



Germination patterns of six herbs invading the Chinese subtropics

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ARTICLE INFO

Keywords:

Annual
Dormancy
Invasion
Light
Seed traits
Temperature

ABSTRACT

Germination is a key step driving biological invasions. We evaluated germination patterns of six herbaceous species that commonly invade natural and agricultural regions of China. Seed fresh mass, shape, and water absorption were measured, and germination was tested under alternating day/night temperature regimes and light/dark treatments. Seeds of *Amaranthus hybridus* were physiologically dormant, exhibiting a low germination percentage (<20 %) under all treatments. Maximum germination of all other study species ranged from 56 % to 95 % in the light-exposed treatments, but optimal temperature requirement was species specific. For instance, *Bidens frondosa* seeds germination rate was highest (70 %) in warm treatments (25/35 and 35/40 °C), while the rate for *Dysphania ambrosioides* was highest (>90 %) in cold treatments (10/20 and 20/30 °C). Germination of the small-seeded *Crassocephalum crepidioides* and *D. ambrosioides* was reliant on light exposure, with nearly null germination in the dark. Seed morpho-physical traits may influence germination patterns of invasive herbs in Chinese landscapes. Observed dormancy may be related to seed after-ripening (or cold stratification) rather than the incubation temperature regimes, as could be the case of *A. hybridus*. Nevertheless, for most species the temperature regimes are key drivers of germination timing, determining the season of recruitment, enabling these invasive species to avoid competition and to colonize and coexist in similar habitats worldwide.

1. Introduction

Invasion by non-endemic species is a major concern globally for its ability to threaten native plant diversity (Mollet et al., 2017). Expanding range and density of invasive species severely impacts agriculture, horticulture and wild ecosystems (Eminniyaz et al., 2013; Early et al., 2016), causing enormous economic losses (Pimentel et al., 2000). Losses are projected to increase with ever-expanding trade and tourism (Pimentel et al., 2000; Fang-Hao et al., 2002), making the control of invasive species a high priority for both food production and biodiversity. Ability to compete, adapt and reproduce in new habitats determines a species' invasiveness (Wolfe, 2002; Pyšek, Richardson, 2007; Bachmann et al., 2012).

Study of traits linked to invasiveness enables us to predict a species' colonization of new habitats (Richardson and Pyšek, 2008), thus predicting which species pose a threat to a given ecosystem (Rejmánek, 2000; Richardson and Pyšek, 2006; Kueffer et al., 2013). Sexual recruitment is an important life stage affecting species success by driving population persistence and expansion (Saatkamp et al., 2014; Gioria and Pyšek, 2016). Hence, germination is a key driver of a species' invasion potential (Gioria and Pyšek, 2017).

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<https://doi.org/10.1016/j.gecco.2023.e02469>

Received 11 January 2023; Received in revised form 1 March 2023; Accepted 9 April 2023

Available online 10 April 2023

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Table 1
Seed collection parameters and characterization of the study species.

Species	Family	Collection month	Locality	GPS Position	Altitude (m asl)	Habit	Habitat	Associated species	Invasion level*	Origin
<i>Amaranthus hybridus</i>	Amaranthaceae	November	Gutang	29°40'N;116°5'E	13.55	Annual	Open area	<i>Artemisia lavandulifolia</i> , <i>Humulus scandens</i> , <i>Solidago canadensis</i> , <i>Conyza canadensis</i>	2	America
<i>Bidens frondosa</i>	Asteraceae	October	Minshan	29°29'N;115°53'E	103.8	Annual	Open area	<i>Solidago canadensis</i> , <i>Ipomoea triloba</i> , <i>Perilla frutescens</i> , <i>Polygonum perfoliatum</i>	1	America
<i>Bidens pilosa</i>	Asteraceae	November	Gutang	29°40'N;116°5'E	13.5	Annual	Open area	<i>Artemisia lavandulifolia</i> , <i>Humulus scandens</i> , <i>Solidago canadensis</i> , <i>Conyza canadensis</i>	1	America
<i>Coreopsis lanceolata</i>	Asteraceae	July	Weijia	26°25'N;114°5'E	8.4	Perennial	Cultivated area	<i>Alternanthera philoxeroides</i> , <i>Erigeron annuus</i> , <i>Cayratia japonica</i> , <i>Trifolium repens</i>	4	America
<i>Crassocephalum crepidioides</i>	Asteraceae	July	Southern road of Lushan	29°31'N;115°53'E	156.8	Annual	Forest	<i>Mallotus apelta</i> , <i>Cyclobalanopsis glauca</i> , <i>Paulownia duclouxii</i> , <i>Trema cannabina</i>	2	Africa
<i>Dysphania ambrosioides</i>	Amaranthaceae	October	Northern road of Lushan	29°36'N;116°0'E	921.6	Annual	Open area	<i>Conyza canadensis</i> , <i>Pennisetum alopecuroides</i> , <i>Setaria faberi</i>	1	America

