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Role of short-term cold stratification on seed dormancy break and germination of alien species in southeastern China

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Abstract Do short periods of relatively low temperatures during winter in subtropical zones promote seed dormancy break and germination of alien species and thus facilitate their spread? To help answer this question, we tested the germination responses to moist storage at low temperatures of seeds of 21 alien species from southeastern China. For each species, fresh seeds and seeds stored dry at room temperature and wet at 15 °C and wet at 4 °C for 30 days were tested for germination at 25 and 25/15 °C in a 12-h daily photoperiod. Fresh seeds of *Bidens pilosa, Eclipta prostrata*, *Hyptis suaveolens*, and *Talinum paniculatum* germinated to 89–96.7 % at both test temperatures, those of *Ageratum conyzoides*, *Mikania micrantha*, and *Wedelia trilobata* to 53–83 % at one or both temperatures, and those of the other 14 species to 0–39 % at one or both temperatures. A 30-day cold stratification pretreatment at 4 °C increased the germination percentage of seeds of *Lantana camara* and *Solanum torvum*, but they germinated to only 10.7–21.3 % and 2–30.7 %, respectively. However, germination speed of seeds of five species increased following moist storage at 4 °C, and that of four species increased following storage at 15 °C. Seeds of 14 species began germinating during wet storage at 15 and/or 4 °C. *B. pilosa* germinated to 87.3 and 57 % at 15 and 4 °C, respectively, and the other 13 species to 0.3–22.3 % at 15 °C. Thus, the short periods at 4 and/or 15 °C increased germination percentages of two species and the rate of six species and permitted early germination of 14 species, leading to the conclusion that the short cool periods during winter in the

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subtropical zone of China may contribute to the success of alien plant species in this region.

Keywords Invasive species · Cold moist storage · Comparative studies · Germination responses · Regeneration strategy · Seed dormancy

Introduction

Due to the increasing influence of humans, many species have invaded and continue to invade new regions at an unprecedented rate, and they exert strong impacts on ecosystems (Didham et al. 2005; Pimentel et al. 2005). Hence, causes of invasiveness of alien plant species are one of the most challenging topics in ecology and invasion biology (Alpert et al. 2000; Pyšek and Richardson 2007; Küster et al. 2008; Prentis et al. 2008). Successful invasion is species specific (Hobbs and Humphries 1995; Wolfe 2002), and it depends on the invader having the capacity to compete, reproduce, and otherwise adapt to the new habitat (Callaway and Aschehoug 2000; Grotkopp and Rejmane 2007; Pyšek and Richardson 2007; Venn et al. 2011; Bachmann et al. 2012).

Seed germination is an important functional trait associated with the survival and establishment of seedlings and the fitness of a species, and hence, it is regarded as a crucial event for successful invasion of a species (Kudoh et al. 2007; Beckmann et al. 2011; Leiblein-Wild et al. 2014). Some studies have found that invasive species have greater germination plasticity, broader germination requirements (Xu and Qiang 2004), and higher germination percentages and rates than native species (Hao et al. 2009; Pan et al. 2012; Wainwright and Cleland 2013). Another aspect of the seed biology of invasive species is that the climate in the new/invaded area may be well suited for breaking seed dormancy and/or facilitating germination.

There are 515 alien invasive plants species in China, and nearly 260 of them are distributed in the subtropical zone of SE China (Yan et al. 2014). Mean minimum winter temperatures in the subtropical zone of SE China range from 7 to 15 °C, and the extreme minimum temperature is −0.5 °C (Wu et al. 2014). Thus, winter temperatures are within the range of those suitable for breaking seed dormancy via cold stratification (*c*. 0–10 °C) (Baskin and Baskin 2014) for varying periods of time in winter, depending on location. We predicted that the short periods of relatively low temperatures during winter in the subtropical zone of China promote seed germination of alien species. To test this prediction, we investigated the effects of storage under cool moist and dry conditions on germination of 21 alien species in SE China. The responses of seeds of alien species in the subtropical zone to low temperatures would provide further information about their regenerative strategies, which would be useful for making future predictions about the spread and control of invading species.

Materials and methods

Study species and study site

Seeds of 21 species (Table 1) were collected from wild populations in the subtropical zone of SE China (Guangdong Province) (114°53′E–117°08′E, 22°31′N–24°15′N). Average annual temperature of this area is 21 °C, with the mean monthly maximum temperature (28 °C) in July, mean monthly minimum temperatures (7–15 °C), and extreme minimum temperature (−0.5 °C) in January (Wu et al. 2014). Mean annual rainfall is 1300–2400 mm, with the wet season extending from April to September and the dry season from October to January (Chen et al. 1997).

