Seed Science Research

cambridge.org/ssr

Research Paper

Cite this article: Hu X-J, Liu C, Li A-R, Yang X-Y, Baskin C (2020). Effect of temperature and moist conditions on seed dormancy cycling of two sympatric limestone species, *Begonia guishanensis* and *Paraisometrum mileense,* in southern China. *Seed Science Research* **30**, 29–36. https://doi.org/10.1017/S0960258520000069

Received: 17 June 2019
Revised: 9 March 2020
Accepted: 9 March 2020
First published online: 14 April 2020

Keywords:
Begonia guishanensis; cold stratification; dry afterripening; endemic species; nitrate; *Paraisometrum mileense* seed dormancy cycling; wet and dry cycles

Author for correspondence:
Xiang-Yun Yang,
E-mail: yxy@mail.kib.ac.cn

Effect of temperature and moist conditions on seed dormancy cycling of two sympatric limestone species, *Begonia guishanensis* and *Paraisometrum mileense,* in southern China

Xiao-Jian Hu[1], Cheng Liu[1], Ai-Rong Li[2], Xiang-Yun Yang[1] and Carol Baskin[3,4]

[1]The Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China; [2]Department of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China; [3]Department of Biology, University of Kentucky, Lexington, KY 40546, USA and [4]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA

Abstract

Information about seed dormancy cycling and germination in relation to temperature and moisture conditions in the natural environment is important for the conservation and restoration of rare species, including *Begonia guishanensis* and *Paraisometrum mileense*, two sympatric perennial limestone (karst) species. Dry afterripening (DAR) and wet and dry (WD) cycles at 15/5 and 25/15°C as well as moist chilling (MC) at 15/5°C were used to mimic the natural environment at different times of the year. A field experiment was conducted to monitor seasonal changes in germination responses of the seeds. About 40–65% of *B. guishanensis* and 5% of *P. mileense* seeds were dormant at maturity. DAR at 25/15 and 15/5°C as well as MC and WD cycles at 15/5°C alleviated dormancy for *B. guishanensis* but not *P. mileense*, and WD cycles at 25/15°C induced a deeper conditional dormancy for both species. Seeds of *B. guishanensis* exhibited dormancy cycling in the field, with increased dormancy under natural WD cycles at relatively high temperatures during the transition from the dry to the wet season in April to May and decreased dormancy during the wet season from June to October. KNO_3 mitigated the dormancy-inducing effect of both artificial and natural WD cycles at relatively high temperatures for *B. guishanensis.* The field experiment indicated that seeds of *B. guishanensis* may be able to form a persistent soil seed bank, while almost all seeds of *P. mileense* germinate at the beginning of the wet season in the field.

Introduction

China has one of the world's largest karst regions, which mainly occurs in Yunnan, Guizhou and Guangxi Provinces (Tong et al., 2018). An abundance of endemic plant species with high extinction risks is found in this region (Chen et al., 2014), and the karst ecosystem is considered to be sensitive and vulnerable (Zhu et al., 2003). The biodiversity and conservation of this ecosystem is of great interest to the government and scientists, and many projects concerning plant conservation and restoration have been conducted (Tong et al., 2018). In order to use seeds in restoration projects, information about seed dormancy and germination is required (Baskin and Baskin, 2014). In addition, seeds in the soil seed bank are considered to play an important role in restoration efforts (Baskin and Baskin, 2014). To date, however, little work has been done on seed ecology, as it relates to habitat preservation/restoration in this region. Further, no studies have been done on germination responses of seeds exposed to natural seasonal environmental changes or on the soil seed bank dynamics of species endemic to the vulnerable karst ecosystem in south-west China.

