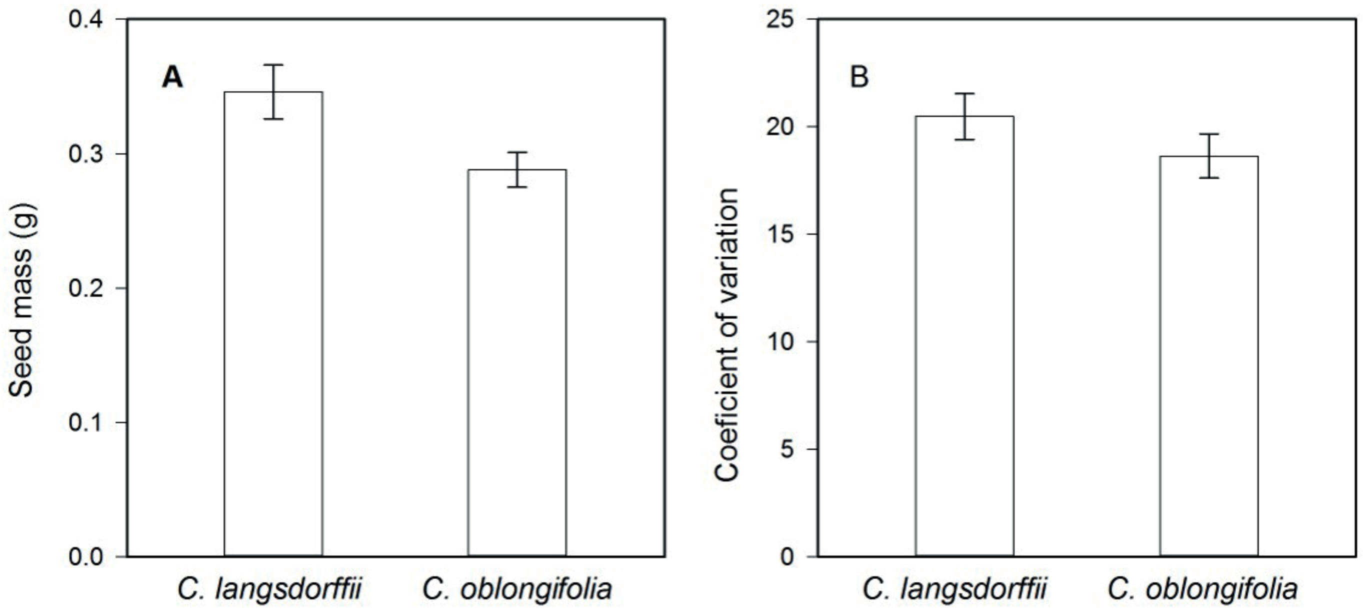

Figure 1: A. variations in seed mass; B. coefficient of variation of seed mass between *Copaifera langsdorffii* Desf. and *C. oblongifolia* Martius species in Minas Gerais, Brazil.

Table 1: Summary of minimal adequate models showing the effects of explicative variables (light intensity, seed mass and plant species and their interactions) on the variables' responses (time required to seed germination and percentage of seed germination) of two *Copaifera* L. species in Minas Gerais, Brazil.

Response variables	Explicative variables	Error distribution	Deviance	DF from residue	Residual deviance	*F*-values	*P*-values
Time to seed germination	Light intensity	Gaussian	12109.1	352	52083	44.899	<0.0001
	Seed mass		1248.5	351	50834	9.258	0.0026
	Species		23.1	350	50811	0.171	0.6793
	Light intensity × seed mass		1960.2	348	48851	7.268	0.0008
	Light intensity × species		2193.4	346	46658	8.1329	0.0003
Percent seed germination	Light intensity	Binomial	12.3916	447	432.37	6.453	0.0017
	Species		4.7335	446	427.64	4.930	0.0269
	Seed mass		5.4008	445	422.24	5.625	0.0181
	Light intensity ×species		18.746	443	403.49	9.763	<0.0001
	Light intensity × species × seed mass		10.545	438	389.59	5.491	0.0044

Moreover, the interaction between light intensity and plant species suggests that *C. langsdorffii* seeds germinate faster under high light intensity (*C. langsdorffii*: 32.72 ± 1.46, *C. oblongifolia*: 39.78 ± 1.82, Fig. 2A), while *C. oblongifolia* seeds required less time to germinate under low light intensity (*C. langsdorffii*: 36.25 ± 2.02 SE, *C. oblongifolia*: 27.63 ± 1.38 SE, Fig. 2B) and darkness (*C. langsdorffii*: 23.27 ± 0.77 SE, *C. oblongifolia*: 20.86 ± 0.72 SE , Fig. 2C). Finally, the interaction between light intensity and seed mass suggested that seed mass affected the time to seed germination, especially under low light intensity and darkness.

The explanatory variables light intensity, plant species, seed mass and the interactions: light × plant species and light × plant species × seed mass affected the percentage of seed germination (Table 1). The time for cotyledons shedding varied in function of light intensity and plant species (Table 2).

