



Liana and vine germination requirements in a subtropical forest zone

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ABSTRACT

Climbing plants may be classified as lianas (woody) or vines (herbaceous). This grouping is not taxonomic but is useful for functional ecology. We evaluated seed germination of three liana and four vine species from the Chinese subtropical forest zone. Seeds were collected from different environments (abandoned agricultural land, broadleaf forest, coniferous mixed forest) and seed traits characterized (seed fresh mass, seed shape index, water gain). Germination tests were conducted under different temperature regimes: 5/10, 10/20, 20/30, 25/35, 35/40 °C under light and dark conditions. Additionally, we tested the relationships of germination responses (germination percentage, mean germination times, and relative light-germination index) with seed traits. The two species from abandoned agricultural land (*Glycine max* subsp. *soja* and *Ipomoea triloba*) had impermeable seed coats and reached only intermediate values of germination percentage, from 20 to 40% in the tested temperatures, with little effect from light regimes. In the broadleaf forest, *Ipomoea nil* also had impermeable seeds, but reached 91% of germination under 25/35 °C. Seeds of *I. nil* were the largest ones (4.8 g per 100 seeds), and there was a positive relationship of seed mass with G%. Seeds of *Kadsura longipedunculata* (liana) had low G% under all tested temperatures, probably related to some dormancy type, which requires further investigation. Species from coniferous mixed forest were greatly affected by temperature regimes, and *Coptosapelta diffusa* (the smallest seeds) had also the most light-dependent germination. Light-germination index had thus a significant/negative relationship with seeds mass. Environmental factors may help to explain the distribution of seed traits in our study, but further research should examine a larger set of species to better understand regeneration ecology of climbing plants in contrasting ecosystems.

1. Introduction

Lianas and vines are climbing plants with woody and herbaceous stems, respectively, but such categorization is unrelated to species taxonomy and phylogenetic relationship. Both groups depend on neighboring plants as external support and play ecological roles in forest ecosystems (Schnitzer and Bongers, 2002; Muoghalu and Okeesan, 2005; Schnitzer, 2018). For instance, lianas compete directly with trees for light, water and nutrients, impacting tree growth and survival and reducing their reproductive success (Wright et al., 2005, 2015; Schnitzer and Carson, 2010; Jones et al., 2017). Morphological plasticity and biomass allocation to branches may be ecological strategies to forage for support, while the supported plants tend to be greater/taller in length (Putz, 1984; den Dobbelen and Oosterbeek, 1995). Vines have been reported to show similar distribution patterns regardless of rainfall

gradients in China (Guo et al., 2012), while liana abundance might be favored by increasing drought frequency, as reported in Amazonian forests, the West African zone and across the isthmus of Panama (Phillips et al., 2002; Swaine and Grace, 2007; Parolari et al., 2019).

Hence, climbing species may traverse multiple habitats from the shaded understory to the upper canopy, thus facing multiple microclimatic conditions. Usually, plants in open habitats face high light intensity and higher daily temperature fluctuations when compared to plants in closed habitats (ten Brink et al., 2013). Heterogeneity of light and temperature within a habitat is thus an important driver of germination strategies (Fenner and Thompson, 2005; ten Brink and Bruun, 2011). Therefore, knowledge of germination parameters is useful for understanding how anthropogenic influences on forest environments may change species composition over time, by influencing recruitment and community assembly. Germination is one of the most sensitive life

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stages, being strongly influenced by light regimes, moisture availability and environmental temperatures (Baskin and Baskin, 2014; Bhatt et al., 2020a). Moreover, temperature is a major environmental factor determining germination timing, signaling seeds to identify the beginning of a favorable season for seedling establishment (Probert, 2000; Baskin and Baskin, 2014). The regeneration ecology of climbing species remains poorly researched, though some studies have been conducted regarding germination patterns and seedling recruitment in tropical vegetation (Sanches and Válio, 2002; Gerwing, 2004; Dupuy and Chazdon, 2006; Schnitzer and Carson, 2010; Roeder et al., 2013).

Forest cover changes the solar radiation that reaches the forest ground (Hutchison and Matt, 1977); thus, canopy gaps provide microhabitat conditions – such as increased light availability and temperature fluctuations – for recruiting climbers and early-succession trees (Dupuy and Chazdon, 2006). Because light availability is heterogeneous in the understory, it may drive the sites where germination occurs (Pons, 2000; Fenner and Thompson, 2005). Light availability then affects seedling establishment and growth (Ricard et al., 2003; Plue et al., 2013; Lin et al., 2014), varying broadly with forest type, season, and disturbance gradient (Frelich, 2002; Tripathi et al., 2020). On the other hand, seed responses to light can be influenced by seed traits (Milberg et al., 2000; Flores et al., 2011). Smaller seeds usually have light-dependent germination, related to forest gaps, while the larger ones can germinate under the shaded forest canopy (Pearson et al., 2002), usually displaying more resources to compete in an environment where light is limited. Although climbing plants have been used to test ecological theories (Schnitzer, 2018), biological aspects of climbing species have been neglected compared to trees, and current knowledge of liana and vine germination requirements appears to be limited in the subtropical Chinese forest zone.

In the present study, we aimed to evaluate seed germination responses to different temperature regimes and light conditions of seven climbing species (three lianas and four vines) from the subtropical forest zone of China. Specifically, we addressed: (i) seed traits – such as seed fresh mass, seed shape and water absorption – to characterize seed morphophysiological features according to their environment of occurrence; (ii) germination parameters – germination percentage (G%) and mean germination time (MGT) – under a range of temperature and light conditions; (iii) the role of seed traits in driving relative light-germination patterns (RLG index). We expected germination requirements and seed functional traits to reflect differences in environmental factors across contrasting habitats including regenerating areas and mixed forests. Because moisture availability is not a constraint for the subtropical monsoonal climate in this subtropical area (Kang et al., 2017), we expected that germination would be mostly limited by temperature, which varies greatly with season, while light dependence could be driven by seed traits (Milberg et al., 2000; Flores et al., 2011). Understanding temperature and light effects on germination is useful for assessing the impact of anthropogenic change on the population distribution of climbing species in forest ecosystems.