Seed collections

Fully ripened fruits or seeds (hereafter seeds) of the 21 species were collected from August to November 2012. Seeds were collected from one to three populations of each species and from at least 20–30 randomly selected individuals. All seeds of each species were pooled. After bulk collection, seeds were air dried at room conditions for 1–2 days and then stored dry at 4 °C until the start of germination tests. Seed mass of each species was determined by weighing three replicates of 100 seeds; the seeds were air dried at 4 °C for about 4 weeks at the time they were weighed.

Seed viability

Viability of fresh seeds was assessed using 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) (Hendry and Grime 1993) prior to initiating the experiments. Three

Table 1 Family, life form, seed mass, seed viability, initial germination (fresh seeds), and germination % during wet storage at 15 °C of the 21 study species

No.	Species	Family	Life form	Single-seed mass (mg)	Seed viability (%)	Initial germination (%)		Germination during wet storage at 15 °C (%)
						25 °C	25/15 °C	
1	*Bidens pilosa*	Asteraceae	Annual herb	1.458 ± 0.037	100	94.7	93.3	87.3
2	*Ageratum conyzoides*	Asteraceae	Annual herb	0.099 ± 0.005	94	78	62.7	0
3	*Eclipta prostrata*	Asteraceae	Annual herb	0.181 ± 0.003	100	96	96.7	10.3
4	*Mikania micrantha*	Asteraceae	Perennial vine	0.055 ± 0.007	90	43.3	52.7	22.3
5	*Wedelia trilobata*	Asteraceae	Perennial vine	1.650 ± 0.022	96	6	83.3	1.0
6	*Indigofera suffruticosa*	Fabaceae	Shrub	3.430 ± 0.049	96	24.7	38.7	4.7
7	*Senna tora*	Fabaceae	Annual herb	17.262 ± 0.175	98	10	7.3	3
8	*Senna alata*	Fabaceae	Shrub	29.245 ± 0.032	100	1.3	0.7	0
9	*Mimosa pudica*	Fabaceae	Shrubby herb	5.669 ± 0.094	96	0.7	1.3	0.3
10	*Mimosa sepiaria*	Fabaceae	Shrub	9.722 ± 0.004	96	12	32	2.7
11	*Leucaena leucocephala*	Fabaceae	Shrub or small tree	40.736 ± 1.159	98	11.3	11.3	8.3
12	*Ipomoea triloba*	Convolvulaceae	Annual vine	13.203 ± 0.136	96	3.7	5.3	6.3
13	*Ipomoea purpurea*	Convolvulaceae	Annual vine	26.973 ± 0.300	100	6	11.3	0
14	*Ipomoea cairica*	Convolvulaceae	Perennial vine	39.327 ± 0.595	100	0.3	0.7	0
15	*Malvastrum coromandelianum*	Malvaceae	Subshrub	2.125 ± 0.033	92	32	14.7	5.3
16	*Lantana camara*	Verbenaceae	Shrub	11.379 ± 0.206	94	4	10.7	0.3
17	*Duranta repens*	Verbenaceae	Shrub	10.879 ± 0.124	98	0	0	0
18	*Hyptis suaveolens*	Lamiaceae	Annual herb	4.773 ± 0.133	100	96	96.7	0
19	*Talinum paniculatum*	Portulacaceae	Annual herb	0.175 ± 0.005	100	89.3	93.3	0.7
20	*Solanum torvum*	Solanaceae	Shrub	0.770 ± 0.072	94	1.3	10	0
21	*Scoparia dulcis*	Scrophulariaceae	Herb or subshrub	0.024 ± 0.011	90	24	26	17.3

replicates of 50 seeds for each species were placed on moist filter paper at room temperature for 24 h and then cut open along the longitudinal axis with a scalpel. Both seed sections were incubated in a 0.1 % aqueous solution of TTC for 24 h at 25 °C in darkness. Seeds with a strongly red-stained embryo were considered to be viable.

Initial germination test

To test germination of fresh seeds of each species, final germination percentage and the number of days to reach 20 % germination (t_{20}) of seeds of each species were determined under laboratory conditions. For each species, three replicates of 50 seeds were placed in 9-cm-diameter Petri dishes on two layers of filter paper moistened with distilled water and incubated at a 12-h photoperiod at a constant temperature (25 °C) and at an alternating temperature regime (25/15 °C). Light was provided by fluorescent tubes (PPFD, 25–30 μmol/m^{-2} s^{-1} at seed level) during the 12-h high-temperature portion of the daily cycle. The temperatures 25 and 15 °C correspond to the daily mean maximum and minimum temperatures, respectively, at the study site during the spring germination period. Seed germination was monitored every 24 h for 30 days, and a seed was considered to be germinated when the radical was visible to the naked eye. Germinated seeds were counted and then discarded. At the end of the germination tests, viability of non-germinated seeds was determined by opening each seed with a needle to determine if the embryo was firm and white (viable) or soft and gray (non-viable) (Baskin and Baskin 2014).