*Level 1: Severely invasive species (invaded in more than one geographical area, causing huge impact on economic and ecological benefits at the national level); Level 2: Highly invasive species (invaded in at least one geographical area and have impact on economic and ecological benefits at the national level); Level 3: Locally invasive species (i.e., distributed in more than one geographical area but only have impact on local scale, not at national level); Level 4: General invasive species (refers to the geographical distribution of a wide or narrow range of invasive plants and their harmful impact is not obvious). Source: Ma and Li (2018).

Many morphological and physiological seed traits play an active role in species dispersal, persistence, germination timing and seedling establishment (Saatkamp et al., 2019). Seed size, seed coat permeability, mass of seed reserves, and hormones, all affect the germination process (Kucera et al., 2005; Lv et al., 2018; Zhang et al., 2020). Previous studies reported functional traits to be linked to a higher invasive success, including the production of a large number of small seeds, an effective dispersal mechanism, a short germination period and the ability to germinate in a wide range of environmental conditions (Higgins et al., 2003; Truscott et al., 2006; Gioria and Pyšek, 2017; Gioria et al., 2018).

Environmental factors such as temperature and light regulate the germination process (Bewley et al., 2013). Changes in day/night temperature throughout the year influences germination periodicity, percentage, speed (time from seed water imbibition to germination), and relative frequency of germination during the incubation time (Baskin and Baskin, 2014; Bhatt, Santo, 2018). Likewise, light availability determines where germination might take place (Fenner and Thompson, 2005). Germination requirements for light can be positive, negative, or neutral (Pons, 2000; Baskin and Baskin, 2014). Seed adaptations to environmental factors are species-specific, determining the species' strategy for competing for resources (Bhatt et al., 2022a, 2022b). However, factors such as seed morphological traits (size, shape) and growth form (herbs, shrubs, trees) can sometimes cluster species with similar germination responses, forming functional groups (Saatkamp et al., 2019). The role of environmental factors on germination could thus be important for developing effective strategies for the management of invasive species (Chauhan and Johnson, 2010).

Previous studies have tested seed responses of commonly occurring invasive species from distinct geographical locations (e.g., *Bidens pilosa* in USA, Australia, and South Africa), showing significant variation in germination patterns (Forsyth and Brown, 1982; Chauhan and Johnson, 2010; Chauhan et al., 2019). Intraspecific populations from distinct geographical regions are exposed to differing local environmental conditions of climate and soil. Differences in the maternal environmental conditions may influence expression of seed dormancy and germination requirements (Guterman, 2000; Tieu et al., 2001; El-Keblawy et al., 2016; Arana et al., 2016; Fang et al., 2017; Bhatt et al., 2020). Germination studies of other invasive herbs include *Amaranthus hybridus* and *Coreopsis lanceolata* in the USA (Banovetz and Scheiner, 1994; Steckel et al., 2004), *Bidens frondosa* in Germany and Korea (Brändel, 2004; Rho and Lee, 2004) and *Crassocephalum crepidioides* in Japan and China (Nakamura and Hossain, 2009; Chen et al., 2009). Nevertheless, there are few germination studies of invasive herb species in Chinese landscapes.

In the present study, we evaluated germination patterns of six herbaceous species that frequently invade natural and agricultural areas in China. Specifically, we tested (a) the influence of temperature regimes and light availability on germination, and (b) the relationships of seed morphological/physical traits (seed fresh mass, size, water absorption) with germination patterns. We expected that seeds of these invasive herbs would germinate in a wide range of environmental conditions, according to their microsites of occurrence. The study species are widely distributed worldwide, and therefore they may display typical regeneration traits related to invasive adaptations (see Pyšek, Richardson, 2007). Investigating the germination requirements of these invasive seeds in Chinese landscapes can enhance our overall understanding of plant population survival and expansion under diverse environmental conditions.