2. Material and methods

2.1. Seed collection

Seeds of the three liana (all perennial) and four vine species (three annuals and one perennial, see Table 1) were collected from three distinct environments, according to their distribution. All three sites are located in a subtropical zone characterized by monsoonal climate type, embracing mixed forest formations around the city of Lushan and the neighboring area of the Jiangxi province, China. Seeds were collected in 2020 from naturally occurring populations at the time of their natural fruit ripening and dispersal (Table 1). Seeds of *Glycine max* subsp. *soja* (hereafter named *G. soja*) and *Ipomoea triloba* were collected from abandoned agricultural land, forming an open vegetation left to naturally regrow in the past many years. *Ipomoea nil* and *Kadsura*

Table 1
Collection details of the studied species.

Environment	Species	Family	Collection date/ 2020	Locality	Lat (N)	Long (E)	Altitude (m asl)	Habit	Microhabitat	Seed color	Seed shape
Abandoned agricultural land	<i>Glycine max</i> subsp. <i>soja</i> (Siebold & Zucc.) H. Ohashi	Fabaceae	November	Gutang	29° 40' 13"	116° 5' 34"	14	Annual vine	Open area	Brown/black	Elliptic
	<i>Ipomoea triloba</i> L.	Convolvulaceae	October	Minshan	29° 29' 43"	115° 53' 16"	98	Annual vine	Open area	Black	Trigonous
Broadleaf forest	<i>Ipomoea nil</i> (L.) Roth	Convolvulaceae	October	Saiyang	29° 31' 56"	115° 53' 44"	135	Annual vine	Forest	Black	Ovoid/trigonous
	<i>Kadsura longipedunculata</i> Finet & Gagnep.	Schisandraceae	October	Halhui	29° 33' 34"	116° 2' 25"	308	Perennial liana	Forest	Grey	Reniform
Coniferous mixed forest	<i>Clematis apiifolia</i> var. <i>argenticulicida</i> (H. Lévl. & Vaniot) W. T. Wang	Ranunculaceae	November	Guling	29° 33' 38"	116° 0' 51"	963	Perennial liana	Rocky slope	Black	Fusiform
	<i>Coprosopelta diffusa</i> (Champ. ex Benth.) Steenis	Rubiaceae	January	Bailu	29° 31' 01"	116° 0' 24"	155	Perennial liana	Forest	Dark-brown	Compressed
	<i>Cynanchum auriculatum</i> Royle ex Wight	Asclepiadaceae	November	Guling	29° 34' 52"	115° 59' 37"	1112	Perennial vine	Open area	Brown	Ovate/fusiform

longipedunculata seeds were collected from a broadleaf forest formation. *Clematis apiifolia* var. *argentilucida* (hereafter named *C. apiifolia*), *Coprosopelta diffusa* and *Cynanchum auriculatum* were collected from a coniferous mixed forest. Approximately 30–35 plants of each species were randomly sampled to represent the genetic diversity of their respective populations. After collection, seeds were stored under laboratory conditions, cleaned and tested for germination within two weeks.

2.2. Seed morphological traits

A Stereo Microscope (Nikon SMZ800N) fitted with a microscope camera IMG-SC600C was used to examine seed morphology, dimensions (length, width, and height), and color. The seed shape index was obtained as the ratio of the three dimensions to the length, and calculating the variance among them (Thompson et al., 1993) on 15 individual seeds per species. Seed fresh mass was determined at the time of collection from three 100-seed replicates per species, using a Sartorius electronic balance (Sartorius Co., Goettingen, Germany).

2.3. Seed permeability

Water absorption (imbibition) was assessed by recording the fresh mass of three 100-seed replicates before and after placing them in moistened (10 ml distilled water) 9 cm-diameter Petri dishes with one sheet of Whatman No. 1 filter paper for 24 h at room temperature (22 ± 2 °C). Water gain (%) was calculated as the proportional increase in fresh weight in relation to seed fresh mass (Baskin et al., 2004).

2.4. Effect of temperature regime and light

Seed surfaces were sterilized to avoid fungus attack by soaking in 0.50% sodium hypochlorite for 1 min, then washing thrice with deionized water. Seeds were placed in incubators (Kesheng incubators, Model-DRX-800C-LED, China) at five temperature regimes (5/10, 10/20, 20/30, 25/35 and 35/40 °C night/day temperatures) and two light regimes (constant darkness or 12 h light per day, hereafter referred to as dark and light treatments, respectively). Lighting of $60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was provided via cool-white-fluorescent tubes, and light exposure coincided temporally with the higher temperatures. Temperature regimes were selected to simulate the average night/day low-altitude temperature for Dec-Feb (5/10 °C), Mar-Apr, Oct-Nov (10/20 °C), May-Jun, Sep (20/30 °C) and Jul-Aug (25/35 °C). The warmest temperature regime (35/40 °C) was applied to investigate seed germination tolerance to climate change.