Seed storage and germination test

Seeds of each species were divided into three storage groups: dry at room temperature (20–25 °C), wet at 15 °C, and wet at 4 °C; three replicates of 50 seeds each for each species were placed at each condition. Regardless of storage condition, all seeds were in darkness for 30 days. During the storage period at 15 and 4 °C, seed germination was monitored every 48 h. After the 30-day storage period, seeds were tested for germination at 25 and 25/15 °C, as described above. The final germination percentage (GP) and number of days to reach 20 % germination (t_{20}) of seeds of each species following various storage conditions were determined based on the number of viable seeds; seeds that germinated during wet storage at 15 or 4 °C were not included in calculations of GP or t_{20}.

Statistical analyses

Two germination attributes were used in the analyses, i.e., GP and t_{20}. GP is germination percentage calculated as GP = GN/SN, where GN is the total number of germinated seeds and SN the total number of test seeds that were viable. T_{20} is the number of days to reach 20 % germination of viable seeds and represents germination speed (Bewley et al. 2013), and was calculated from the first day of incubation. Univariate analysis of variance at the 5 % level of probability was performed to test the main effect of species, storage condition, and incubation temperature on GP and t_{20}. Then, one-way ANOVAs and the least significant difference test (LSD) were carried out to test the difference in GP and t_{20} between various storage conditions and both incubation temperatures for each species. The original GP data were arcsine transformed before analysis. All statistical tests were performed using SPSS 16.0 software.

Fig. 1 Final germination percentages at two test temperatures ▶ of fresh and stored seeds of 15 species. The *six* species (*Bidens pilosa*, *Talinum paniculatum*, *Senna tora*, *Senna alata*, *Mimosa pudica*, and *Ipomoea cairica*) for which differences were not significant are not included. The *clear* and *solid boxes* represent 25 and 25/15 °C, respectively. *Different lowercase letters* for a species indicate significant differences (LSD, 0.05) between 25 °C and 25/15 °C after each storage condition, and *different uppercase* letters for a species indicate significant differences (LSD, 0.05) between different storage conditions at the same incubation temperature. Each *box* represents the distribution of germination percentages: minimum, maximum, and median, which is indicated by line across the *box*

Results

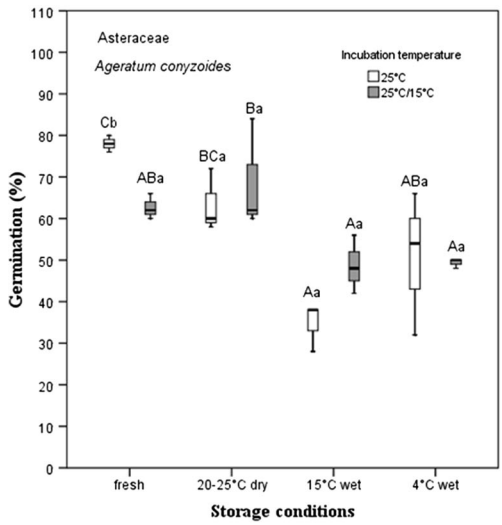
Seed mass, viability, and germination of fresh seeds

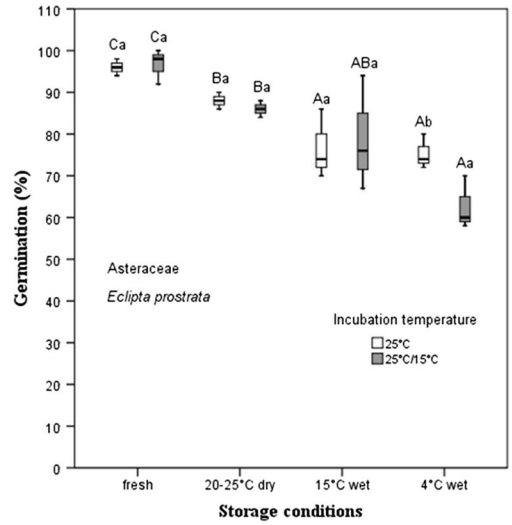
Seed mass of the 21 species ranged from 0.024 mg to 40.736 mg, and initial viability of seeds was >90 %. Fresh seeds of *Bidens pilosa, Eclipta prostrata*, *Hyptis suaveolens*, and *Talinum paniculatum* germinated to 89–96.7 % at both test temperatures. Fresh seeds of the other 17 species had high viability, but they germinated to relatively low percentages at one or both of the test temperatures (those of *Ageratum conyzoides*, *Mikania micrantha* and *Wedelia trilobata* to 53–83 % and the other 14 species to 0–39 %) (Table 1). For fresh seeds of the 21 species, there was a significant negative correlation between germination percentage and seed mass (25 °C: $r = -0.498$*, $P < 0.05$; 25/15 °C: $r = -0.565$**, $P < 0.01$). The small seeds had higher germination percentages than the large ones.