2. Materials and methods

2.1. Seed collection

Seeds of the six invasive species were collected during 2020 at the time of their natural dispersal to ensure seed maturity. All study species except *Coreopsis lanceolata* are annuals, and most originated from America (Table 1). The selected invasive species have been categorized into four levels of invasiveness, based on severity of impact (Ma and Li, 2018). Seeds for each species were collected from 25 to 30 randomly chosen mother plants to represent the genetic diversity of the population. Seeds were cleaned immediately after collection and tested for germination within one week.

2.2. Microscopy

A Stereo Microscope (Nikon SMZ800N), fitted with a microscope camera IMG-SC600C, was used to examine seed shape, seed dimensions (length, width, and height), and color. Fifteen seeds were examined for each species, attaching them ventrally to filter paper using double-sided sticky tape. Seed size (fresh mass) was determined at the time of collection from three replicates of 100 seeds per species, using a Sartorius electronic balance (Sartorius Co., Goettingen, Germany).

2.3. Water absorption

Water absorption was assessed by recording the mass of three replicates of 100-seed of each species before and after placing them in 9-cm-diameter Petri dishes containing, two sheets of Whatman No. 1 filter paper for 24 h at room temperature (22 ± 2 °C), moistened with 10 mL distilled water. The gain in seed fresh mass after water absorption was calculated using the formula below:

$$\text{Water gain (\%)} = 100 [(W2 - W1) / W1]$$

where W1 and W2 represent the seed mass before and after water imbibition for 24 h (Baskin et al., 2004).

2.4. Seed germination

To determine the effect of temperature and light, seed germination was conducted in incubators (Kesheng incubators, Model- DRX-800C- LED, China) set at different alternating temperature regimes (5/10 °C, 10/20 °C, 20/30 °C, 25/35 °C, and 35/40 °C) in either constant darkness (dark treatment) or 12 h light per day (light treatment). Incubators were fitted with cool-white fluorescent tubes (60 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). The tested temperature regimes were chosen to simulate day/night temperature at different months of the year at the locations that seeds were collected (5/10 °C = December to February; 10/20 °C = March to April and/or October to November; 20/30 °C = May to June and/or September; 25/35 °C = July to August). A higher temperature regime (35/40 °C) was added to investigate the influence of climate change on germination. Light exposure of the light regimes was concurrent with the warmer temperature conditions in each treatment.

Seed surfaces were sterilized in 0.50 % sodium hypochlorite for 1 min, then washed thrice with deionized water to avoid fungal infections. Seeds were then placed in 9-cm Petri dishes containing three disks of Whatman No. 1 filter paper moistened with 10 mL of distilled water and placed in incubators. Darkness was achieved by wrapping Petri dishes in two layers of aluminium foil. Four replicates of 25 seeds each were used for each treatment. A germinated seed was defined as one with a radicle emerged ≥ 2 mm. Germinated seeds in the light treatments were counted and removed daily for a 30-day period, but seeds in the dark treatments were checked only at the end of the 30-day period. At the end of the tests, all ungerminated seeds from the light treatments were dissected under a stereoscope to determine embryo integrity and viability. Intact and white embryos were classified as viable; turgid or visibly damaged and brown were classified as dead).

2.5. Data analysis

Germination data were evaluated for each species individually using GLM models with a binomial distribution, with the *lme4* package in R version 4.1.1.2 (Bates et al., 2015; R Core Team, 2021). The model considered the proportion of germinated seeds as a function of the interaction between temperature x light treatments. Post-hoc Tukey HSD tests were applied to evaluate differences within treatment combinations using the *emmeans* function (Lenth et al., 2022). Likewise, mean germination times were calculated for seed treatments exposed to light (see Ranal and Santana, 2006) and tested as a function of the temperature regimes using linear models.