Begonia guishanensis S. H. Huang & Y. M. Shui (Begoniaceae) is endemic to mixed forests on limestone hills in Yunnan, China (Huang and Shui, 1994), and *Paraisometrum mileense* W. T. Wang (Gesneriaceae), belonging to the monotypic genus *Paraisometrum,* is endemic to south-west China (Yunnan, Guizhou and Guangxi) (Weitzmann et al., 1997). According to Shimizu (1963) and Chin (1977), these two species are 'plants exclusive to limestone habitat', thus persistence of their populations is dependent on the stability of their natural habitat (Zhu, 2007). Due to increased agriculture activities and urbanization, the natural limestone habitats are fragmented. Those two species coinciding in one place (Shilin County, Yunnan Province). Both species are herbaceous perennials but have different growth habits. The aboveground organs of *B. guishanensis* emerge only in the wet season during late May to late October. On the other hand, plants of *P. mileense* are evergreen and can survive extreme water loss down to below 10% relative water content; thus, they are considered to be resurrection plants (Li et al., 2014). Despite the drought tolerance of *P. mileense*, it is listed as one of the 'plant species with extremely small populations' and is critically endangered (Ma et al., 2013). Seeds of *B. guishanensis* are obovoid, c.a. 372 µm in length and 236 µm in width (Yang et al., 2015) and are considered to have little or no endosperm (McDonald and Kwong, 2005; Tebbitt and Garden, 2005). The seed length and width of *P. mileense* are 590 ± 60 and 280 ± 20 µm, respectively (Liu et al., 2015), and have only one or several cell layers of endosperm (Imaichi et al., 2000). Embryos of both species are fully developed (Hu et al., 2012; Liu et al., 2015). Seeds of both species are dispersed at the end of the wet season, and they are known to have non-deep physiological dormancy (PD) (Hu et al., 2012; Liu et al., 2015). However, dormancy levels of *P. mileense* seeds are usually low and are expressed only at temperatures below 20°C (Liu et al., 2015). GA_3, dry afterripening (DAR) and moist chilling (MC) are able to break the dormancy, and KNO_3 significantly enhances the germination of both species (Hu et al., 2012; Liu et al., 2015).

Depending on the species, seeds with non-deep PD may cycle between full dormancy (no germination at any condition) and non-dormancy (germination over a wide range of conditions) or between conditional dormancy (germination over a limited range of conditions) and non-dormancy several times before they eventually germinate or die (Baskin and Baskin, 2014). Many studies have been carried out to determine the environmental factors that influence dormancy cycles. Temperature, light or darkness, soil moisture condition, gaseous environment, and organic and inorganic chemicals may play a role in dormancy cycling, but temperature and soil moisture are the most critical (Baskin and Baskin, 2014). However, the main factors driving dormancy cycling vary with the species, and only a small study has examined the interactions of two or more factors (Cao et al., 2013).

Based on the meteorological data from 1990 to 2008 for Shilin County, Yunnan Province (the area of common distribution for the two species), we summarized several possible combinations of temperature and moisture conditions that seeds may experience in the field after dispersal (Table 1). From November to February, rainfall is very scarce (98 mm) and is accompanied by low temperatures (5.5–17.8°C); thus, seeds during this period may experience MC, DAR and wet and dry cycles (WD cycles) at low temperatures, and the WD cycles can be considered as MC interrupted by DAR events. From late April to early June, temperatures range from 15.0 to 24.8°C, and precipitation is still sporadic. During this time, seeds in the field may experience DAR and WD cycles at relatively high temperatures. Although the temperature is as high as that during the wet season and is favourable for germination, the imbibition phases are not long enough to promote germination. After the start of the wet season, temperatures do not change much (17.3–24.6°C), but rainfall becomes abundant, at which time seeds will experience suitable temperature and moisture conditions for germination. However, based on our observations, seedlings of *B. guishanensis* are rarely found, while those of *P. mileense* are relatively abundant in their natural habitat during the wet season.

We speculated that seed dormancy might play a role in the rarity of *B. guishanensis* seedlings during the wet season, and this dormancy could be induced during the transition between the dry and the wet season. DAR at room temperature and MC at 5°C are able to break dormancy for seeds of *B. guishanensis* (Hu et al., 2012), but the effects of WD cycles at relatively high temperatures are unknown for both species. Also, the effects of DAR and MC under the natural temperatures (5.5–17.8°C) that occur at the beginning of the dry season have not been determined for either species. Depending on the species, WD cycles may promote, depress or have no effect on seed germination (Kastner et al., 1981; Bradford et al., 1990; Allen et al., 1993; Ren and Tao, 2003; Santini et al., 2017; Lima and Meiado, 2018). Hoyle et al. (2008) showed that WD cycles under high summer temperatures alleviated dormancy for seeds of several species from south-west Queensland, Australia, and the effects of WD cycles on some species were better for breaking dormancy than dry afterripening (DAR) and MC under the same temperature regimes. These results suggest that mimicking the temperature and moisture conditions that seeds experienced in the field after dispersal can provide a better understanding of dormancy-breaking and germination requirements.

For non-deep physiologically dormant (PD) seeds that exhibited dormancy cycling, if dormancy is alleviated by low winter temperatures, then high summer temperatures may induce dormancy or conditional dormancy. Thus, the primary (conditional) dormancy of *B. guishanensis* and *P. mileense* seeds should be alleviated during the cool, dry season, resulting in germination in the warm, wet season. In support of this idea, the previous work has shown the dormancy releasing effect of MC at 5°C and DAR at room temperature (Hu et al., 2012; Liu et al., 2015). Thus, we hypothesized that MC, DAR and WD cycles under low temperatures (e.g. 15/5°C) would break the dormancy *B. guishanensis* and *P. mileense* seeds, while WD cycles under high temperatures (e.g. 25/15°C) will induce conditional dormancy.