For incubation, seeds were placed in 9 cm petri dishes layered with three disks of Whatman No. 1 filter paper moistened with 10 mL distilled water. The dark treatment was achieved by wrapping Petri dishes in two layers of aluminum foil. Four 25-seed replicates were used for each treatment (7 species, 5 temperature regimes, 2 light regimes). Seeds were classified as germinated when observed with an emerged radicle of ≥ 2 mm. Germinated seeds were counted and removed daily from the light treatments for 30 days, but seeds in the dark treatments were counted once on day 30. After completion, ungerminated seeds from the light treatment were dissected to evaluate the embryo status and viabilities of the non-germinated seeds (living and therefore white; turgid and brown and therefore dead) under a stereoscope.

2.5. Data analysis

Seed traits (seed fresh mass, seed shape index, water gain) were compared among the study species using linear models (LMs). Germination percentage (G%) was tested as a function of habit, temperature and light regimes (and interactions) using generalized linear mixed effect models (GLMMs) with a binomial distribution and species as a random effect (Zuur et al., 2009), firstly across all environments. The effect of temperature and light regimes were also evaluated for each

environment (vegetation type) separately. Additionally, G% was separately evaluated within each species as a function of temperature and light interactions. The GLM and LM analyses were conducted using *lme4* and *nlme* packages (Bates et al., 2015; Pinheiro et al., 2021) in R software (Core Team, 2020). Multiple comparisons were performed among treatment combinations using post-hoc Tukey tests performed with the *emmeans* package (Lenth et al., 2022). Log-transformed mean germination times (MGT, days) were compared among temperature regimes within species using a linear model, but only for seeds in the light treatments.

Germination parameters (G% and MGT) were then evaluated as a function of seed traits: seed fresh mass and seed shape index, separately under each temperature regime. G% was compared using a GLMMs with binomial distribution and species as a random effect. Values of log-transformed MGT were evaluated as a function of the same seed traits, under each temperature regime, using linear mixed effect models (LMMs). Regarding the effect of light on G%, we calculated the relative light-germination index (RLG), by dividing G% in the light treatment by the sum of G% in the light + dark treatment (Milberg et al., 2000). The RLG index varies from zero (seeds only germinated in the dark) to 1 (seeds only germinated in the light); a value of 0.5 indicates seed germination independent of light/dark regimes. RLG was evaluated as a function of log-transformed seed fresh mass and shape index using LMMs. To do so, we pooled RLG data from three temperature regimes (10/20, 20/30, 25/35 °C); the coolest and warmest conditions were excluded due to low G%. Species and temperature regimes were considered as random effects in the analyses.

3. Results

3.1. Seed traits

The studied species had small seeds, with mean fresh seed mass (for 100 seeds) of 1.2 g in the abandoned agricultural land and varying from 0.03 to 0.5 g in the coniferous mixed forest (Fig. 1a). In the broadleaf forest, seeds of *I. nil* (vine) and *K. pedunculata* (liana) showed comparatively larger seeds, with 4.8 and 2.2 g for 100 seeds, on average, respectively. Regarding seed shape, seeds from abandoned agricultural land and broadleaf forest were relatively rounder (shape index from 0.03 to 0.06) compared to the coniferous mixed forest (shape index >0.15 ; Fig. 1b), where seed morphology tended to be fusiform or compressed (Table 2). Seed imbibition (water gain, %) reached the greatest values in seeds from coniferous mixed forest, from 40 to 83%, while *G. soja* and both *Ipomoea* species had a lower imbibition, from 8 to 10% (Fig. 1c).

3.2. Temperature and light

Temperature regime significantly affected germination of all species, and interactions that included temperature were also significant (Table 2). This influence was weaker for species on abandoned agricultural land than other environments but remained significant (Table 2).

Seeds of *G. soja* and *I. triloba* (from abandoned agricultural land, both vines) presented a G% varying from 6 to 43% irrespective of temperature and light regimes (Fig. 2). Light regime affected germination of *I. nil* seeds (broadleaf forest, vine), significantly increasing G% in the light compared to dark treatments. Additionally, G% of *I. nil* seeds reached 91 and 74%, respectively, under 25/35 and 35/40 °C (Fig. 2). Seeds of *K. longipedunculata* (broadleaf forest, liana) had the lowest G%, reaching only 20% at 25/35 °C in the light, and germination $\leq 8\%$ under all other conditions (Fig. 2), indicating the presence of dormancy, given the seeds presented intact embryos by the end of the trials (Supplementary material, Table S1).

In the coniferous mixed forest, *C. apiifolia* seeds (liana) germinated from 74 to 89% at 10/20 and 20/30 °C, under both light and dark