To assess the relationships of seed morpho-physical traits with germination patterns, we applied a Pearson's correlation using the package *corrplot* (Wei and Simko, 2021). We considered seed fresh mass (g), water gain after 24 h of imbibition (%), seed length (mm), and seed shape (index). Seed shape index was calculated as described in Thompson et al. (1993) by dividing each seed dimension by the largest value (seed length) and then calculating the variance among them. Germination percentage (GP in the light), mean germination time (MGT, days) and relative-light germination (RLG index, calculated by dividing germination in the light by the sum of G % in light + dark treatment (see Milberg et al., 2000) were evaluated considering a mild temperature regime (20/30 °C) and the warmest tested condition (35/40 °C).

3. Results

3.1. Seed traits

Seed fresh mass varied from 0.005 g in *Dysphania ambrosioides* to 0.177 g in *Bidens frondosa* (Table 2). Water imbibition for 24 h increased seed fresh mass by 60–274 % among studied species. Seed length varied greatly among species, with the smallest seeds (of *D. ambrosioides*) measuring 0.68 mm and the largest (*Bidens pilosa*) around 9.16 mm (Table 2). Shape index values were close to zero (0.059 and 0.043) for the round-seeded *Amaranthus hybridus* and *D. ambrosioides*, while all other species had a shape index from 0.165 (*Coreopsis lanceolata*) to 0.292 (*B. pilosa*). Hence, seed morphological aspects ranged from globose to flat-spherical in three species (*A. hybridus*, *C. lanceolata*, *D. ambrosioides*) and from broad oval to cylindrical in the other three (*B. frondosa*, *B. pilosa*, *Crassocephalum crepidioides*). The seed coat was mostly dark-colored, varying from brown to black, and dispersal mode of each species was mediated

Table 2
Seed traits of the study species. Data in the columns are presented as means \pm SD.

Species	Seed fresh mass (g)	Water gain (24 h, %)	Seed length (mm)	Seed shape index	Seed color	Seed shape	Dispersal mode
<i>Amaranthus hybridus</i>	0.011 \pm 0.002	111 \pm 19	0.99 \pm 0.04	0.059 \pm 0.022	Black	Sub-globose	–
<i>Bidens frondosa</i>	0.177 \pm 0.023	60 \pm 4	5.70 \pm 0.86	0.220 \pm 0.023	Dark brown	Broad oval	Zoochory
<i>Bidens pilosa</i>	0.149 \pm 0.012	70 \pm 2	9.16 \pm 1.27	0.292 \pm 0.011	Dark brown	Cylindrical	Zoochory
<i>Coreopsis lanceolata</i>	0.133 \pm 0.010	61 \pm 9	2.67 \pm 0.37	0.165 \pm 0.048	Brown	Flat-spherical	Anemochory
<i>Crassocephalum crepidioides</i>	0.021 \pm 0.002	274 \pm 13	2.25 \pm 0.15	0.234 \pm 0.009	Black	Cylindrical	Anemochory
<i>Dysphania ambrosioides</i>	0.005 \pm 0.002	83 \pm 29	0.68 \pm 0.04	0.043 \pm 0.011	Black	Globose	–

either by wind or animals.

3.2. Germination

Germination percentage was affected by temperature regimes in all study species (Fig. 1). Seeds of *A. hybridus* exhibited a low G % (<20 %) under all tested treatments, even though seeds remained viable (see Supplemental material), indicating physiological dormancy. Germination percentage of both *Bidens* species was zero under the coolest temperature (5/10 °C) but *B. frondosa* increased to 70 % under 25/35 and 35/40 °C and *B. pilosa* reached ~90 % under 20/30 and 25/35 °C in the light treatments (Fig. 1). In the dark, G % of *B. frondosa* remained around 50 % from 20/30–35/40 °C, while *B. pilosa* seeds reached 69 % under 20/30 °C.