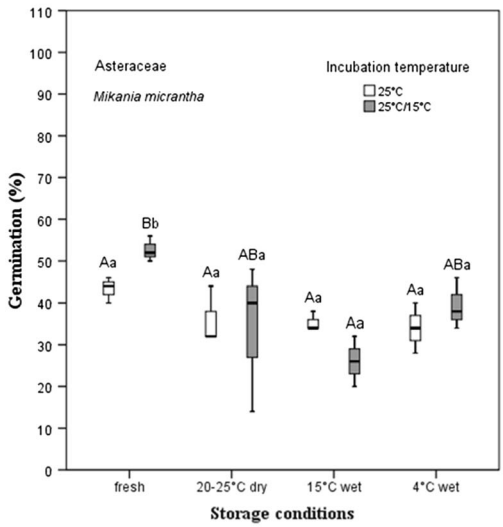
Table 2 Univariate analysis of variance of germination percentage (GP) and t_{20} for 21 alien species at two incubation temperatures following storage at various conditions

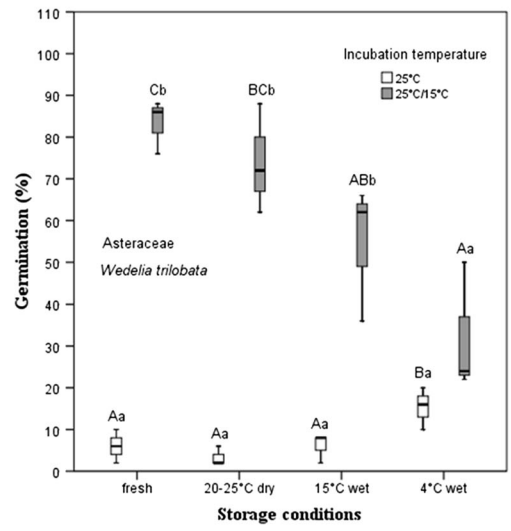
	Germination percentage				T_{20}			
	df	*F*	*P*	Partial eta squared	df	*F*	*P*	Partial eta squared
Corrected model	24	135.669	***	0.872	11	160.290	***	0.685
Intercept	1	2360.0	***	0.831	1	969.662	***	0.861
Species	20	160.523	***	0.870	7	31.735	***	0.587
Storage conditions	3	12.511	***	0.073	3	11.389	***	0.180
Incubation temperature	1	8.050	**	0.017	1	60.052	***	0.278

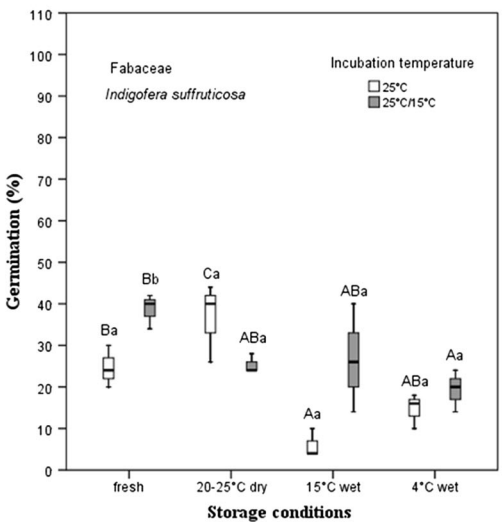
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

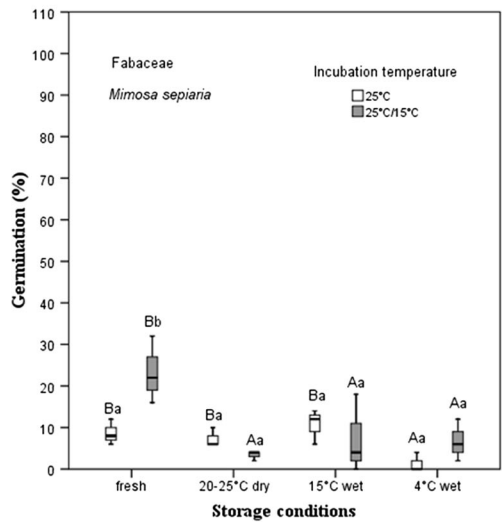

Asteraceae
Ageratum conyzoides
Incubation temperature
25°C
25°C/15°C
Germination (%)
Cb
ABa
BCa
Ba
Aa
Aa
ABa
Aa
fresh
20-25°C dry
15°C wet
4°C wet
Storage conditions


Ca
Ca
Ba
Ba
Aa
ABa
Ab
Aa
Asteraceae
Eclipta prostrata
Incubation temperature
25°C
25°C/15°C
Germination (%)
fresh
20-25°C dry
15°C wet
4°C wet
Storage conditions