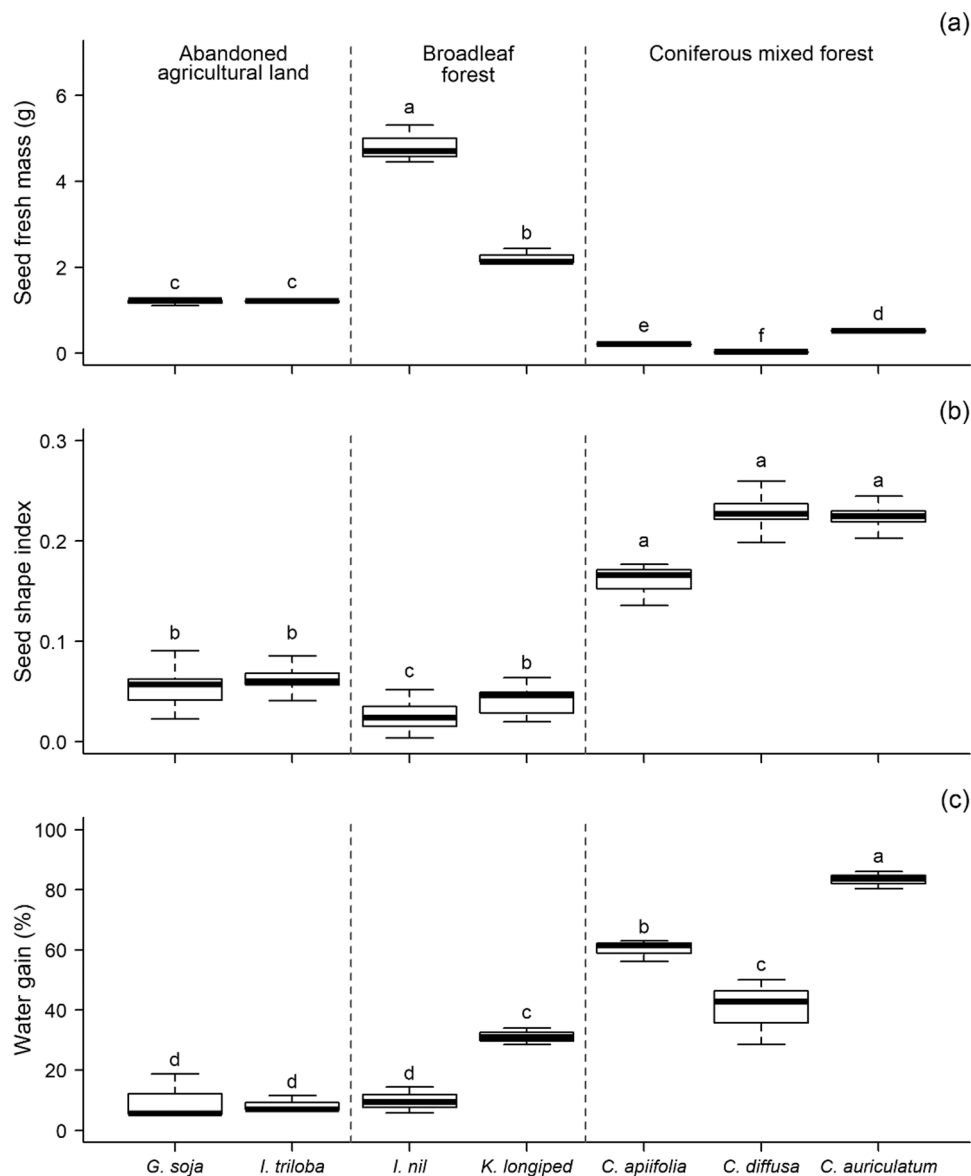


Fig. 1. Seed fresh mass for 100 seeds, seed shape index, and water gain of the studied species from different environments and habits. Lower case letters indicate significant differences ($P \leq 0.05$; Tukey's test). For collection details and information on species habit/growth form, check [Table 1](#).

treatments, but decreased to 39% at 25/35 °C (in the light), with a germination $\leq 2\%$ in the other treatments ([Fig. 2](#)). G% of *C. diffusa* (liana) was stimulated in the light treatments, varying from 38 to 57% at 20/30, 25/35, and 35/40 °C, with a germination $\leq 5\%$ under cooler treatments and dark conditions ([Fig. 2](#)). Germination of *C. auriculatum* (vine) was also enhanced by light, reaching highest G% (60 to 80%) under 20/30 and 25/35 °C ([Fig. 2](#)).

Mean germination times were affected by temperature regimes in all species ([Table 3](#)). For *G. soja* seeds, germination occurred within 8 to 13 days, except at the coolest temperature (5/10 °C = 20 days; [Table 3](#)). Seeds of *I. triloba* mostly germinated within 8 to 12 days, with a decrease to 6 days (faster germination) under 20/30 °C ([Table 3](#)). For *I. nil* seeds, germination lasted from 11 to 15 days to occur, irrespective of temperature regime. Most MGT values could not be determined for *K. longipedunculata* seeds in the tested temperatures due to low G%. MGT varied from 17 to 20 days in *C. apiifolia* seeds, and from 11 to 22 days in *C. diffusa* (which had the fastest germination, of 11 to 12 days, in the warmest conditions). *C. auriculatum* seeds also had faster MGT in warmer temperature regimes, varying from 5 to 7 days at 20/30, 25/35, and 35/40 °C, while 10/20 °C delayed germination to 17 days ([Table 3](#)).

3.3. Relationship of germination parameters with seed traits

Little to no relation was found regarding seed traits (seed fresh mass and seed shape index) with germination percentage ([Table 4](#)). The exception was under the temperature regime of 25/35 °C, where a positive correlation suggested that larger seeds may show higher G%, as is the case of *I. nil* seeds (relatively large seeds that reach their highest G% under this temperature). Seed shape index also seem to influence on G% under the temperature regime of 25/35 °C ([Table 4](#)), where more flattened/compressed seeds (such as *C. diffusa* and *C. auriculatum*) reached some of their highest G%, ~60%.

The RLG index was significantly influenced by seed fresh mass, with smaller seeds tending to be more light-dependent compared to the larger ones ($P = 0.016$; [Fig. 3a](#)). In contrast, no relationship was found regarding RLG and seed shape index ([Fig. 3b](#)).