Germination percentage of *C. lanceolata* seeds was 51 % in the coolest temperature, rising to 86–91 % under light treatments and 10/20, 20/30, and 25/35 °C, but decreasing to 33 % in the warmest temperature regime (35/40 °C, Fig. 1). In the dark, G % of

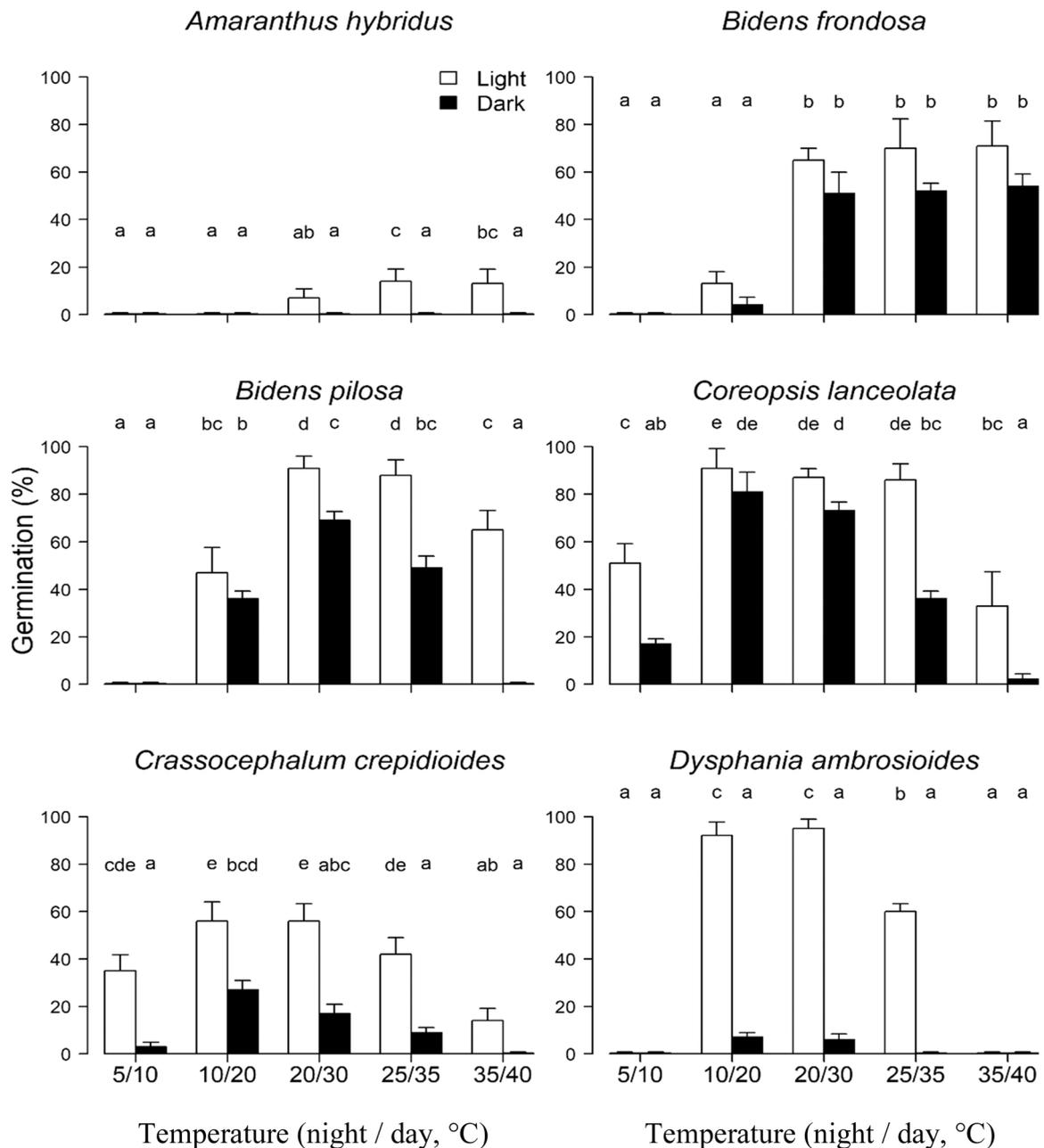


Fig. 1. Germination percentage (mean ± SD) across night / day temperature regimes and under light and dark conditions. Letters above the bars indicate significant differences among treatments, separately for each species.

C. lanceolata seeds ranged from 73 % to 81 % in the temperatures of 10/20 and 20/30 °C, with a significant decrease in all other dark treatments. Seeds of *C. crepidioides* and *D. ambrosioides* were the most affected by light, displaying low G % (<30 %, mostly null) in all dark treatments. However, both species showed enhanced G % in the light: from 42 % to 56 % for *C. crepidioides* from 10/20–25/35 °C and > 90 % for *D. ambrosioides* under 10/20 and 20/30 °C (Fig. 1).