Asteraceae
Mikania micrantha
Incubation temperature
25°C
25°C/15°C
Germination (%)
Aa
Bb
Aa
ABa
Aa
Aa
Aa
ABa
fresh
20-25°C dry
15°C wet
4°C wet
Storage conditions


Incubation temperature
25°C
25°C/15°C
Asteraceae
Wedelia trilobata
Germination (%)
Cb
BCb
ABb
Aa
Aa
Aa
Ba
Aa
fresh
20-25°C dry
15°C wet
4°C wet
Storage conditions


Fabaceae
Indigofera suffruticosa
Incubation temperature
25°C
25°C/15°C
Germination (%)
Ba
Bb
Ca
ABa
Aa
ABa
ABa
Aa
fresh
20-25°C dry
15°C wet
4°C wet
Storage conditions


Fabaceae
Mimosa sepiaria
Incubation temperature
25°C
25°C/15°C
Germination (%)
Ba
Bb
Ba
Aa
Ba
Aa
Aa
Aa
fresh
20-25°C dry
15°C wet
4°C wet
Storage conditions

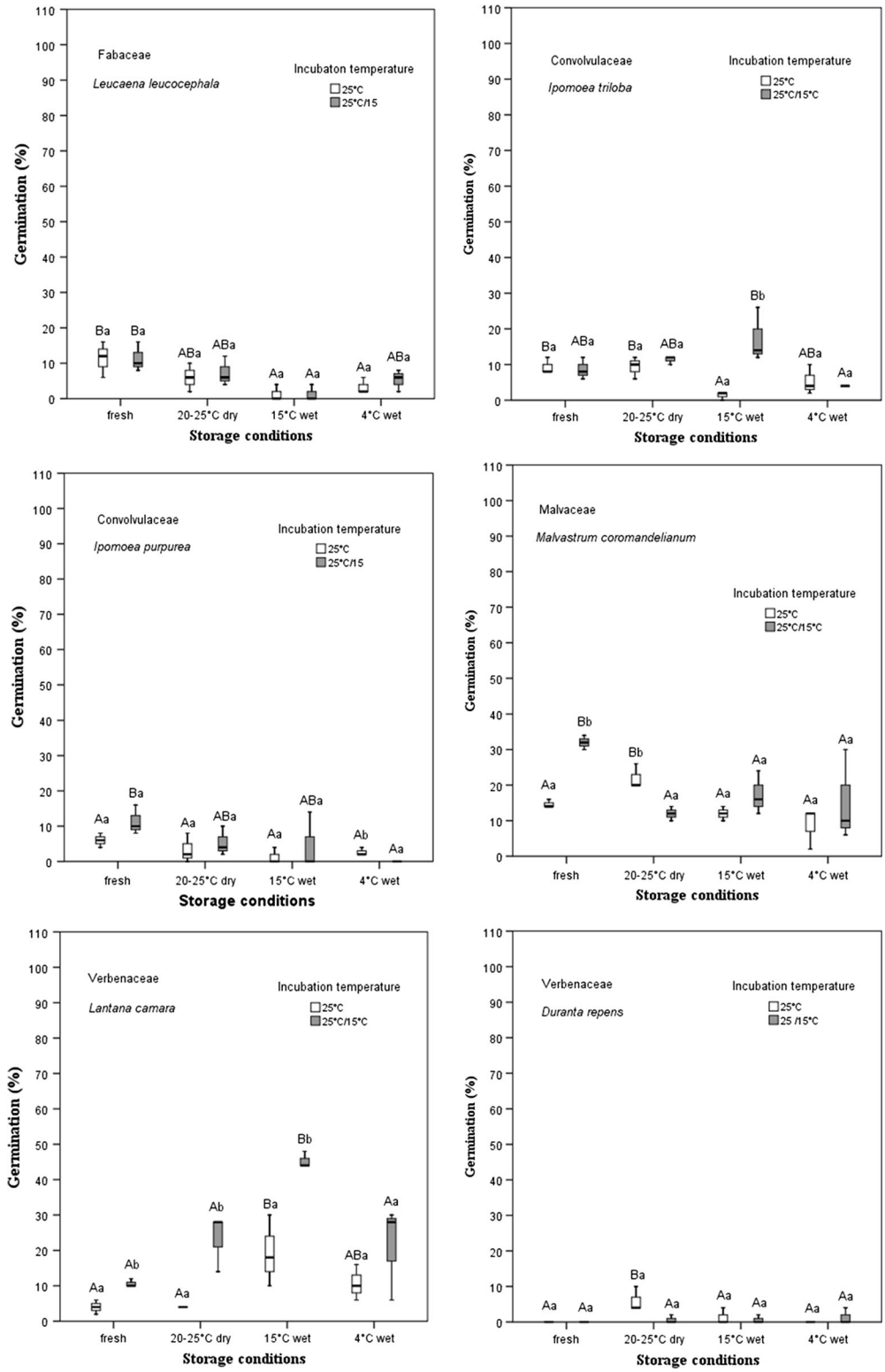


Fig. 1 continued

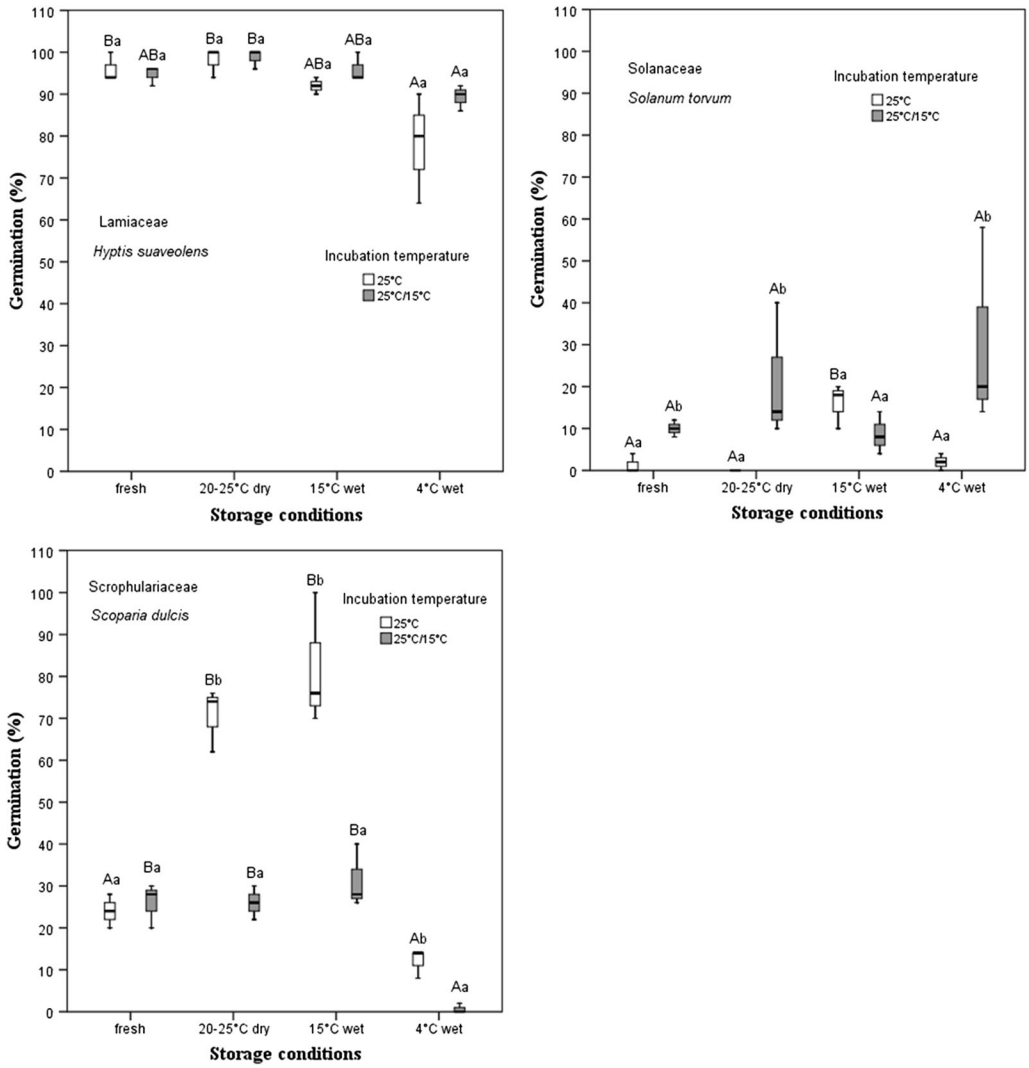


Fig. 1 continued

Effect of storage on GP and t_{20}

Overall, storage condition had a significant effect on GP ($F = 12.511$, $P < 0.001$) and t_{20} ($F = 11.389$, $P < 0.001$) (Table 2). The effect of storage condition on GP varied with the species. For *Lantana camara* (4–10.7 %), *Solanum torvum* (1–10 %), and *Scoparia dulcis* (24–26 %), whose fresh seeds had low germination, storage conditions (dry and/or wet storage) significantly increased germination to 19–45 %, 2–30.7 %, and 31–82 %, respectively (Fig. 1). Storage had little or no effect on germination percentages of seeds of *B. pilosa*, *H. suaveolens*, and *T. paniculatum* (fresh seeds germinated to 89–96.7 %), *M. micrantha* (43–52.7 %); five Fabaceae species; and three Convolvulaceae species, *Malvastrum coromandelianum*, and *Duranta repens* (0–32 %). For *A. conyzoides*, *E. prostrata*, and *Indigofera suffruticosa*, whose fresh seeds germinated to 62.7–78 %, 96–96.7 %, and 24.7–38.7 %, respectively, the various wet storage treatments decreased germination to 34.7–48.7 %, 62.7–75 %, and 6–26.7 %, respectively. Seeds of 14 species began germinating during wet storage at 15 or 4 °C, with those of *B. pilosa* reaching 87.3 and 57 %, respectively, and those of *E. prostrata*, *M. micrantha* and *S. dulcis* reaching 10.3–22.3 % at

