

Article category: Notes and comments

Germination responses to light of four Neotropical forest tree species along an elevational gradient in the southern Central Andes

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Abstract

Seed germination is a key part of plants' life cycle and is mostly affected by the genetic background, the environmental conditions experienced by the mother plant and the seedbed conditions. The germination response to light is essential to optimize germination and seedling establishment in space and time. In addition, the germination response to light is a trait often related to the response of the seeds to their position in the soil (uncovered/buried). Here, we studied the germination response to light of four key tree species of the Yungas forest (*Anadenanthera colubrina*, *Enterolobium contortisiliquum*, *Jacaranda mimosifolia* and *Handroanthus impetiginosus*) sampled along an elevational and environmental gradient with contrasting vegetation cover and disturbance. Relative Light Germination (RLG) and mean germination time (MGT) were determined. Final germination was tested under cycles of light (8 h) and darkness (16 h) vs. complete darkness (24 h) and elevation, and MGT was tested as a function of elevation of the provenance. The RLG increased from smaller to larger-seeded species. The MGT of three of the studied species was affected by the elevation of the provenance. Complete darkness negatively affected final germination, while two species exhibited a significant interaction between the provenance and light. The variable germination responses to light along the elevational gradient highlights the influence of the environment on germination as a key factor that should be considered for forest management, conservation and restoration projects.

Keywords:

Seeds, regeneration, Yungas forest, mountain forest, subtropical tree species

Introduction

Seed germination is a stage of high-risk in the life cycle of plants species that leads to the establishment of new individuals in a population (Augspurger, 1984; Hoffmann et al., 2010; Kanegae, Braz, & Franco, 2000). Germination time and amount are affected by several aspects including the genetic background, the environmental conditions experienced by the mother plant during seed production (e.g. temperature, radiation and precipitation) and the seedbed conditions (e.g. temperature, light and soil moisture content; Baskin and Baskin, 2014; Carón et al., 2015; De Frenne et al., 2012; Karssen, 1970).

Light is an important factor determining germination of seeds on the seedbed. Seeds of some species only germinate in light (photoblastic positive), others only in darkness (photoblastic negative) and others are indifferent to light conditions (photoblastic indifferent) (Baskin & Baskin, 2014). However, the ultimate effect of light on seeds varies with the genotype and the dominant environmental factors during seeds' maturation, dormancy and germination itself (Pons, 2000). Therefore, seeds produced under contrasting environmental conditions can exhibit variable responses to light (Pons, 2000). Frequently, small seeded species in temperate biomes were reported as photoblastic positive while in other environments such as tropical and Mediterranean environments some studies point out the opposite relationship (Leishman, Wright, Moles, & Westoby, 2000; Metcalfe & Grubb, 1995, 1997; Milberg, Andersson, & Thompson, 2000). Consequently, it is important to interpret the response to light in the specific environment under study. The presence of light provides important information about the time and place for seedling establishment increasing the chances of successful germination and seedling establishment. For example, the presence/absence of light can be an indication of seeds buried in the soil vs at the soil surface which has a strong influence on the emergence above the soil and on the seedlings' early survival.

Knowledge about the response of seed germination to light in some forest types such as montane forest is especially limited (Baskin & Baskin, 2014). According to Baskin and Baskin (2014) the light requirements for germination of only 20 tropical montane tree species has been determined. However, such knowledge is of major importance because many forest types including tropical and subtropical montane forests are under strong pressure due to degradation, deforestation, and forest

fragmentation mainly caused by agricultural expansion (FAO & JRC, 2012; Gasparri, Grau, & Manghi, 2008). In South America, land-use change resulted in a loss of 4.3 million ha of forest per year between 2000 and 2005 (FAO & JRC, 2012). Land-use change is predicted to continue in tropical and temperate forests of Latin America, mainly caused by expanding agriculture and increasing demand of woody biomass and biofuel (Grau & Aide, 2008). In addition, selective logging is rising in the region. Therefore, larger gaps are created in the forest canopy. Simultaneously, the forest soil conditions are altered through changes in the amount of light that reaches the soil and by soil movements that modify the position of the seeds in the soil, i.e. seeds get buried (excluded from light) or uncovered (exposed to light).