Contrast analysis showed that seed germination percentage was lower in darkness, but germination percentage did not change between treatments of low and high light intensity (Table 3). In general, seed mass showed a negative relationship with germination percentage (Residual DF = 448, *F* = 10.264, *P* <0.001), and seeds of *C. oblongifolia* (0.83 ± 0.02: mean ± SE) had greater percentage germination than those of *C. langsdorffii* (0.74 ± 0.03: mean ± SE). However, the significance of the interaction between light intensity × plant species indicate that the effects of light intensity on seed germination is species-dependent. In fact, in high light intensity, germination percentage of *C. langsdorffii* and *C. oblongifolia* seeds did not vary (Residual DF = 148, *F* = 3.625, *P* = 0.062), but *C. oblongifolia* seeds presented greater germination percentage than those of *C. langsdorffii* in low light intensity (Residual DF = 148, *F* =10.982, *P* <0.001) and darkness (Residual DF = 148, *F* = 10.907, *P* <0.001). Finally, the significance of the interaction of third order suggests the effect of light available on the relationship between germination and seed mass is species-dependent (Fig. 3A-C).

Contrast analysis showed that the time to cotyledons shedding was different among the three treatments of light intensity, being greater in darkness, followed by low light intensity and high light intensity treatments (Table 3). Moreover, the time to cotyledons shedding varied between species (Residual DF = 239, *F* = 24.27, *P* <0.001), with cotyledons of *C. langsdorffii* and *C. oblongifolia* presenting a mean delay of 30.24 ± 1.3 SE and 36.25 ± 1.1 SE days to their fall, respectively (Fig. 4).

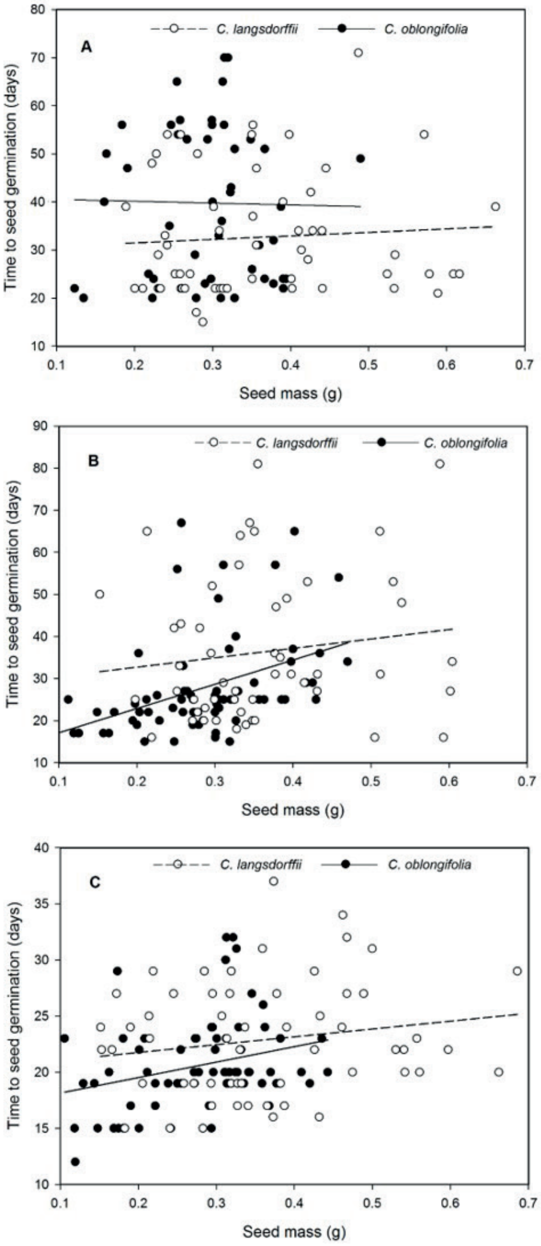

Figure 2: A. relationship between seed mass and time required to seed germination of *Copaifera langsdorffii* Desf. and *C. oblongifolia* Mart. plants in high light intensity in Minas Gerais, Brazil; B. low light intensity; C. darkness.

The shoot dry mass of seedlings was affected by light intensity, plant species, seed mass, and by interaction light

Table 2: Summary of minimal adequate models showing the effects of explicative variables (light intensity, seed mass and plant species and their interactions) on the variables' responses (aerial mass, root mass and root:shoot ratio) of seedlings of *Copaifera langsdorffii* Desf. and *C. oblongifolia* Mart. in Minas Gerais, Brazil.

Response variables	Explicative variables	Error distribution	Deviance	DF from residue	Residual deviance	*F*-values	*P*-values
Time to cotyledon shedding	Light intensity	Gaussian	2249.28	234	20808	13.733	<0.0001
	Species		1560.79	233	19247	19.059	<0.0001
	Light intensity × species		329.45	231	18918	2.011	0.1361
Shoot mass	Light intensity	Gaussian	0.049	232	0.408	43.671	<0.001
	Species		0.053	231	0.356	92.869	<0.001
	Seed mass		0.198	230	1.158	347.15	<0.001
	Light intensity ×species		0.001	228	0.157	0.698	0.4991
	Light intensity ×seed mass		0.013	226	0.144	11.385	<0.001
Roots mass	Species	Gaussian	0.001	240	0.015	22.07	<0.001
	Seed mass		0.006	239	0.014	104.72	<0.001
Root:shoot ratio	Species	Gaussian	0.036	238	2.123	4.126	0.0433

Table 3: Results of contrast analysis showing the effects of different light intensity treatments on response variables (time to seed germination, percent of seed germination, time required to cotyledons shedding, and shoot mass) of *Copaifera langsdorffii* Desf. and *C. oblongifolia* Mart. in Minas Gerais, Brazil. *The levels of low light intensity and darkness were grouped and then compared with the high light intensity treatment.