Mean germination times (MGT) were strongly influenced by temperature in the study species, except for the dormant seeds of *A. hybridus* (Table 3). Seeds of *B. frondosa* displayed faster germination times with the temperature increase, from ~24 days at 10/20 °C to around five days under 25/35 and 35/40 °C. For *B. pilosa*, germination lasted ~19 days at 10/20 °C, decreased to 4.6 and 4.7 days under 20/30 and 25/35 °C but was delayed to ~18 days under the warmest condition, of 35/40 °C (Table 3). MGT of *C. lanceolata* and *C. crepidioides* lasted from ~26–24 days in the coolest temperature (5/10 °C), being reduced to seven to eight days under 20/30 and 25/35 °C then delayed again to ≥ 20 days under the warmest condition. For seeds of *D. ambrosioides*, MGT lasted from ~13–15 days under 10/20 and 25/35 °C, but had a decrease (7.6 days) at the mild temperature of 20/30 °C.

3.3. Relationships of seed traits with germination

Germination percentage was positively correlated with seed mass, length, and seed shape index, mostly in the warmest condition, showing that larger seeds are more tolerant to high temperatures (r values >0.70; Fig. 2). Otherwise, the relative light-germination index (RLG) had a strong inverse relationship with seed mass, meaning that larger seeds were less dependent on light for germination, particularly in the mild temperature regime ($r = -0.93$). A similar pattern was found with RLG x seed length and shape (Fig. 2). Hence, morpho-physical traits were positively correlated with each other (seed mass x length x shape), with the exception of water gain, which was negatively related to seed mass ($r = -0.55$). Since larger seeds reached higher G % in the warmest condition and mostly had low RLG values (being non-photoblastic at most treatments), the RLG index was negatively correlated to G % at 35/40 °C ($r = -0.79$; Fig. 2).

4. Discussion

Germination patterns varied significantly with temperature regimes among the tested species. For instance, seeds of *D. ambrosioides* germinated over 90 % at 10/20 and 20/30 °C, followed by *C. lanceolata* (ranging from 86 % to 91 % of germination at 10/20, 20/30, and 25/35 °C). Germination rate of *B. pilosa* seeds was 88–91 % under the temperatures of 20/30 and 25/35 °C, and 71 % for *B. frondosa* at the warmest temperature of 35/40 °C. These species exhibited low levels of innate seed dormancy, reaching relatively high G % values in one or more of the tested temperatures. Prompt germination has been suggested as a strategy found in many invasive species to colonize new habitats, as reported in Australia and Switzerland (Pérez-Fernández et al., 2000; Chrobock et al., 2011). In contrast, *A. hybridus* seeds showed null/low G % under all tested treatments (maximum germination up to 14 %), indicating the presence of a deep physiological dormancy (Baskin and Baskin, 2022). Seeds of *C. crepidioides* exhibited a maximum germination of 56 %, indicating an intermediate level of dormancy, although the species has been reported to bear nondormant seeds at the time of maturity (Chen et al., 2009). Our results indicate that at least a fraction of seeds within our tested population show some level of physiological dormancy.

Physiological dormancy is one of the most common types of seed dormancy, found in many plant families worldwide, including Asteraceae, Amaranthaceae and Chenopodiaceae (Baskin and Baskin, 2014, 2020). Previous studies reported that *A. hybridus* seeds are strongly dormant at the time of maturation, requiring a storage period or burial in soil to alleviate the dormancy (Gallagher and Cardina, 1998a, 1998b; Steckel et al., 2004; Cristaudo et al., 2007). Physiologically dormant seeds usually require certain environmental conditions to achieve after-ripening and thus break dormancy (Nadella et al., 2003). Physiological dormancy has been subcategorized as nondeep, intermediate and deep dormancy, with each species typically demonstrating one subcategory (Baskin and Baskin, 2004, 2014, 2022). *Dysphania ambrosioides* (previously known as *Chenopodium ambrosioides*) and *B. frondosa* may also display nondeep seed dormancy at certain temperatures (Vázquez-Yanes and Orozco-Segovia, 1990; Rho and Lee, 2004). Similarly, *B. pilosa* seeds collected from distinct geographical locations have shown a significant variation in germination responses (Forsyth and Brown, 1982; Chauhan and Johnson, 2010; Chauhan et al., 2019). *Coreopsis lanceolata* seeds have been reported to require dry storage to alleviate dormancy (Norcini and Aldrich, 2007), although we found a nondormant pattern under most tested treatments. These differences in dormancy and germination may be related to environmental conditions in the maternal environment. Physiological

Table 3

Mean germination time (days, mean ± SD) of seeds exposed daily to light, across temperature regimes. Letters within each row indicate significant differences among treatments. n.s = non-significant. Traces indicate missing values due to a low germination percentage.