4. Discussion

There was a considerable variation in seed traits across the studied environments, and a species-specific variation in the temperature

Table 2

Seed germination as a function of habit (lianas vs. vines) and treatments (temperature regime, light conditions and their interactions) using a generalized linear mixed model with binomial distribution for all the studied environment (= seven species). The effect of treatments and their interactions was also tested within each environment separately (abandoned agricultural land, broadleaf forest, coniferous mixed forest). n.s = non-significant ($P > 0.05$)

Environment	Treatments	Estimate	s.e	P-value
All	Intercept	-2.130	0.487	–
	Habit (H)	0.502	0.639	n.s
	Temperature (T)	0.032	0.006	<0.001
	Light (L)	0.214	0.249	n.s
	H x T	0.015	0.008	0.049
	H x L	0.026	0.304	n.s
	T x L	-0.053	0.010	<0.001
	H x T x L	0.025	0.012	0.040
Abandoned agricultural land	Intercept	-1.410	0.179	–
	Temperature (T)	0.015	0.007	0.025
	Light (L)	0.117	0.252	n.s
	T x L	-0.007	0.010	n.s
Broadleaf forest	Intercept	-3.628	1.202	–
	Temperature (T)	0.093	0.009	<0.001
	Light (L)	0.457	0.331	n.s
	T x L	-0.055	0.013	<0.001
Coniferous mixed forest	Intercept	-1.434	0.377	–
	Temperature (T)	0.039	0.005	<0.001
	Light (L)	0.209	0.205	n.s
	T x L	-0.048	0.008	<0.001

requirements for germination of lianas and vines. Environmental factors drive regeneration traits, such as seed dispersal, formation of a soil seed bank, germination, seedling emergence, establishment, growth and fitness (Metzner et al., 2017; Saatkamp et al., 2019). Seeds of *I. nil* (vine) and *K. longipedunculata* (liana), for instance, were found in the understory of broadleaf forests, where light availability is considerably lower. Both species displayed larger and ovate seeds, irrespective of the growth form, suggesting that the environment could work as an ecological filter driving seed traits in the subtropical forest zone. Larger seeds typically display more resources to warrant seedling growth through the litter layer above the soil seed banks (Lönnberg and Eriksson, 2013), also providing nutrients for the establishing seedling before it becomes a self-reliant plant (Salisbury, 1974; Mazer, 1989).

In contrast, species growing in the coniferous mixed forest had the lowest seed masses, from 0.03 to 0.5 g for 100 seeds. Higher light penetration in the forest understory and less litter on the forest floor (personal observation) could be linked to the lower seed mass and light-dependent germination (see Milberg et al. 2000), as observed for *C. diffusa* seeds. Despite their small seed masses, seeds of *C. apiifolia* and *C. auriculatum* were still able to germinate from 60 to 80% in the dark, depending on temperature regime, but achieving significantly higher G% in the light treatments under optimal conditions. The limitation to seedling establishment of smaller seeds could also be related to gap size and increasing drought in forest edges. Larger seeds of neotropical pioneer species have been reported to germinate under lower water potentials, which may lead them to take more risks for recruitment of the emerging seedlings in large gaps (Daws et al., 2008). Conversely, even though water stress might inhibit germination, many small-seeded species could show a relatively high germination recovery with the onset of favorable conditions, as recently shown for a Chinese wetland (Bhatt et al., 2022). Once germination occurs, gap size could still hamper the establishment of climbing plants due to the lack of neighboring support plants in larger clearings. Notwithstanding, seeds of many climbers seem to show biomechanical features to keep them upright before requiring support to grow, and morphological changings (investment in number of searcher shoots, for instance) can be related to strategies for foraging in order to ascend towards the forest canopy (Putz and Holbrook, 1991; den Dubbelden and Oosterbeek, 1995).

Our observed patterns of light requirements for germination

indicated that the small seeds of *C. diffusa* were the most light-dependent (positive photoblastic), while all other species were able to germinate equally well under both light and dark in at least one of the tested temperatures. However, seeds of most species had a significant enhancement in G% in the light treatments, except the vines from open habitats (*G. soja* and *I. triloba*) whose G% was mostly not affected by photoperiod. Interactions of light and temperature can regulate G%, mostly under the warmer temperature regimes (Pons, 2000). For instance, seeds of *C. apiifolia* and *C. auriculatum* behaved as light-dependent respectively under 25/35 and 35/40 °C, therefore requiring light only at certain temperature regimes. Usually, light requirement during germination is linked to the seed size (see Milberg et al. 2000, Flores et al. 2011), given small seeds need to avoid germination under dark conditions (e.g. belowground) due to fewer reserves that cannot support seedling emergence from the deeper layers of the soil. Hence, we have found a significant relationship between seed mass and light requirement (RLG index, Fig. 3). Nevertheless, previous studies reported that factors other than the seed size such as phylogeny, plant size, plant growth form and life form also play an important role in determining the light requirement during germination (de Villiers et al., 2002; Fenner and Thompson, 2005; Flores et al., 2011; 2016; Cheib and Garcia, 2012).

Concerning the species' life histories, all three species from coniferous mixed forest (*C. apiifolia*, *C. diffusa*, and *C. auriculatum*) showed a perennial life cycle, therefore running fewer risks to the population of a failed germination season as compared to annual species, because they can produce multiple batches of seeds within a life cycle and, therefore, may allocate less resources to seed size. Annual vines from abandoned agricultural land (*G. soja* and *I. triloba*) displayed a relatively larger seed fresh mass (~1.2 g for 100 seeds) compared to the coniferous mixed forest, suggesting that species from open habitats may not necessarily produce small seeds (Foster and Janson, 1985; Mazer, 1989). Species growing in open habitats have a high probability of perceiving shade from neighboring herbaceous vegetation (ten Brink and Bruun, 2011). Hence, producing relatively larger seeds could be advantageous for annual species to provide enough resources for fast seedling growth to catch up with or overtop their neighbors.