Response	Interaction levels (mean ± standard error)			Deviance	Residual DF	*F*	*P*
variables	Darkness	Low light	High light				
Time to seed germination	21.93 ± 0.45	31.42 ± 1.27		5586.3	250	45.306	<0.001
		31.42 ± 1.27	35.87 ± 1.27	1125.9	221	5.101	0.024
Percent seed germination		0.80 ± 0.03	0.69 ± 0.03	5.082	298	5.048	0.025
	0.88 ± 0.04	0.80 ± 0.03		2.972	298	2.953	0.087
Time to cotyledon shedding		33.15 ± 1.02	29.72 ± 1.19	589.96	140	6.793	0.010
	36.20 ± 0.98	33.15 ± 1.02		391.21	170	4.537	0.035
Shoot mass	0.092 ± 0.003	0.091 ± 0.004		0.0006	170	0.051	0.821
		0.092 ± 0.003*	0.124 ± 0.006	0.051	239	29.005	<0.001

intensity × seed mass (Table 2). Shoot dry mass of *C. langsdorffii* seedlings was 29.4% greater than dry mass of those of *C. oblongifolia* (Residual DF = 239, *F* = 44.52, *P* <0.001). According with contrast analysis, seedling growth under higher light intensity showed greater shoot dry mass in comparison with seedling growth on low light intensity and darkness (Table 3). Furthermore, shoot dry mass showed a positive relationship with seed mass (Residual DF = 239, *F* = 322.83, *P* <0.001, y = 0.002 + 0.305x). Finally, the significant interaction between light intensity and seed mass suggests that curve slopes vary among the treatments of light intensity. In fact, the contrast analyses suggest that the curve slope of low and high light intensity treatments did not vary between them (Residual DF = 169, *F* = 0.6460, *P* = 0.422), but was greater than that of darkness treatment (Residual DF = 237, *F* = 25.783, *P* <0.01) (Fig. 5).

Root dry mass of seedlings was affected by plant species and seed mass (Table 2). Root mass of *C. langsdorffii* seedlings was 22.2% greater than root mass of *C. oblongifolia*. Besides, we found a positive relationship between seedling mass and seed mass (Fig. 6A). Finally, we observed that root:shoot biomass ratio of *C. oblongifolia* was approximately 13.2% greater than root:shoot biomass of *C. langsdorffii* (Table 2, Fig. 6B).

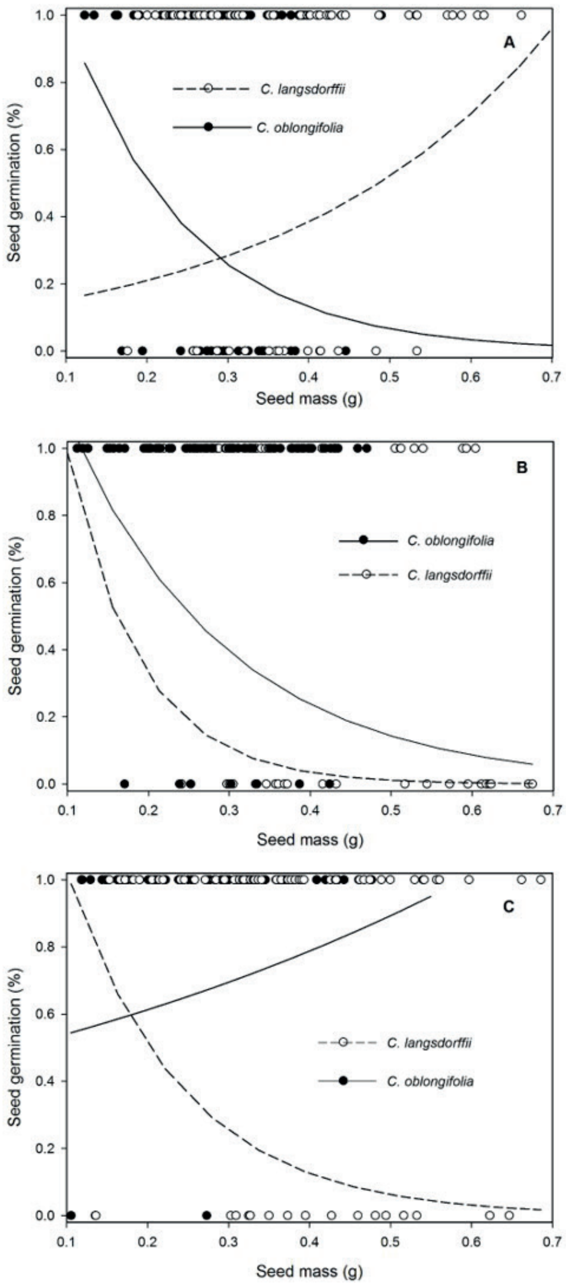

Figure 3: A. effects of seed mass on percent of seed germination of *Copaifera langsdorffii* Desf. and *C. oblongifolia* Mart. plants in high light intensity in Minas Gerais, Brazil; B. low light intensity; C. darkness.