Species	Night / day temperature (°C)				
	5/10	10/20	20/30	25/35	35/40
<i>Amaranthus hybridus</i>	–	–	6.0 ± 0.8n.s	4.4 ± 1.9n.s	4.2 ± 1.6n.s
<i>Bidens frondosa</i>	–	23.7 ± 2.5 a	8.3 ± 1.1 b	5.4 ± 0.9 bc	4.8 ± 1.5c
<i>Bidens pilosa</i>	–	19.5 ± 1.5 a	4.6 ± 0.7 b	4.7 ± 0.5 b	17.6 ± 2.4 a
<i>Coreopsis lanceolata</i>	23.7 ± 1.2 a	10.4 ± 0.6 b	7.3 ± 0.4c	7.8 ± 0.9c	23.6 ± 1.3 a
<i>Crassocephalum crepidioides</i>	25.9 ± 0.3 a	13.5 ± 1.6c	7.0 ± 1.2 d	6.8 ± 1.0 d	20.5 ± 1.4 b
<i>Dysphania ambrosioides</i>	–	12.6 ± 0.4 a	7.6 ± 0.8 b	14.6 ± 2.2 a	–

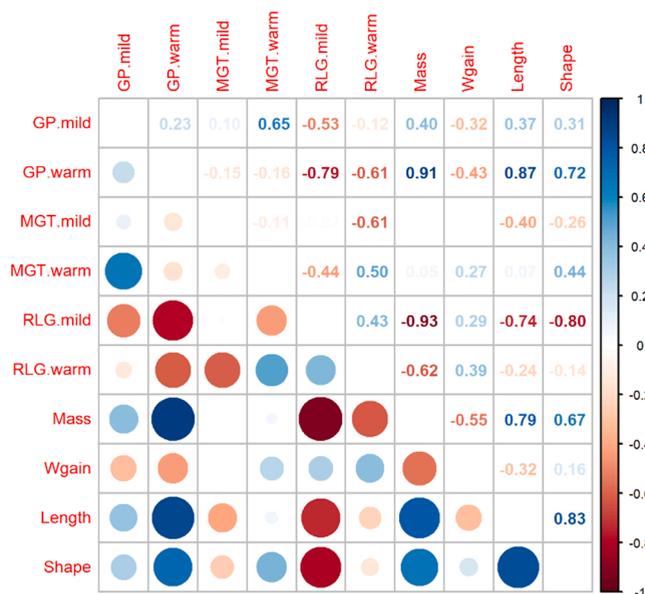


Fig. 2. Pearson's correlation among seed germination and seed morpho-physical traits. Germination percentage (GP in the light), mean germination time (MGT, days) and relative-light germination (RLG index) were each evaluated under “mild” (20/30 °C) and “warm” (35/40 °C) temperature regimes. “Wgain” is the seed weight gain during moisture imbibition, and “Shape” is the seed shape index.

dormancy is altered in seeds according to regulatory environmental cues, such as thermal environment (Baskin and Baskin, 2022). Seeds have evolved highly efficient environmental sensors that respond to their prevailing environment and also to their environmental history (Huang et al., 2015), thus reflecting the adaptation to different habitat/climatic conditions (Baskin and Baskin, 2014; Willis et al., 2014).