To determine if the seeds of *B. guishanensis* and *P. mileense* undergo dormancy cycling and the possible driving factors, we carried out experiments on DAR, MC and WD cycles in the laboratory and conducted a field experiment in the Kunming Botanical Garden (Yunnan Province), where the climate is quite similar to that in the natural habitat of *B. guishanensis* and *P. mileense*. The aims of this research were to determine: (1) the effect of WD cycles, constant dry (can be considered as DAR) and constant wet (can be considered as incubation or MC) under different temperature regimes on seed dormancy and germination; and (2) if seeds undergo dormancy cycling in the natural environment.

Materials and methods

Ripe capsules of *B. guishanensis* and *P. mileense* were collected in Shilin County in Yunnan Province on 22 October 2013. All seeds were given 3 weeks of DAR at room temperature before initiation of experiments. Only round, fully developed seeds were used in the experiments.

WD cycles, DAR and MC treatment

To determine the effects of WD cycles, DAR and MC treatment on seed dormancy, seeds of both species were incubated at 15/5°C and/or 25/15°C. The 15/5°C regime simulates field temperatures during the early part of the dry season and 25/15°C the latter part of the dry season and wet season (Table 1). For WD cycles, seeds were first sown on water-saturated filter papers in 90-mm Petri dishes with the lid on and sealed in transparent plastic bags at both temperature regimes. After 2 d, the Petri dishes were taken out of the plastic bag, the lids removed, and seeds were allowed to dry at each temperature for 5 d. The duration of one WD cycle was 1 week. For the DAR treatment, seeds were placed on dry filter paper in 90-mm Petri dishes with lid

Table 1. Summary of maximum and minimum air temperatures and rainfall data in Shilin County, Yunnan Province, China, from 1990 to 2008 (China Meteorological Scientific Data Sharing System)

Period	Average maximum air temperature (°C)	Average minimum air temperature (°C)	Average number of days with rain	Average precipitation (mm)
11.1–3.1	17.8	5.5	12.8	98
4.21–6.10	24.8	15.0	14.1	155
6.11–8.30	24.6	17.3	39.0	512

off at both temperatures. For the MC treatment, seeds were sowed on 1% agar water medium in 90-mm Petri dishes with lid on and were sealed in transparent plastic bags at only 15/5°C because some seeds of both species can germinate at 25/15°C (Hu et al., 2012; Liu et al., 2015). For all treatments, the photoperiod was 12 h light (22.2 µmol $m^{-2}s^{-1}$ illumination by cool white fluorescent light) and 12 h dark. All treatments were given for 0, 2, 4 and 8 weeks. One 90-mm Petri dish containing 1500 (for *B. guishanensis*) or 750 seeds (for *P. mileense*) was used, and after 2, 4 and 8 weeks, 480 (for *B. guishanensis*) or 240 seeds (for *P. mileense*) were picked out for each treatment and tested for germination (see below).

Field experiment

For each species, 60 (for *B. guishanensis*) or 30 (for *P. mileense*) seeds were placed in each of 33 nylon cloth bags and on 14 November 2013 were placed on top of six pots (about 20 cm in dimension) filled with soil taken from the natural habitat of the two species. The pots were set placed in the Kunming Botanical Garden, which is located about 100 km from the seed collection site. The altitude of the Garden and the seed collection was about 2000 m a.s.l. A double-layer of black mash net was set 2.5 m above the pots to reduce sunshine and the impact of falling raindrops. At 30-day intervals, three bags of seeds for each species were retrieved and tested for germination in the laboratory (see below).

Germination tests

After WD cycles, DAR and MC treatments and various periods in the garden, seeds of both species were tested for germination in light (12 h of 22.2 µmol $m^{-2}s^{-1}$ cool white fluorescent light and 12 h dark each day) at 20, 25, 15/25 (night/day) and 20/30°C (night/day). Seeds of *B. guishanensis* were placed on 1% agar/water medium (plain agar) or 1% agar/water medium with 90 mg l^{-1} KNO_3, while those of *P. mileense* were placed on plain agar. Three replicates of 20 seeds each were used for each test condition. The Petri dishes were placed in transparent plastic bags to prevent desiccation.