In South America, one of the forest types most strongly affected by deforestation, fragmentation and selective logging is the Yungas' forest (Brown & Pacheco, 2006; Grau & Brown, 2000; Volante, Alcaraz-Segura, Mosciaro, Viglizzo, & Paruelo, 2012). This forest type, extending from Venezuela to Argentina (Brown & Kappelle, 2001) between 400 and 3000 m a.s.l., is one of the most biodiverse ecosystems of the continent (Brown & Kappelle, 2001) and is extremely important for the provisioning of essential ecosystem services, including watershed protection, production of wood and biofuel and recreation (Brown, 1995). The strong land-use change, forest degradation and fragmentation combined with the lack of knowledge on germination responses to light make the future dynamics and composition of this forest type difficult to predict. However, the deforestation and forest degradation pressures are highly variable along its elevational range. The areas at lower elevations are the most threatened due to their accessibility and less steep slopes. Therefore, the forest soil has a variable disturbance regime and the seeds of trees produced at lower elevations are more likely to be moved in the forest soil due to human trampling and machine manipulation resulting in a variable exposure of seeds to light. For this reason, studying the germination of important forest tree species of this highly threatened forest under contrasting light conditions is important to understand and forecast the composition of the forest under different management regimes and to develop restoration and conservation actions.

Here, we studied the variation in seed mass and germination response (germination amount and time) of four important, tree species of the Yungas forest, *Anadenanthera colubrina*, *Enterolobium contortisiliquum*, *Jacaranda mimosifolia* and

Handroanthus impetiginosus sampled along an elevational gradient with variable deforestation and forest degradation pressures under contrasting light conditions (cycles of light and darkness vs. complete darkness). We expect that the germination responses to light will vary among species and that the provenance of the seeds will influence the germination response of the species to light. A variable germination along the gradient is expected because the species response to light is known to be affected by the environmental conditions during ripening (Pons, 2000) which are highly variable along the gradient considered here. We also expect that the species with the heavier seeds will germinate better under complete darkness as recorded for many species around the globe (Metcalf & Grubb, 1995; Milberg et al., 2000).

Material and Methods

Four common and widespread distributed tree species (typical elements of Yungas but also frequent in the transition with Chaco) with potential high economic value were selected: *Jacaranda mimosifolia* (Bignoniaceae), *Handroanthus impetiginosus* (Bignoniaceae), *Enterolobium contortisiliquum* (Fabaceae) and *Anadenanthera colubrina* (Fabaceae). For each of these four species, we collected seeds in four or five provenances located along an elevational-climatic gradient from 349 to 1567 m a.s.l. (corresponding to a mean annual temperature gradient of 16.3 °C to 22.3 °C, Table 1). In each provenance, seeds from at least ten randomly selected dominant individuals were collected at seed maturity (between September - November of 2015 depending on the species; see Carón et al. 2018 for details). Immediately after collection the seeds were extracted from the fruits and the seeds were kept at room temperature and humidity in paper bags until the beginning of the experiment (November 2015). The seeds of all the species are considered orthodox therefore, it is unlikely the conservation conditions affected the experimental results.

Four replicates of 25 seeds per provenance and species were germinated at a constant temperature of 25 °C in a growth chamber at the National University of Salta. This temperature was chosen since this is the optimum temperature for germination of *Jacaranda mimosifolia*, *Handroanthus impetiginosus* and *Enterolobium contortisiliquum* and frequently used for germination trials of *Anadenanthera colubrina*; Santos Rego et al. 2011; Barboza Nogueira et al. 2014; Baskin and Baskin 2014), under cycles of 8/16 h of light/darkness (30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 400-700 nm of cool white fluorescent light) vs. complete darkness. The germination was conducted in petri-dishes of 9-cm diameter containing one disk of Whatman No. 1 filter paper moistened with distilled water. For the complete darkness treatment, the petri-dishes were wrapped in aluminium foil and placed in black polyethylene bags to avoid any exposure to light. The seeds germinated under cycles of light and darkness were counted and removed daily during 30 days while the seeds incubated under complete darkness were only checked at the end of the experiment (30 days after initiation). The emergence of the radicle was considered as 'germination'. Because the seeds of *Enterolobium contortisiliquum* have physical dormancy, seeds were mechanically scarified by making a small cut on the seed coat distal to the embryo prior to the experiment (Galíndez et al., 2010; Galíndez et al., 2016). For the

other species no dormancy was reported; therefore no pretreatment was applied (see Fredericksen et al. 2000; Socolowski and Takaki 2004; Baskin and Baskin 2014).