Temperature requirements for germination could be linked to a seasonal variation in germination timing, which may enable species to coexist in similar environmental conditions by reducing competition for resources (Fenner and Thompson, 2005; Baskin and Baskin, 2014). Seeds of *C. lanceolata* were the only ones able to germinate under the coolest tested temperature (51 % at 5/10 °C), and maintained germination at the warmest tested temperature (33 % at 35/40 °C). As a perennial species, this may indicate a broad germination niche and therefore a more generalist strategy of being recruited from soil seed banks at any time through the year. Previous studies linked the species' ability to germinate over a wide range of temperatures with species persistence under different environmental conditions (Ferreras et al., 2015; Gioria and Pyšek, 2017; Farooq et al., 2021). Conversely, the other tested species (all annuals) displayed specific thermal requirements for germination and were classified into more aggressive categories of invasion (levels 1 and 2). These species dispersed from July to October (summer to autumn), but seeds may need to survive through the winter to germinate under more favorable conditions in the following seasons.

Seeds were mostly unable to germinate at the coolest temperature, thus avoiding recruitment in the winter season (December to February). This could be an adaptative strategy, given that the chances of seedling survival would be much lower due to extreme cold and frost. Similarly, higher temperatures (35/40 °C) decreased G % and delayed germination of most species, except for *B. frondosa* seeds, which preferably germinated in the warmest conditions. The tolerance to high temperatures seems to be linked to the seed size, since larger seed dimensions were positively correlated with G % (see Fig. 2). Tolerance to short bursts of 48–86 °C temperatures for short periods (a few seconds) has been related to seed size in some weeds (Vidotto et al., 2013). Seed tolerance to fire-related heat shocks has been linked to seed mass in fire-prone ecosystems, such as the Brazilian savannas (Ribeiro et al., 2015; Daibes et al., 2019). Seed mass may provide protection to the embryo, aiding in survival under conditions of heat shock. Nevertheless, little is known about the role of seed size in predicting germinability patterns under incubation temperatures in the context of global change (but see Fernández-Pascual et al., 2019). Minimum, optimum and maximum temperatures can be used to describe the thermal ranges favorable for germination (Labouriau, 1978; Bewley et al., 2013). Testing the relationships of seed traits with germination under a wide range of temperatures enables the identification of species' tolerance to low and high temperatures during the early stage of regeneration, enabling us to predict the possibility of invasion under different climatic scenarios.

Seed size is predictive of the relationships of germination dependency to light availability (Milberg et al., 2000; Flores et al., 2013). Seeds of the tested species tended to germinate at a significantly higher rate in light as compared to the dark treatments. Two of the species with the smallest seeds, *C. crepidioides* and *D. ambrosioides*, were the most inhibited by constant darkness. Small seeds appear to avoid germinating when buried in deep soil, presumably due to limited reserves of nutrients to maintain seedling growth before they can photosynthesize (Pons, 2000). Temperature also affects the light requirement during germination. For example, *C. lanceolata* seeds showed G % ≥ 70 % in the dark at 10/20 and 20/30 °C, but germination was significantly inhibited both below and above these optimal temperatures. Hence, the interactions of temperature and light also play an important role in determining the capacity of seeds to germinate under light or dark, depending on the species. Therefore, seeds may require light at a certain temperature but not at others

(Pons, 2000; Bewley and Black, 2012).

5. Conclusion

Each of the studied species exhibits its own temperature requirement for germination, despite the species appearing in similar environments. Temperature is a key driver of germination timing, determining the month of recruitment. Different temperature requirements may reduce interspecific competition, allowing these invasive species to colonize and coexist in nature. The variation in seed morpho-physical traits is crucial to driving seed tolerance to high temperatures, and also predicting germination-light requirements. Examining these traits could thus be used as an indicator of the invasion potential of species at the earlier stage of plant life (Molina-Montenegro et al., 2018). These germination patterns are essential for better understanding of ecological relationships that drive species distribution and community assembly, as well as for determining the most appropriate management practices in Chinese landscapes and other invaded habitats worldwide.

Author contribution

AB conceived, designed, AB and XC, performed the experiments. AB, LFP analyzed the data. AB, LFP and DJG wrote the manuscript. All authors approve this submission.

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

Data will be made available on request.

Acknowledgements

A.B. thanks to the Talents Program of Jiangxi Province (PR China) (Grants jxsq2020104003) for funding. LFD thanks the Sao Paulo Research Foundation (FAPESP) for the postdoctoral fellowship (grant #2022/01560-9).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.gecco.2023.e02469](https://doi.org/10.1016/j.gecco.2023.e02469).

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