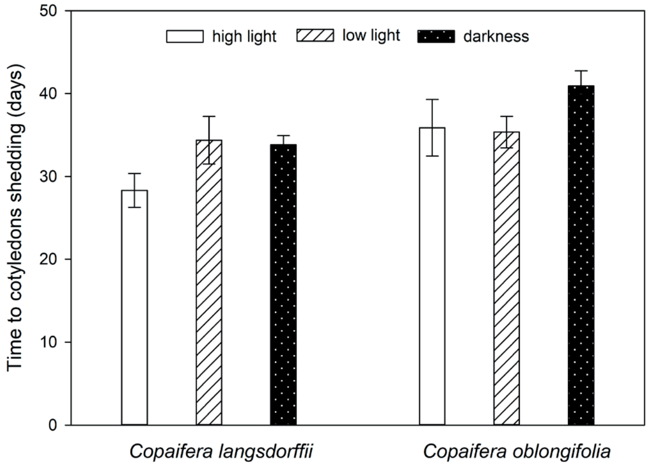

Figure 4: Variations in time required to cotyledons fall (mean ± ED) of *Copaifera langsdorffii* Desf. and *C. oblongifolia* Mart. seedling in high light intensity, low light intensity, and darkness in Minas Gerais, Brazil.

Discussion

Our results showed that light intensity and seed size were important factors that interact to shape the regeneration niche of *Copaifera langsdorffii* and *C. oblongifolia*. It is generally accepted that the niche of an adult plant species reflects its regenerative niche (Quero et al., 2009). According to this approach, the wide geographical distribution of *C. langsdorffii* suggests that its seeds can germinate successfully under a wide range of environmental conditions as observed experimentally in our study. On the other hand, *C. oblongifolia* occurs in open areas under conditions of high incidence of sun radiation. However, in our study, we found that its seeds had high germinability under low light intensity and darkness. These different physiological responses between seeds and adults of *C. oblongifolia* suggest a regenerative-adult niche conflict during the ontogenetic stages of these plant species. This agrees with other studies that have shown that seeds of some species of weeds adapted to environments of high incidence of solar radiation present high levels of seed germination in the absence of light or after being buried in the soil (Ohadi et al., 2010; Batlla and Benech-Arnold, 2014; Wang et al., 2016; Hu et al., 2017).

Seed mass showed a positive relationship with the time required for seed germination and conversely, presented a negative relationship with germination percentage in both plant species. Seed size is directly related with volume ratio and coat thickness of the seeds. Hence, greater seeds have low capacity of water absorption, resulting in increase of time for germination (Souza et al., 2015b). In contrast, increase in time to seed germination can raise the probability of attack by microorganisms, resulting in loss of seed viability (Fagundes et al., 2011; Souza and Fagundes, 2014).

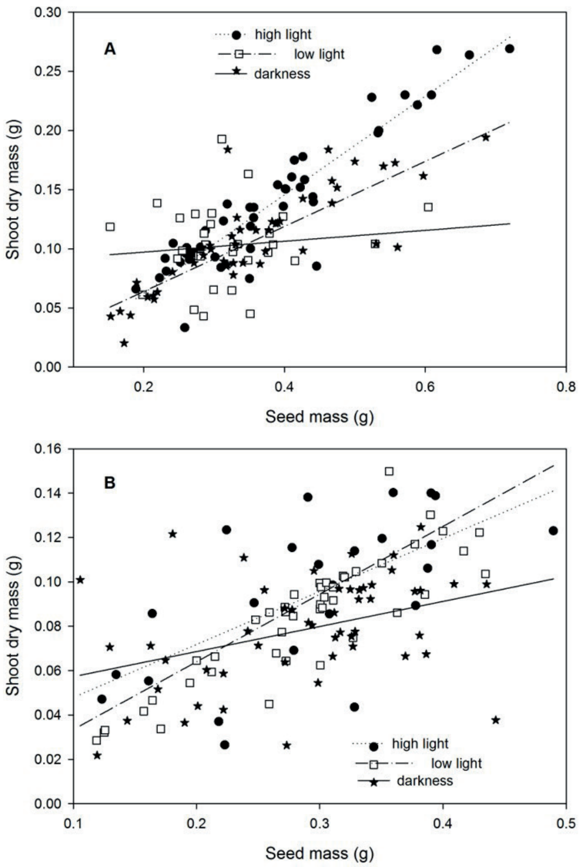

Figure 5: A. relationship between seed mass and shoot dry mass of *Copaifera langsdorffii* Desf.; B. *C. oblongifolia* Mart. seedling in high light intensity, low light intensity, and darkness in Minas Gerais, Brazil.