At the end of the experiment, the germination percentage under each light treatment was determined for petri-dish. Germination percentages recorded in light/darkness vs. in complete darkness were used to calculate an index of light requirement for germination referred as the “relative light germination” index (RLG; see Milberg et al. 2000) according to the following formula $RLG = GI / (Gd + GI)$; where GI is the germination percentage in light/darkness cycles and Gd the germination percentage in darkness. RLG ranges from 0 (germination only in darkness) to 1 (germination only in light/darkness cycles). Additionally, the mean germination time (MGT) was calculated (this was only done for the seeds incubated under cycles of light and darkness where germination was recorded daily), following the formula: $MGT = \sum DN / \sum N$; where D is the number of days counted from the date of sowing and N is the number of seeds germinated on day D (Ellis & Roberts, 1980).

The viability of the seeds was not checked at the end of the experiment. However, viability was tested in 30 seeds of the same seed lots before the experiment with Tetrazolium salt solution (The tetrazolium Subcommittee of the Association of Official Seed Analysts, 2000). After coloration (for approximately 24 h), the seeds were categorized, based on the pattern of coloration (The tetrazolium Subcommittee of the Association of Official Seed Analysts, 2000), into viable or non-viable (including seeds with necrotic tissue).

Data analysis

For each species separately, the proportion of germinated seeds under cycles of light and darkness vs. complete darkness (total amount of the four replicates of 25 seeds per provenance) was analysed using mixed effect models with binomial error distribution (glmer function, lme4 library in R Core Team 2017) with the standardized elevation of the provenance of the seeds (ratio between the provenance-specific value and the overall mean), the light treatment (cycles of light and darkness vs. complete darkness) and their interaction as explanatory factors and the provenance of origin as random factor. The mean germination time (MGT) was analysed with GLM with Gaussian error structure and with the standardized elevation of the

184 provenance of the seeds as explanatory factor. To fulfil normality and
185 homoscedasticity assumptions, the MGT was log transformed. For each variable
186 analysed, first the full model was fitted (all the fixed effects and their interactions),
187 after which models were simplified by dropping first the least significant interaction
188 and then the least significant individual variable at each step. The comparison
189 between models was based on the likelihood ratio test (LRT) for the binomial data
190 and on the scaled deviance for the Gaussian data, until all the remaining terms were
191 significant (Zuur, Ieno, Walker, Saveliev, & Smith, 2009).

Table 1: Population's characteristics, seed mass; seed mass class, germination in light, and relative light germination (RLG) of *Jacaranda mimosifolia*, *Handroanthus impetiginosus*, *Anadenanthera colubrina* and *Enterolobium contortisiliquum* from populations along an elevational gradient.

Species	Population (Code)	Elevation (m.a.s.l)	Temperature (MAT °C †)	Viability with tetrazolium test (%)	Seed mass (mg)	Seed mass class ‡	Germination in light (%)	RLG
<i>Jacaranda mimosifolia</i> (Bignoniaceae)	Orán	349	22.29	76.7	31.48 ± 0.49	Small	93 ± 2.564	0.59 ± 0.025
	Salta Capital	1230	16.68	66.7	12.03 ± 0.25		97 ± 1.714	0.50 ± 0.004
	Coronel Moldes	1247	17.19	76.7	14.52 ± 0.28		97 ± 1.714	0.51 ± 0.008
	La Caldera	1251	16.34	70	12.71 ± 0.23		96 ± 1.969	0.61 ± 0.038
	Rosario de Lerma	1567	15.90	66.7	15.19 ± 0.25		99 ± 1.000	0.55 ± 0.003
<i>Handroanthus impetiginosus</i> (Bignoniaceae)	Coronel Moldes	1130	17.99	43.3	100.10 ± 3.93	Intermediate	84 ± 3.685	0.73 ± 0.024
	Vaqueros	1265	16.58	26.7	164.75 ± 2.82		80 ± 4.020	0.70 ± 0.094
	Chicoana	1295	16.69	36.7	180.32 ± 8.40		64 ± 4.824	0.71 ± 0.078
	Salta Capital	1341	16.98	40	147.28 ± 4.67		52 ± 5.021	0.61 ± 0.019
<i>Anadenanthera colubrina</i> (Fabaceae)	Güemes	719.1	16.27	56.7	148.05 ± 3.20	Intermediate	51 ± 5.024	0.81 ± 0.016
	La Caldera	1254	16.34	33.3	144.44 ± 3.14		54 ± 5.009	1.00 ± 0.000
	Chicoana	1272	17.18	70	159.80 ± 2.39		98 ± 1.407	0.96 ± 0.014
	Salta Capital	1396	16.84	56.7	179.18 ± 3.97		77 ± 4.230	0.98 ± 0.019
<i>Enterolobium contortisiliquum</i> (Fabaceae)	Rosario de la Frontera	868.9	18.59	86.7	314.73 ± 5.60	Large	53 ± 5.016	1.00 ± 0.000
	Güemes	1027.05	19.28	30	384.90 ± 5.75		23 ± 4.230	1.00 ± 0.000
	Jujuy	1027.5	17.58	73.3	295.83 ± 4.07		51 ± 5.024	1.00 ± 0.000
	El Carril	1174	17.42	86.7	327.71 ± 5.11		78 ± 4.163	1.00 ± 0.000
	Lesser	1300.4	16.48	86.7	0.285 ± 0.005		60 ± 4.924	1.00 ± 0.000