Germination was monitored every 7 d, and seeds with a radicle that exceeded one-third the total seed length were considered to be germinated. The Petri dishes were randomly rearranged on checking days. The germination test ran for a minimum of 4 weeks, after which if no additional seeds germinated for 2 continuous weeks, the test was terminated. At the end of each test, all non-germinated seeds were cut open to determine if they contained an embryo and if it was viable; firm white embryos were recorded as viable and soft and/or rotten ones as nonviable.

Statistical analysis

Germination percentage (GP) used for dormancy assessment was calculated as follows:

$$\mathrm{GP} = \sum \frac{ni}{(N - Ne - Nd)} \times 100\%$$

and viability was calculated as follows:

$$V = \frac{(N - Ne - Nd)}{N} \times 100\%$$

where N is the total number of seeds tested, Ne the number of empty seeds, Nd the number of decayed seeds and ni the number of germinated seeds. If the viability was below 70% (which means more than 30% of the seeds tested in that treatment were empty and\or decayed), germination data for that treatment were excluded from dormancy assessment and analysis. Germination data were analyzed using one-way ANOVA for different treatments at different germination temperatures and on different medium separately to determine the effect of different treatments on germination at a certain incubation temperature on each medium. Data were arcsine square-root transformed if necessary to meet the assumptions of ANOVA for normality and homogeneity of variance. A least significant difference post-hoc test was used for multiple comparisons. Differences obtained at a level of $P < 0.05$ were considered to be significant. All statistical analyses were performed using SPSS 16.0 for windows (SPSS, Chicago, IL).

Results

Effect of WD cycles, MC and DAR

Seed viability for all treatments was above 70% for both species. The initial germination of *B. guishanensis* seeds ranged from 23.8% at 25/15°C to 84.2% at 25°C on plain agar (Fig. 1) and from 81.1% at 30/20°C to 94.8% at 20°C on agar with KNO_3 (Fig. 2). WD cycles at 15/5°C significantly promoted germination at 20, 25/15 and 30/20°C on plain agar and at 25, 25/15 and 30/20°C on agar with KNO_3. However, WD cycles at 25/15°C significantly decreased germination at 20, 25, 25/15 and 30/20°C on plain agar (Fig. 1) and at 25/15°C on agar with KNO_3 (Fig. 2). MC at 15/5°C significantly promoted germination at 20, 25/15 and 30/20°C on plain agar and at 20, 25, 25/15 and 30/20°C on agar with KNO_3. DAR at 15/5°C promoted germination at 25 and 30/20°C on agar with KNO_3 but had no effect at 20 and 25/15°C regardless of media. DAR at 25/15°C significantly