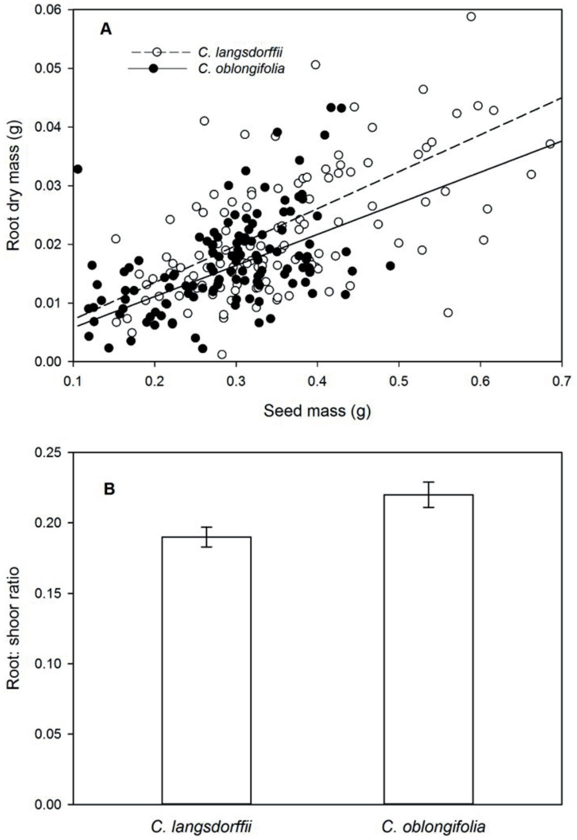

Figure 6: A. relationship between seed mass and root dry mass of *Copaifera langsdorffii* Desf. and *C. oblongifolia* Mart. seedling; B. variation in root:shoot ratio of *C. langsdorffii* Desf. and *C. oblongifolia* Mart. seedlings in Minas Gerais, Brazil.

This fact may explain the negative relationship between seed size and germination percentage observed in this study. In fact, four saprophytic fungus species (*Aspergillus niger* P.E.L. Van Tieghem, *Aspergillus flavus* Link, *Mucor* sp. and *Rhizopus stolonifer* Vouillemin) were observed developing on seeds during the final phases of the seed germination test.

Following germination, light had a positive effect on time required to cotyledons shedding. In the absence of light, all nutrients available for seedling growth come from cotyledon reserves as photosynthesis is light limited. Therefore, we can expect that seedlings growing under low light intensity and darkness are more dependent of cotyledon reserve, exhausting this resource early. However, despite *C. langsdorffii* had heavier seeds, which implies higher cotyledon reserves than *C. oblongifolia*, the seedlings of *C. oblongifolia* retained their cotyledons for a longer time. These results suggest that *C. langsdorffii* can use cotyledon reserves more intensely in order to reach greater initial growth. In several seedlings of arboreal species, a fast-initial growth generally is considered as an adaptive strategy which promotes greater light capture at understory level, increasing the competitive ability and therefore, more probability of survival in environments of low light availability (Aud and Ferraz, 2012).

Seedlings of both species, under conditions of high light intensity, presented greater shoot mass than those subjected to low light intensity and darkness, suggesting that both study species share the same needs for light availability (i.e. high light intensity) for initial development. Therefore, similar physiological processes should be regulating this pattern of initial development in both species. Moreover, seed mass affected positively aerial plant growth (i.e. seedlings shoot mass), where seedlings of *C. langsdorffii* had more mass than seedlings of *C. oblongifolia*. Our results support the general predictions that seeds with greater mass develop larger and vigorous seedlings (He et al., 2007). The interspecific variation in seed/seedling size observed in this study probably reflects the adult niche of each plant species. In fact, plant species producing larger seeds have more reserves, having vigorous seedlings with greater competitive ability in predictable habitats. In contrast, plants developing smaller seeds should present greater percentage of germination and germinate more quickly in order to colonize more efficiently habitats in initial stages of succession (Moles and Westoby, 2006; Sõber and Ramula, 2013).

Root dry mass of both species was not affected by light intensity but was positively affect by seed mass, suggesting that initial growth of root system is dependent of seed reserves. Moreover, greater root mass of *C. langsdorffii* probably is associated with greater seed mass of this species. Finally, we observed that *C. oblongifolia* allocated proportionally more resource to root development than *C. langsdorffii*. It is important to highlight that resource allocation for roots or shoots is seed size dependent and smaller seeds must allocate proportionally a greater amount of resource to root development (Yang and Midmore, 2005). High investment in the root allows the root system to reach deeper levels of substrate with more water and nutrients, increasing the seedling survivorship with poor cotyledon reserves in stressful environmental conditions (Canadell and Zedler, 1995).

We conclude that light represents a key resource that interacts with seed mass to shape the regenerative niche of two *Copaifera* species. This information allows the understanding of the factors that affect the distribution of adult plants. Our results show the importance of seed size and light availability as factors affecting germination rates, as well as aerial and below ground growth of two *Copaifera* species. In the case of *C. langsdorffii*, our study shows experimental evidence of the importance of these factors for their ability to colonize different environments, which explains their wide geographic distribution. Adult shrubs have restricted distribution and occur in disturbed areas under high level of light incidence, while their seeds present high germinability under available low light. In order to justify this apparent seed-adult conflict and local pattern of plant distribution, we hypothesize that the technique of revolving soil before sowing of seed crops could bury and stimulate seed germination of the seed bank of *C. oblongifolia*.