Highest germination percentages varied from species to species, depending on the tested temperature regimes. Three study species – *I. nil*, *C. apiifolia*, and *C. auriculatum* – reached germination percentages higher than 70 and up to 90% in at least one of the tested temperatures. In other three cases (*C. diffusa*, *I. triloba*, and *G. soja*) the species showed intermediate levels of germination, from 40 to ~60%; seeds of *K. longipedunculata* had the lowest germination percentages (around 20%). Despite G% values, three species showed low imbibition in the permeability tests (*G. soja* and both *Ipomoea* species). Seeds can be considered water-impermeable when seed fresh mass increases less than 20% after soaking in water for a 24 h period (Baskin and Baskin, 2003; Baskin et al., 2006). This indicates the presence of physical dormancy, caused by impermeable layers of palisade cells in the seed coat (Baskin et al., 2000; Baskin and Baskin, 2014; Smýkal et al., 2014). *Glycine* seeds, for instance, usually show physical dormancy related to the palisade layer of the seed coats and the presence of phenolic compounds (Ma et al., 2004; Xu et al., 2009; Zhou et al., 2010). Permeability of the seed coats may also vary with subspecies and species varieties, given seeds of the soybean wild type (*G. soja*) are mostly impermeable, while the cultivated soybean (*G. max*) has been selected for permeable seeds (Qutob et al., 2008; Chandra et al., 2017; Kumar et al., 2019).

Previous studies reported that *Ipomoea* spp. show impermeable seed coats, requiring scarification or alternating temperatures to germinate (see Jayasuriya et al. 2007, Chauhan and Abughho 2012). We show that *I. nil* seeds germinated up to 91% under 25/35 °C, and 74% at 35/40 °C, with a significant enhance in G% caused by warmer temperature fluctuations, as reported for some legume trees from Brazilian tropical forests (see de Paula et al. 2012, Geisler et al. 2017). The same pattern has been found in the literature for *Ipomoea lacunosa* seeds at 20/35 °C,

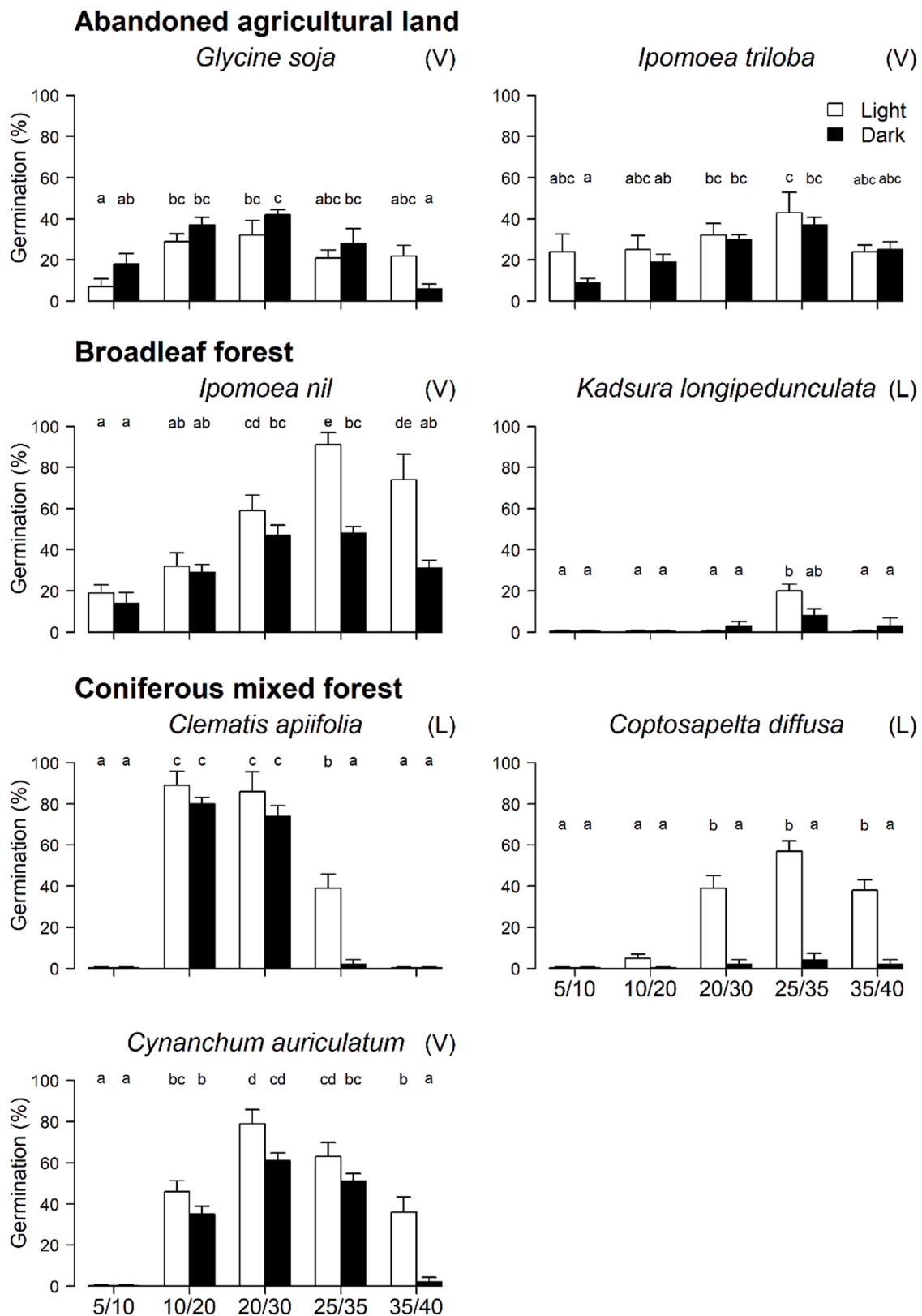


Fig. 2. Germination percentage (means \pm SD) of the studied species, grouped by their respective environments, related to night/day temperatures and light/dark regimes. L = liana; V = vine. Lower case letters indicate significant differences ($P \leq 0.05$; HSD Tukey's test).

showing that seed coat permeability is controlled by the opening of water gap structures that may be disrupted by specific temperature regimes, allowing imbibition to occur (Jayasuriya et al., 2007). Yet, seeds of *G. soja* and *I. triloba* reached intermediate G% (~20 to 40%) under all

tested temperatures, therefore showing at least a fraction of non-dormant (permeable) seeds in their populations. Temperature regime may not be the most limiting factor for germination of vines from open habitats, but the presence of dormancy seems to control the

Table 3

Species' mean germination times (days; mean \pm SD) under the light treatments and alternating temperature regimes. Letters indicate significant differences among temperature regimes ($P < 0.05$; Tukey's multiple range test). Dashes indicate insufficient data due to low germination percentage. V = vine; L = liana.