†: Data extracted from Worldclim version 2.0 (Hijmans et al., 2005)

196 ‡ The seed mass classes were compared with a Kruskal-Wallis test and the categories were significantly different ($\chi^2 = 838.45$, $df = 2$, p-
197 value < 0.001)

Results

Germination under cycles of light and darkness vs. complete darkness

The germination of all species was negatively influenced by the absence of light compared to the germination under cycles of light and darkness (Table 2 and Fig. 1). This response was especially clear for the two Fabaceae species (*Anadenanthera* and *Enterolobium*), these species virtually did not germinate under complete darkness (Fig. 1). The elevation of the provenance of the seeds did not significantly affect the germination of any of the species (Table 2). However, the interaction between the elevation of provenance of the seeds and the light treatment (cycles of light and darkness vs. complete darkness) showed to be significant for *Handroanthus* and *Anadenanthera*. For *Handroanthus*, the difference between the germination recorded under cycles of light and darkness compared with the recorded under complete darkness decreased with increasing the elevation of the provenance of the seeds. On the other hand, the difference between the germination of *Anadenanthera* under cycles of light and darkness vs. complete darkness increased with the elevation of the provenance of the seeds (Table 2 and Fig. 1).

Relative light germination (RLG) increased from smaller-seeded species (*Jacaranda*) to intermediate (*Handroanthus* and *Anadenanthera*) to large-seeded species (*Enterolobium*) (Table 1).

Table 2: Seed germination of *Jacaranda mimosifolia*, *Handroanthus impetiginosus*, *Anadenanthera colubrina* and *Enterolobium contortisiliquum* as a function of the elevation of the provenance of the seeds and the light treatment

Species	Variable	LRT	P-value (Chi ² -test)
<i>Jacaranda mimosifolia</i>	Elevation	2.322	n.s.
	Light treatment (darkness)	107.741	<0.001 ***↓
	Elevation*Light treatment (darkness)	1.996	n.s.
<i>Handroanthus impetiginosus</i>	Elevation	3.305	n.s.
	Light treatment (darkness)	98.144	< 0.001 ***↓
	Elevation*Light treatment (darkness)	12.260	0.001 ***↑
<i>Anadenanthera colubrina</i>	Elevation	0.620	n.s.
	Light treatment (darkness)	445.930	<0.001 ***↓
	Elevation*Light treatment (darkness)	40.057	0.001 ***↓
<i>Enterolobium</i>	Elevation	0.970	n.s.

contortisiliquum

Light treatment (darkness)	486.71	<0.001 ***↓
Elevation*Light treatment (darkness)	4.708E-09	n.s.

LRT: likelihood ratio test. The direction of the effect is indicated by arrows: ↑ and ↓ correspond to an increase or decrease in germination, respectively. The significance codes are: n.s.: $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