Author contributions

MF, PCR, WSA and MLF contributed to the design and original ideas of the study and wrote the manuscript. HMV, MAP, LAD, RRJ contributed to the data collection in the field and performed the statistical analysis. JSA and HTD contributed to the data collection in the laboratory and experimental design. All authors reviewed and approved the final manuscript.

Funding

The Coordination of Scientific Research and academic secretary (Universidad Michoacana de San Nicolás de Hidalgo, UMSNH), awarded a research grant to P. Cuevas-Reyes. This project was supported by Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG APQ 01926.11) and Programa de Pós-Graduação *Stricto Sensu* em Biodiversidad e Uso dos Recursos Naurais (BURN) da UNIMONTES. The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) has awarded a scholarship for H. T. Santos' studies of Master in Biodiversity of PPG-BURN (State University of Montes Claros).

Acknowledgements

The authors thank Postgraduate Program of Biodiversity (PPG-BURN) of Unimontes and the Projeto Jequitaí (CODEVASF/SEAPA-MG) for the logistical support during field work.

Literature cited

Aud, F. F. and I. D. K. Ferraz. 2012. Seed size influence on germination responses to light and temperature of seven pioneer tree species from the Central Amazon. Anais da Academia Brasileira de Ciências 84(3): 759-766. DOI: https://doi.org/10.1590/S0001-37652012000300018

Bartoń, K. 2015. MuMIn: Multi-Model Inference. R package version 1.15.1. 311. http://CRAN.R-project.org/package=MuMIn (consulted June, 2019).

Batlla, D. and R. L. Benech-Arnold. 2014. Weed seed germination and the light environment: implications for weed management. Weed Biology and Management 14(2): 77-87. DOI: https://doi.org/10.1111/wbm.12039

Benvenuti, S., M. Macchia and S. Miele. 2001. Light, temperature, and burial depth effects on *Rumex obtusifolius* seed germination and emergence. Weed Research 41(2): 177-186. DOI: https://doi.org/10.1046/j.1365-3180.2001.00230.x

Boyd, N. and R. Van Acker. 2004. Seed germination of common weed species as affected by oxygen concentration, light, and osmotic potential. Weed Science 52(4): 589-596. DOI: https://doi.org/10.1614/WS-03-15R2

Brown, J., N. J. Enright and B. P. Miller. 2003. Seed production and germination in two rare and three common co-occurring *Acacia* species from south-east Australia. Austral Ecology 28(3): 271-80. DOI: https://doi.org/10.1046/j.1442-9993.2003.t01-4-01287.x

Buckley, Y. M., P. Downey, S. V. Fowler, R. Hill, J. Memmot, H. Norambuena, M. Pitcairn, R. Shaw, A. W. Sheppard, C. Winks, R. Wittenberg and M. Rees. 2003. Are invasives bigger? A global study of seed size variation in two invasive shrubs. Ecology 84(6): 1434-1440. DOI: https://doi.org/10.1890/0012-9658(2003)084[1434:AIBAGS]2.0.CO;2

Canadell, J. and P. H. Zedler. 1995. Underground structures of woody plants in mediterranean ecosystems of Australia, California, and Chile. In: Arroyo M. T. K., P. H. Zedler and M. D. Fox (eds.). Ecology and Biogeography of mediterranean Ecosystems in Chile, California, and Australia. Springer-Verlag. New York, USA. Pp. 177-210. DOI: https://doi.org/10.1007/978-1-4612-2490-7_8

Costa, F. V., A. C. M. Queiroz, M. L. B. Maia, R. Reis-Junior and M. Fagundes. 2016. Resource allocation in *Copaifera langsdorffii* (Fabaceae): how supra-annual fruiting affects plant traits and herbivory? Revista de Biología Tropical 64(2): 507-520. DOI: https://doi.org/10.15517/rbt.v64i2.18586

Coutinho, R. D., P. Cuevas-Reyes, G. W. Fernandes and M. Fagundes. 2019. Community structure of gall-inducing insects associated with a tropical shrub: regional, local and individual patterns. Tropical Ecology 60: 74-82. DOI: https://doi.org/10.1007/s42965-019-00010-7

Crawley, M. J. 2007. The R book. Imperial College London at Silwood Park. London, UK. Pp. 527-528.

Davidson, A. M., M. Jennions and A. B. Nicotra. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology Letters 14(4): 419-431. DOI: https://doi.org/10.1111/j.1461-0248.2011.01596.x

Delgado, J. A., M. D. Jiménez and A. Gómez. 2009. Samara size versus dispersal and seedling establishment in *Ailanthus altissima* (Miller) Swingle. Journal of Environmental Biology 30(2): 183-186.