Species	Temperature (night/day)				
	5/ 10 °C	10/ 20 °C	20/ 30 °C	25/ 35 °C	35/ 40 °C
<i>Glycine soja</i> (V)	20 \pm 2 a	13 \pm 2 b	10 \pm 1 bc	8 \pm 2 c	11 \pm 2 bc
<i>Ipomoea triloba</i> (V)	12 \pm 1 a	8 \pm 2 ab	6 \pm 1 b	8 \pm 2 ab	9 \pm 2 a
<i>Ipomoea nil</i> (V)	15 \pm 1 a	11 \pm 1 b	13 \pm 3 ab	13 \pm 1 ab	13 \pm 1 ab
<i>Kadsura longipedunculata</i> (L)	–	–	–	20 \pm 0.4	–
<i>Clematis apiifolia</i> (L)	–	17 \pm 0.2 b	18 \pm 1 b	20 \pm 1 a	–
<i>Coptosapelta diffusa</i> (L)	–	22 \pm 1 a	15 \pm 1 b	12 \pm 1 c	11 \pm 1 c
<i>Cynanchum auriculatum</i> (V)	–	17 \pm 2 a	5 \pm 0.2 c	6 \pm 2 bc	7 \pm 1 b

proportion of seeds able to germinate at a time.

All other species have shown water gain values from 31 to 83%, thus showing permeable seed coats. Although *K. longipedunculata* produces permeable seeds, their low G% probably indicates the presence of some other type of dormancy, such as physiological restraints (see Baskin and Baskin 2004). Because the dissected embryos from the germination tests were found to be intact (see Supplementary material, Table S1), we argue that the lack of germination in *K. longipedunculata* can be ascribed to seed dormancy rather than viability loss of the freshly-harvested seeds. Moreover, dormancy type can be associated to phylogenetic relatedness in ancient groups of plants, such as Schisandraceae (Austrobaileyales). Morphophysiological dormancy has been described in early-divergent clades, as *Trithuria* (Hydatellaceae, see Tuckett et al. 2010), and in a close related Chinese species of *Schisandra* in the ANA grade (Chien et al., 2011). Further analysis of the embryo size and growth (or a move-along experiment, see Baskin and Baskin 2003) would be required to fully understand the presence and type of dormancy in *K. longipedunculata* seeds. Other basal clades, including Magnoliaceae and Annonaceae, have also been reported to show morphological (or morpho-physiological) dormancy, related to embryo growth and development (Corral-Aguirre and Sánchez-Velásquez, 2006; da Silva et al., 2007).

Previous studies have found that germination requirements can be species-specific, and strongly influenced by seasonal changes in the local temperature patterns (Yi et al., 2019; Bhatt et al., 2020b). Such

interspecific variation in thermal requirements for germination might be important to avoid germination under unfavorable conditions. Hence, our results show that temperature regimes significantly affected germination responses of species from the coniferous mixed-forest species. Seeds of *C. auriculatum* reached maximal germination at 20/30 °C, while seeds of *C. apiifolia* germinated relatively well at both 10/20 and 20/30 °C. Such results indicate that seeds of these species may achieve their highest germination levels under moderate temperatures, which could be found right after dispersal (November), in the autumn, but then subjecting the recently-emerged seedlings to the imminent winter. Alternatively, seeds incorporated into the soil seed banks could probably survive until moderate temperature conditions to arise again. In contrast, *C. diffusa* seeds germinated better at the temperature regime of 25/35 °C, which is likely to occur during summer (July and August) in the collection sites. Poor germination in winter (December to February), when temperature is around 5/10 °C, could thus be an adaptative strategy to prevent emergence under cold and frost conditions, in which seedlings would not be able to survive. Therefore, treatments both below and above the optimal temperatures significantly reduced the germination percentage. Germination inhibition at temperature extremes may often result in induced secondary dormancy, as observed in some *Euphorbia* species from Mediterranean ecosystems (Cristaudo et al., 2019). To test this hypothesis, further studies should transfer the non-germinated seeds from the coolest conditions to an optimal temperature and observe whether germination recovers. As well, a decrease of G% at the hottest temperature regimes (35/40 °C) indicates the species' sensitivity to high temperatures but may result in seed death due to protein denaturation. Temperature has long been recognized as one of the main factors regulating the germination process (Labouriau, 1978), especially where the availability of water is not a constraint (Fenner and Thompson, 2005). In addition, it is important to highlight that our collection sites in the Chinese subtropical forest zone were located in a monsoonal climatic region, where water availability is not a constraint (Kang et al., 2017).

Understanding seed dormancy and germination and how these processes are affected by environmental factors is fundamental to unveiling plant reproduction strategies and consequently the population persistence under natural conditions. In this context, germination time is an important functional trait regulating the germination process as a whole, being greatly affected by temperature regime (Alvarado and Bradford, 2002; Bewley et al., 2013). For instance, most vine species reached their fastest germination times (from 5 to 13 days) under temperatures of 20/30 and 25/35 °C. Lianas, on the other hand, tended to germinate within 12 to 20 days in the same temperature regimes. Differences in MGT and dormancy level could be ecologically important for determining the germination timing under natural conditions, reducing

Table 4

Statistical tests regarding the relationship of seed traits (seed fresh mass and seed shape index) vs. germination parameters (germination percentage and mean germination time) in the different tested temperature regimes. Significant P -values (< 0.05) highlighted in bold. n.s = non-significant.