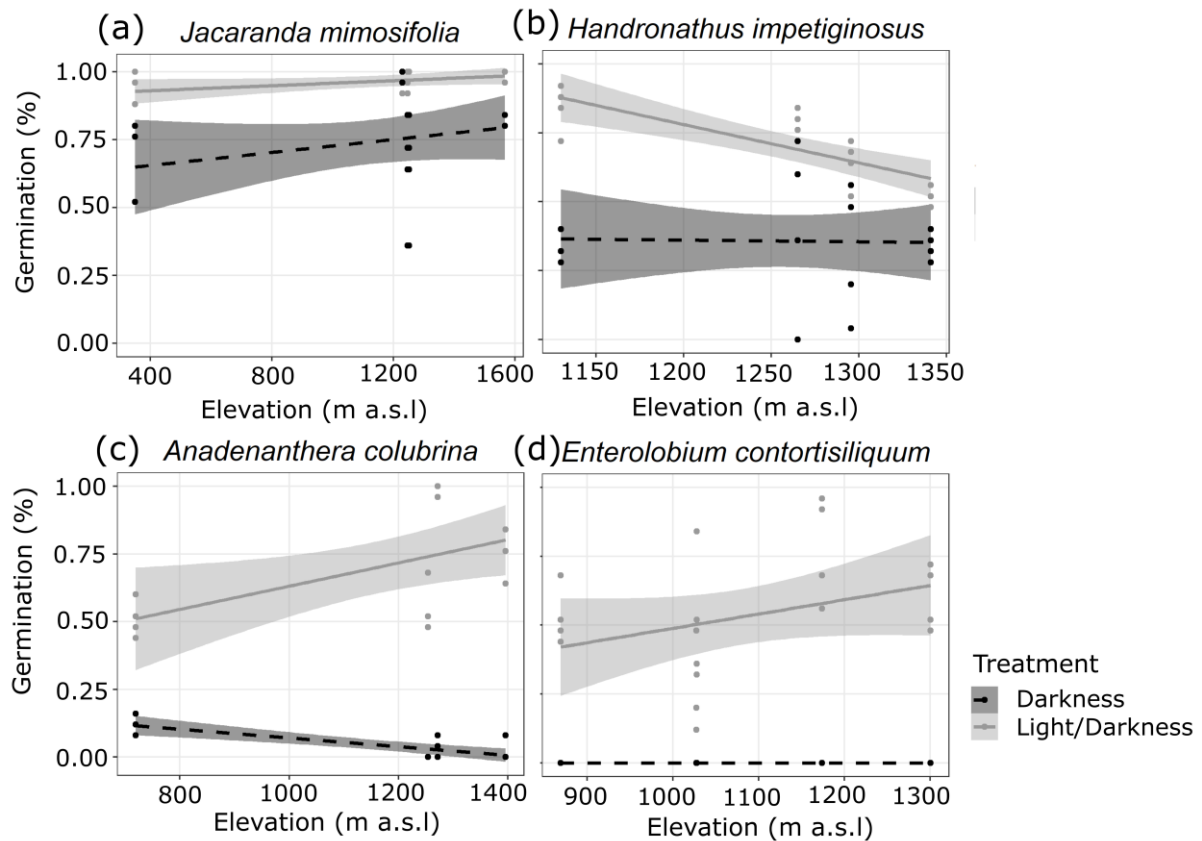


Figure 1: Seed germination of *Jacaranda mimosifolia* (a), *Handronathus impetiginosus* (b), *Anadenanthera colubrina* (c) and *Enterolobium contortisiliquum* (d) as a function of the elevation of provenance of the seeds and the light treatment (n= 4 replicates of 25 seeds per provenance under each treatment). The shaded areas indicate the 95% confidence interval.

Mean germination time

The mean germination time of *Handronathus* and *Anadenanthera* increased with the elevation of the provenance of the seeds, such that seeds from populations located at higher elevations germinated slower than seeds from populations located at lower

elevations. A decrease in germination time with increasing elevation of the provenance was observed in *Enterolobium* (Table 3).

Table 3: Mean germination time of *Jacaranda mimosifolia*, *Handroanthus impetiginosus*, *Anadenanthera colubrina* and *Enterolobium contortisiliquum* as a function of the elevation of the provenance of the seeds

Species	scaled dev.	P-value (Chi ² -test)
<i>Jacaranda mimosifolia</i>	1.775	n.s.
<i>Handroanthus impetiginosus</i>	7.678	0.006 **↑
<i>Anadenanthera colubrina</i>	4.381	0.036 *↑
<i>Enterolobium contortisiliquum</i>	5.867	0.015 *↓

scaled dev: scaled deviance. The direction of the effect is indicated by arrows: ↑ and ↓ correspond to an increase or decrease in mean germination time, respectively. The significance codes are: n.s.: $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Discussion

The four species studied here germinate significantly better under cycles of light and darkness compared to dark conditions. The germination of the two Fabaceae species was virtually zero under complete darkness, which was not expected because species of the Fabaceae family tend to germinate well in the dark regardless of their seed size (Fenner, 2006). Still, to confirm the results of this study, the germination of the two Fabaceae species should be analysed under different light and temperature regimes (including alternating temperatures) and under natural forest conditions. It is important to note that, despite the different results between germination percentages and viability, it is expected that the same proportion of viable seeds have been incubated under both light conditions and because of this, the results have not been affected.