Fagundes, M. 2014. Galling Insect Community associated with *Copaifera langsdorffii* (Fabaceae): the role of Inter- and Intra-annual host plant phenology. In: Fernandes, G. W. and J. C. Santos (eds.). Neotropical Insect Galls. Springer. Dordrecht, The Netherlands. Pp. 163-174. DOI: https://doi.org/10.1007/978-94-017-8783-3_11

Fagundes, M., M. G. Camargos and F. V. Costa. 2011. A Qualidade do solo afeta a germinaçao das sementes e o desenvolvimento das plântulas de *Dimorphandra mollis* Benth. (Leguminosae: Mimosoidae). Acta Botanica Brasilica 25(4): 908-915. DOI: https://doi.org/10.1590/S0102-33062011000400018

Fagundes, M., E. M. Barbosa, J. B. B. S. Oliveira, B. G. S. Brito, K. T. Freitas, K. F. Freitas and R. Reis-Junior. 2019. Galling inducing Insects associated with a tropical shrub: the role of resource concentration and species interactions. Ecología Austral 29(1): 12-19. DOI: https://doi.org/10.25260/ea.19.29.1.0.751

Fenner, M. and K. Thompson. 2005. The ecology of seeds. Cambridge University Press. Cambridge, UK. DOI: https://doi.org/10.1017/CBO9780511614101

Fernandes, E. G., E. M. Valério, K. L. R. Duarte, L. M. N. Capuchinho and M. Fagundes. 2018. Fungi associated with *Copaifera oblongifolia* (Fabaceae) seeds: occurrence and possible effects on seed germination. Acta Botanica Brasilica 33(1): 179-183. DOI: https://doi.org/10.1590/0102-33062018abb0100

Ferreras, A. E., G. Funes and L. Galetto. 2015. The role of seed germination in the invasion process of honey locust (*Gleditsia triacanthos* L., Fabaceae): comparison with a native confamilial. Plant Species Biology 30(2): 126-136. DOI: https://doi.org/10.1111/1442-1984.12041

Gallagher, R. V., R. P. Randall and M. R. Leishman. 2014. Trait differences between naturalized and invasive plant species independent of residence time and phylogeny. Conservation Biology 29(2): 360-369. DOI: https://doi.org/10.1111/cobi.12399

Geritz, S. A. 1995. Evolutionarily stable seed polymorphism and small-scale spatial variation in seedling density. The American Naturalist 146(5): 685-707. DOI: https://doi.org/10.1086/285820

Gonçalves, J. F. C., D. C. S. Barreto, U. M. Santos-Junior, A. V. Fernandes, P. T. B. Sampaio and M. S. Buckeridge. 2015. Growth, photosynthesis and stress indicators in young rosewood plants (*Aniba rosaedora* Ducke) under different light intensity. Brazilian Journal of Plant Physiology 17(3): 325-334. DOI: https://doi.org/10.1590/S1677-04202005000300007

Grubb, P. J. 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. Biological Reviews of the Cambridge Philosophical Society 52(1): 107-145. DOI: https://doi.org/10.1111/j.1469-185X.1977.tb01347.x

Guerrero, P. C., D. Mardones, N. Viveros, F. T. Peña-Gómez and R. O. Bustamante. 2016. Evolutionary change in the germination niche between related species within *Neoporteria* clade (Cactaceae) is idiosyncratic to habitat type. Gayana Botánica 73(2): 177-182. DOI: https://doi.org/10.4067/S0717-66432016000200177

He, Y., M. Wang, S. Wen, Y. Zhang, T. Ma and G. Du. 2007. Seed size effect on seedling growth under different light conditions in the clonal herb *Ligularia virgaurea* in Qinghai-Tibet Plateau. Acta Ecologica Sinica 27(8): 3091-3108. DOI: https://doi.org/10.1016/S1872-2032(07)60063-8

Herrera, L. P. and P. Laterra. 2008. Do seed and microsite limitation interact with seed size in determining invasion patterns in flooding Pampa grasslands? In: Van der Valk, A. G. (ed.). Herbaceous Plant Ecology. Springer. Dordrecht, The Netherlands. Pp. 93-105. DOI: https://doi.org/10.1007/978-90-481-2798-6_8

Hu, X. W., J. Pan, D. D. Min, Y. Fan, X. Y. Ding, S. G. Fan, C. C. Baskin and J. M. Baskin. 2017. Seed dormancy and soil seedbank of the invasive weed *Chenopodium hybridum* in north-western China. Weed Research 57(1): 54-64. DOI: https://doi.org/10.1111/wre.12237

Jelbert, K., I. Stott, R. A. McDonald and D. Hodgson. 2015. Invasiveness of plants is predicted by size and fecundity in the native range. Ecology and Evolution 5(10):1933-1943. DOI: https://doi.org/10.1002/ece3.1432

Knüsel, S., A. De Boni, M. Conedera, P. Schleppi, J.-J. Thormann, M. Frehner and J. Wunder. 2017. Shade tolerance of *Ailanthus altissima* revisited: novel insights from southern Switzerland. Biological Invasions 19(2): 455-461. DOI: https://doi.org/10.1007/s10530-016-1301-4

Leishman, M. R. 2001. Does the seed size/number trade-off model determine plant community structure? An assessment of the model mechanisms and their generality. Oikos 93(2): 294-302. DOI: https://doi.org/10.1034/j.1600-0706.2001.930212.x

Longas, M. M., G. R. Chantre and M. R. Sabbatini. 2016. Soil nitrogen fertilisation as a maternal effect on *Buglossoides arvensis* seed germinability. Weed Research 56(6): 462-469. DOI: https://doi.org/10.1111/wre.12229

Milberg, P., L. Andersson and A. Noronha. 1996. Seed germination after short-duration light exposure: implications for the photo-control of weeds. Journal of Applied Ecology 33(6): 1469-1478. DOI: https://doi.org/10.2307/2404785