Temperature	Seed trait	Germination percentage (G%)			Mean germination time (MGT)		
		Estimate	s.e	P -value	Estimate	s.e	P -value
5/10 °C	Intercept	-1.965	1.344	–	9.592	2.114	–
	Seed fresh mass	0.101	0.365	n.s	-0.846	0.264	n.s
	Seed shape index	-13.469	8.061	n.s	-104.302	32.062	n.s
10/20 °C	Intercept	-1.663	2.358	–	2.068	0.389	–
	Seed fresh mass	-0.025	0.668	n.s	0.039	0.104	n.s
	Seed shape index	4.861	12.629	n.s	3.982	2.058	n.s
20/30 °C	Intercept	-2.830	1.865	–	2.089	0.957	–
	Seed fresh mass	0.397	0.527	n.s	0.054	0.257	n.s
	Seed shape index	16.780	9.964	n.s	0.919	5.058	n.s
25/35 °C	Intercept	-3.188	0.669	–	2.581	0.734	–
	Seed fresh mass	0.990	0.200	<0.001	0.001	0.209	n.s
	Seed shape index	14.982	3.540	<0.001	-1.412	3.929	n.s
35/40 °C	Intercept	-4.657	1.910	–	2.335	0.365	–
	Seed fresh mass	1.018	0.539	n.s	0.038	0.098	n.s
	Seed shape index	14.429	10.131	n.s	-0.796	1.872	n.s

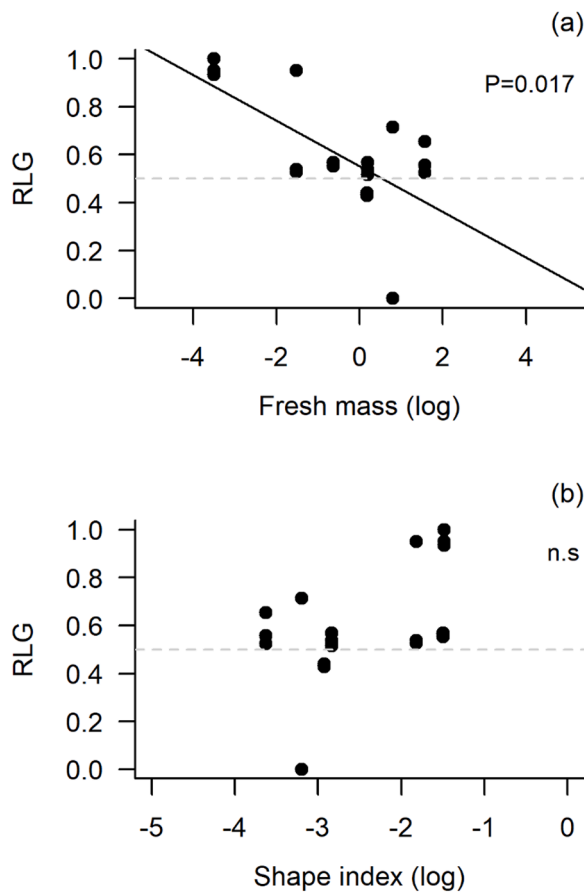


Fig. 3. Relative light-germination index (RLG) as a function of seed fresh mass (a) and seed shape index (b). n.s = non-significant.

the competition for resources, especially in the early stage of life. Early germination can be particularly important to drive colonization events related to biological invasions (Gioria and Pyšek, 2017).

Little to no relationship was found between seed traits and germination parameters, such as germination percentage, but the relatively larger seeds of *I. nil* greatly influenced the positive correlation between seed mass and G% under 25/35 °C. Seed shape index may as well influence on this relationship under the same temperature. However, we did not find any correlation between seed mass and shape with MGT values under any of the tested temperatures. Because *I. nil* seeds have the largest seed mass compared to the other study species, they may also have a higher probability of post-dispersal predation (Moles and Westoby, 2006; Xiao et al., 2006). Therefore, higher germination could be a strategy to avoid risks of mortality under warmer conditions. Besides this, higher germination percentage by large/heavy seeds has been associated with their larger reserves (Bonfil, 1998; Wu and Du, 2007). However, future studies should consider more species and a phylogenetic perspective in order to untangle some ecological questions about the role of seed traits on germination patterns. Our study focused on seven species belonging to different environments, life histories (annual and perennial), habits/growth forms (lianas and vines), and families, showing different germination requirements that might be the reason for the contrasting results. Addressing the effects of temperature changes over time could also help us to understand dormancy cycling and seasonal variations in germination levels throughout the year.

5. Conclusion

Our results show that the environment plays an important role in determining seed traits rather than the habit/growth form. Seed

germination was strongly influenced by temperature, long recognized as one of the main factors regulating the germination process. Light dependency may vary with species, but seed mass is a good predictor of the light requirement for germination. Differences in germination responses are ecologically important for determining the timing of plant recruitment in the natural environment, reducing competition for resources in the early stage of plant life.

CRedit authorship contribution statement

Arvind Bhatt: Conceptualization, Resources, Visualization, Formal analysis, Writing – review & editing. **L. Felipe Daibes:** Writing – review & editing. **Xingxing Chen:** Visualization, Writing – review & editing. **David J Gallacher:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated during and/or analysed during this study are available from the corresponding author on request.

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Supplementary materials

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