The significance of the interaction between light and the elevation of the provenance of the seeds even though was negative for *Handroanthus* and positive for *Anadenanthera* evidence the relevance of the provenance on the response of the seeds to light. Previous studies reported that the effect of light on seeds depends on the genotype and on the environmental factors experienced during ripening of the seeds, during dormancy and during germination itself (Pons, 2000). Therefore, taking into account that along the elevational gradient the environmental conditions are highly variable (e.g. variable temperature and forest cover), it is likely that this variation in environmental conditions resulted in variable response to light. Moreover, Bewley and Black (1982) pointed out that that seed responses to light are a control of light over dormancy rather than over germination. It is known that the induction and release of dormancy are controlled by various regulators including plant hormones and dormancy proteins; the relative importance of these regulators is influenced by environmental factors during seed maturation and storage (Nee, Xiang, & Soppe, 2017). Considering that the storage conditions were identical for all the seed lots used in this experiment it is likely that the differences are caused by the environmental conditions. It is likely that *Anadenanthera* is more adapted to more open habitats with lower temperatures at higher elevations (relative to *Handroanthus*). On the other hand, the response of *Handroanthus* might be linked to other aspects such as the prevention of germination when exposed to very open conditions to avoid desiccation at the soil surface. Additionally, this variation in the

response to light as a function of the provenance might indicate that the ability of the species to colonize gaps might change not only with the life stage as indicated by Fenner (2000) but also with the provenance of the seeds. Traditionally, *Jacaranda*, *Enterolobium* and *Anadenanthera* are classified as pioneers while *Handroanthus* is a typical climax species. Our findings support the previous classification of *Handroanthus*, *Enerolobium* and *Anadenanthera* but not of *Jacaranda* since the latter germinated well under complete darkness (although this species also produces a high number of small seeds, a typical trait of pioneer tree species).

The mean germination time of all the species except *Jacaranda* varied as a function of the elevation of the provenance of the seeds. This might be linked to the variation in genetic and environmental characteristics at the provenance (Baskin & Baskin, 2014; Castro, Reich, Sánchez-Miranda, & Guerrero, 2008; Michael Fenner & Thompson, 2005) and with different competition pressures along the elevational gradient. For instance, higher competition at lower elevations may trigger faster germination. Additionally, the variable temperature along the elevational gradient might have an influence on the mean germination time. Lower temperatures at higher elevations might condition the seeds to germinate slower to avoid early germination and frost-induced seedling damage.

Finally, we found a positive relationship between the RLG index and the seed mass. This does not confirm to previous findings: species that produce heavier seeds tend to exhibit higher germination percentages and require less light for germination (Leishman et al., 2000; Milberg et al., 2000; Pons, 2000). The Fabaceae species studied here produce larger seeds and needed light to germinate while the two Bignoniaceae species produced lighter seeds that germinated in relative high percentages under complete darkness. However, results in line with our findings were reported in some vegetation types such as the Mediterranean region and tropical rainforests (Leishman et al., 2000; Metcalfe & Grubb, 1995, 1997; Milberg et al., 2000). Recent studies have shown that the relationship between seed mass and germination response to light is not as simple as previously proposed (Cartas 2014) and might change according to the environment. It is important to consider that the seed mass is not only important for its relationship with the germination response to light, but it is also important for other aspects of the species' ecology including the

species' strategy to deal with herbivory, seed water relation (Green & Juniper, 2004) and the competitive hierarchies between seedlings (Leishman et al., 2000).

In sum, we demonstrate a negative relationship between seed mass and the germination responses to light which can be an indication of a possible variable relationship between seed mass and germination responses to light in different environments. The germination of all the species studied here was negatively affected by complete darkness. The germination success of *Handroanthus* and *Anadenanthera* was influenced by the provenance of the seeds and this variation should be considered when trying to classify the species along the pioneer – climax continuum. However, further research on the germination of the tree species studied here under different light regimes and alternating temperatures in a growth chamber and in the field simulating different degrees of forest canopy cover and light qualities is needed to fully understand the seed germination ecology of these species and the position of the species along the pioneer – climax continuum.

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