Milberg, P., L. Andersson and K. Thompson. 2000. Large-seeded spices are less dependent on light for germination than small-seeded ones. Seed Science Research 10(1): 99-104. DOI: https://doi.org/10.1017/S0960258500000118

Moles, A. T. and M. Westoby. 2006. Seed size and plant strategy across the whole life cycle. Oikos 113(1): 91-105. DOI: https://doi.org/10.1111/j.0030-1299.2006.14194.x

Murray, B. R., B. P. Kelaher, G. C. Hose and W. F. Figueira. 2005. A meta-analysis of the interspecific relationship between seed size and plant abundance within local communities. Oikos 110(1): 191-195. DOI: https://doi.org/10.1111/j.0030-1299.2005.13943.x

Ohadi, S., H. R. Mashhadi, R. Tavakkol-Afshari and M. B. Mesgaran. 2010. Modelling the effect of light intensity and duration of exposure on seed germination of *Phalaris minor* and *Poa annua*. Weed Research 50(3): 209-217. DOI: https://doi.org/10.1111/j.1365-3180.2010.00769.x

Onyekwelu, J. C., B. Stimm, R. Mosandl and J. A. Olusola. 2012. Effects of light intensities on seed germination and early growth of *Chrysophyllum albidum* and *Irvingia gabonensis* seedlings. Nigerian Journal of Forestry 42(2): 58-67.

Poorter, L. 2007. Are species adapted to their regeneration niche, adult niche, or both? The American Naturalist 169(4): 433-442. DOI: https://doi.org/10.1086/512045

Quero, J. L., L. Gómez-Aparicio, R. Zamora and F. T. Maestre. 2009. Shifts in the regeneration niche of an endangered tree (*Acer* *opalus* ssp. *granatense*) during ontogeny: Using an ecological concept for application. Basic and Applied Ecology 9(6): 635-644. DOI https://doi.org/10.1016/j.baae.2007.06.012

R Core Team. 2020. R: A language and environment for statistical computing, version 3.6.3. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/

Ranieri, B. D., F. F. Pezzini, K. S. Garcia, A. Chautems and M. G. C. França. 2012. Testing the regeneration niche hypothesis with Gesneriaceae (tribe Sinningiae) in Brazil: Implications for the conservation of rare species. Austral Ecology 37(1): 125-133. DOI: https://doi.org/10.1111/j.1442-9993.2011.02254.x

Ribeiro, L. C. and F. Borghetti. 2014. Comparative effects of desiccation, heat shock and high temperatures on seed germination of savanna and forest tree species. Austral Ecology 39(3): 267-278. DOI: https://doi.org/10.1111/aec.12076

Simão, E. and M. Takaki. 2008. Effect of light and temperature on seed germination in *Tibouchina mutabilis* (Vell.) Cogn. (Melastomataceae). Biota Neotroprica 8(2): 63-68. DOI: https://doi.org/10.1590/S1676-06032008000200006

Sõber, V. and S. Ramula. 2013. Seed number and environmental conditions do not explain seed size variability for the invasive herb *Lupinus polyphyllus*. Plant Ecology 214(6): 883-892. DOI: https://doi.org/10.1007/s11258-013-0216-8

Souza, M. L. and M. Fagundes. 2014. Seed size as key factor in germination and seedling development of *Copaifera langsdorffii* (Fabaceae). American Journal of Plant Sciences 5(17): 2566-2573. DOI: https://doi.org/10.4236/ajps.2014.517270

Souza, M. L. and M. Fagundes. 2017. Seed predation of *Copaifera langsdorffii* (Fabaceae): a tropical tree with supra-annual fruiting. Plant Species Biology 32(1): 66-73. DOI: https://doi.org/10.1111/1442-1984.12128

Souza, M. L., R. R. Solar and M. Fagundes. 2015a. Reproductive strategy of *Copaifera langsdorffii* (Fabaceae): more seeds or better seeds? Revista de Biología Tropical 63(4): 1161-1167.

Souza, M. L., D. P. Silva, L. B. Fantecelle and J. P. Lemos Filho. 2015b. Key factors affecting seed germination of *Copaifera langsdorffii*, a Neotropical tree. Acta Botanica Brasilica 29(4): 473-477. DOI: https://doi.org/10.1590/0102-33062015abb0084

Souza, A. D. G., O. J. Smiderle, V. M. Spinelli, R. O. D. Souza and V. J. Bianchi. 2016. Correlation of biometrical characteristics of fruit and seed with twinning and vigor of *Prunus persica* rootstocks. Journal of Seed Science 38(4): 322-328. DOI: https://doi.org/10.1590/2317-1545v38n4164650

Wang, H., B. Zhang, L. Dong and Y. Lou. 2016. Seed germination ecology of Catch weed Bedstraw (*Galium aparine*). Weed Science 64(4): 634-641. DOI: https://doi.org/10.1614/WS-D-15-00129.1

Warton, D. and F. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92(1): 3-10. DOI: https://doi.org/10.1890/10-0340.1

Yang, Z. and D. J. Midmore. 2005. Modeling plant resource allocation and growth partitioning in responses to environmental heterogeneity. Ecological Modelling 181(1): 59-77. DOI: https://doi.org/10.1016/j.ecolmodel.2004.06.023