15 °C, and the other 10 species germinated to <10 % during wet storage at 15 °C (Table 1).

Storage condition had a significant effect on t_{20} of seven of the eight species incubated both at 25 °C and 25/15 °C; the exception was *A. conyzoides* seeds at 25/15 °C. T_{20} at one or both test temperatures decreased for seeds of *B. pilosa*, *E. prostrata*, *M. micrantha*, *H. suaveolens*, and *T. paniculatum* following wet storage at 4 °C; for *E. prostrata*, *T. paniculatum*, and *S. dulcis* following wet storage at 15 °C; and for *H. suaveolens, S. dulcis* and *T. paniculatum* following dry storage (Table 3).

Effect of test temperature on GP and t_{20}

Overall, incubation temperature had a significant effect on GP ($F = 8.05$, $P < 0.01$) and t_{20} ($F = 60.052$, $P < 0.001$) (Table 2), but it significantly changed GP of only *W. trilobata*, *L. camara* and *S. dulcis* seeds (Fig. 1). After seeds were stored at various conditions, the GP of *W. trilobata* and *L. camara* seeds was significantly higher at 25/15 °C than at 25° and that of *S. dulcis* at 25 °C than at 25/15 °C. Incubation temperature had a significant effect on t_{20} of *B. pilosa*, *H. suaveolens*, *T. paniculatum*, and *S. dulcis* seeds when they were fresh and following all storage conditions, and it had a significant effect on *A. conyzoides*, *E. prostrata*, and *M. micrantha* seeds, depending on treatment (Table 3).

Discussion

Information about the germination responses of seeds is important in understanding the invasiveness of alien species in new habitats such as the subtropical region of China. That is, do the environmental conditions of the habitat promote dormancy break, and do seeds germinate at a time when conditions are favorable for seedling establishment? Cold stratification of seeds of many temperate region species ensures that dormancy break occurs during winter and that seeds can germinate in spring or early summer, at the beginning of the favorable season for seedling growth (Meyer and Kitchen 1994; Schütz and Rave 1999; Baskin and Baskin 2014). However, for species in subtropical and tropical regions, it has been suggested that low temperatures may be a limiting factor for survival and establishment of seedlings (Hacker and Ratcliff 1989). In our study of 21 alien species in SE China, low temperatures had different effects on dormancy break and germination, depending on the species and dormancy state of fresh seeds.

Fresh seeds of *B. pilosa, E. prostrata*, *H. suaveolens*, and *T. paniculatum* had high viability and germinated to high percentages at both test temperatures. Fresh seeds of the other 17 species had high viability, but they germinated to relatively low percentages at one or both of the test temperatures, indicating the presence of dormancy. Ten species belong to genera in Fabaceae, Convolvulaceae, and Malvaceae that are known to have physical dormancy (PY), i.e., water impermeable seed coat, and the other seven species belong to families with physiological dormancy (PD), i.e., low growth potential of the fully developed embryo (Baskin and Baskin 2014).

PY can be broken artificially using different treatments such as mechanical or acid scarification (Baskin and Baskin 2014), cooling at very low temperatures (Pritchard et al.1988) or brief exposures to high temperatures (Martin et al. 1975; Herranz et al. 1998). In nature, PY often is broken in response to the changes in temperature that occur when the seasons change, a canopy gap is formed, or buried seeds are brought to the soil surface (Baskin and Baskin 2014). Van Assche et al. (2003) found that PY could be broken in a group of annual legumes by exposing seeds to winter conditions (5 °C for 2–8 months) or two cycles of 5 and 20 °C for 2 months and then moving them to alternating (15/6 °C) spring temperatures. Although we exposed seeds of six Fabaceae, three Convolvulaceae, and one Malvaceae *(M. coromandelianum*) species to low temperatures for 30 days and then moved them to 25/15 °C, germination percentages were not increased significantly. Thus, we suggest that seeds of these species may form a persistent soil seed bank.

Cold stratification at 4 °C was not a very effective method to increase germination of seeds with PD, and it significantly increased germination percentages for seeds of only *L. camara* and *S. torvum*. However, incubation at 4 °C significantly increased the germination speed for seeds of *B. pilosa*, *E. prostrata*, *M. micrantha, H. suaveolens*, and *T. paniculatum*, suggesting that some breaking of PD had occurred. On the other hand, cold stratification at 4 °C decreased germination percentages of *A. conyzoides*, *E.*

Table 3 T_{20} of eight species at two incubation temperatures following various storage conditions

Storage conditions	Incubation temperature			
	25 °C	25/15 °C	25 °C	25/15 °C
	Bidens pilosa		*Wedelia trilobata*	
FS	3.192 ± 0.252Ba	5.911 ± 0.083Bb	–	7.333 ± 0.642B
RT	3.151 ± 0.068Ba	6.057 ± 0.684Bb	–	10.781 ± 0.709C
15 °C	–	–	–	6.50 ± 0.601A
4 °C	1.233 ± 0.251Aa	2.80 ± 0.608Ab	–	8.733 ± 0.568B
	Ageratum conyzoides		*Hyptis suaveolens*	
FS	2.571 ± 0.418Aa	8.033 ± 0.251ABb	4.623 ± 0.509Cb	2.969 ± 0.403Aa
RT	6.151 ± 0.1.685Ba	9.588 ± 1.655Ba	1.365 ± 0.138Aa	2.961 ± 0.297Ab
15 °C	4.722 ± 0.1.548ABa	7.711 ± 0.772Ab	2.239 ± 0.045Ba	3.607 ± 0.204Bb
4 °C	4.741 ± 0.2.179ABa	7.388 ± 0.346Ab	2.625 ± 0.904Ba	7.10 ± 0.360Cb
	Eclipta prostrata		*Talinum paniculatum*	
FS	3.433 ± 0.404Ba	2.841 ± 0.194Ca	8.143 ± 0.213Ba	14.767 ± 0.493Cb
RT	3.056 ± 0.335Ba	5.385 ± 0.103Db	4.314 ± 0.042Aa	9.145 ± 0.816Bb
15 °C	1.254 ± 0.0943Aa	2.414 ± 0.272Bb	2.553 ± 0.600Aa	6.148 ± 1.466Ab
4 °C	1.327 ± 0.017Aa	1.322 ± 0.282Aa	4.217 ± 1.188Aa	9.340 ± 0.0318Bb
	Mikania micrantha		*Scoparia dulcis*	
FS	4.385 ± 0.334Ba	4.251 ± 0.231Ba	13.466 ± 0.509Ca	15.733 ± 0.642Bb
RT	3.701 ± 0.200Ba	5.697 ± 0.091Cb	7.106 ± 0.386Ba	20.666 ± 2.081Cb
15 °C	5.33 ± 0.850Ca	5.866 ± 0.493Ca	3.983 ± 0.419Aa	8.714 ± 0.1.115Ab
4 °C	1.779 ± 0.112Aa	1.772 ± 0.300Aa	–	–

T_{20} values for various storage conditions were compared by the least significant difference test (LSD) at the 5 % level. The data are the mean value of t_{20}. Different lowercase letters for a species indicate significant differences between 25 and 25/15 °C after each storage condition, and different uppercase letters for a species indicate significant differences between different storage conditions at the same incubation temperature

FS fresh seeds, *RT* seeds stored dry at room temperature

prostrata, M. micrantha, and *H. suaveolens,* but it is not known if incubation at 4 °C decreased viability and/or induced some of the seeds into secondary dormancy.

Seeds of 14 species started germinating while they were being wet stored at 15 or 4 °C. The implication of seeds germinating at 15 and/or 4 °C is that germination could occur in the field during cool days of winter or early spring (if soil moisture was not limiting), thereby allowing seedlings to become established at the beginning of the growing season.

In addition, we also found that seed mass of 21 species had a negative correlation with fresh seed germination, suggesting that species with smaller seeds have strong seed reproductive capacity. However, although germination of fresh seeds of the large-seeded species was relatively low, it has been suggested that some large-seeded species (e.g., *Ipomoea cairica*) may be able to reproduce via cloning and thus rapidly colonize in new habitats (Wang et al. 2012).

In conclusion, although we predicted that cold stratification would break dormancy in seeds of alien species, we did not find such a response, except for seeds of *L. camara* and *S. torvum* in which cold stratification at 4 °C significantly increased germination but to low percentages. Cold stratification at 4 °C increased seed germination speed of five species, and germination of 14 species began while seeds were being stored wet at 15 and 4 °C. Thus, while the relatively low temperatures of winter in SE China significantly increased germination percentages for only two species in our study, they would play an important role in synchronizing the timing of germination of some species in the field to occur in the cool season. Germination during the cool season would

allow seedlings to become established early in the growing season. The responses of seeds of the 21 alien species to cold stratification suggest that low winter temperatures, i.e., cold stratification, would not be a major factor in promoting the northward spread of these species in China.

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