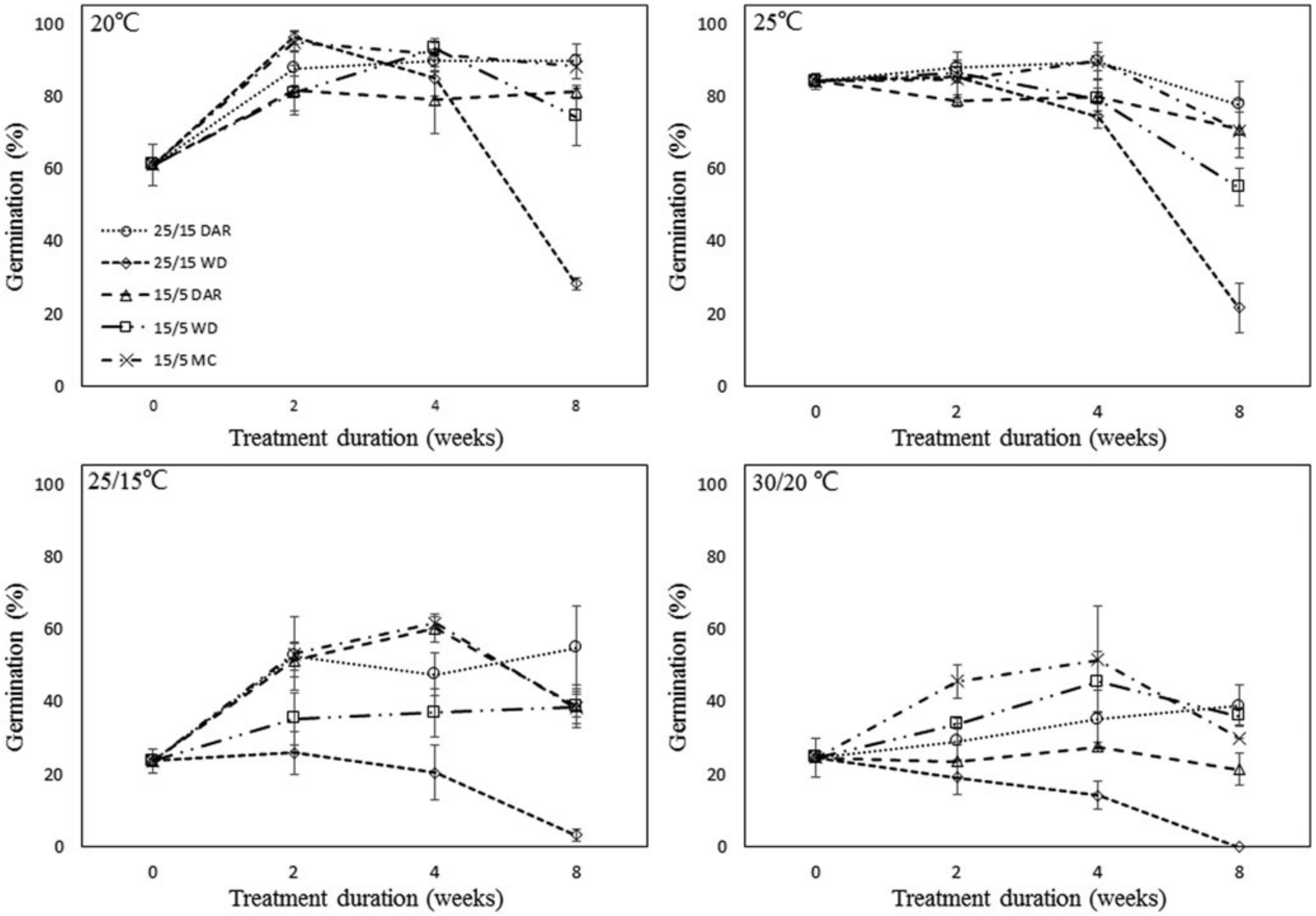

Fig. 1. Final germination (mean ± s.e.) of *B. guishanensis* seeds on plain agar in light at 20, 25, 25/15 and 30/20°C after different durations of DAR, WD and MC treatment under 25/15 and 15/5°C.

promoted germination at 20 and 25/15°C on plain agar (Fig. 1) and at 20, 25 and 30/20°C on KNO_3 agar (Fig. 2).

The initial germination of *P. mileense* was 100% at 20, 25 and 30/20°C and 96.7% at 25/15°C. Most of the treatments had no significant effect on GP at any test temperature, and only 8 weeks of WD cycles at 25/15°C showed a significant depressing effect on germination at 20 and 25/15°C (Fig. 3).

Field experiment

For *B. guishanensis*, the viability of seeds retrieved from the field on day 120 (14 March 2014) and then tested for germination ranged from 0% at 30/20°C to 63.3% at 20°C. Also, the viability of *P. mileense* seeds retrieved from the field on day 180 (13 May 2014) and tested at 25 and 30/20°C was 28.3% and 20.0%, respectively. The loss of viability during field burial may distort the effect of dormancy change on germination, thus germination data for seeds with <70% viability were excluded from the analysis. For *B. guishanensis*, no seeds germinated in the cloth bags during field burial, while for *P. mileense*, no germinated seeds were found until day 210 (12 June 2014). On day 210, although a few seeds had germinated in the cloth bag, there were at least 60 non-germinated seeds that could be used for the germination test. On day 240 (12 July 2014), most of the *P. mileense* seeds had germinated, so germination tests could not be performed in the laboratory.

Germination percentages of *B. guishanensis* seeds retrieved before mid-May were not significantly changed compared to the control on agar with KNO_3, but the germination of the seeds retrieved on 13 May 2014 (day 180) was significantly decreased at all temperatures. Germination on agar with KNO_3 of seeds retrieved after 12 June 2014 at all temperatures had returned to the initial level, which was around 90% (Fig. 4).

Germination percentages of *B. guishanensis* seeds retrieved on day 30 (14 December 2013) to day 90 (12 February 2014) and placed on plain agar were increased significantly at 20°C but were depressed significantly at 25°C with germination ranging from 52.8% to 71.7%. GP of *B. guishanensis* seeds retrieved on day 180 (13 May 2014) was significantly decreased at all temperatures on plain agar, and only germination at 25°C recovered gradually to the initial level, which was around 80% after day 270 (11 August 2014) (Fig. 4).

Germination percentages of *P. mileense* retrieved on 12 February 2014 and tested at 25°C, on 13 May at 20°C and on 12 June at 30/20°C were significantly lower than those of the control (Fig. 5).

Discussion

Our hypothesis that WD cycles, MC and DAR at low temperatures can alleviate dormancy, while WD cycles at high temperatures can induce conditional dormancy that was supported by

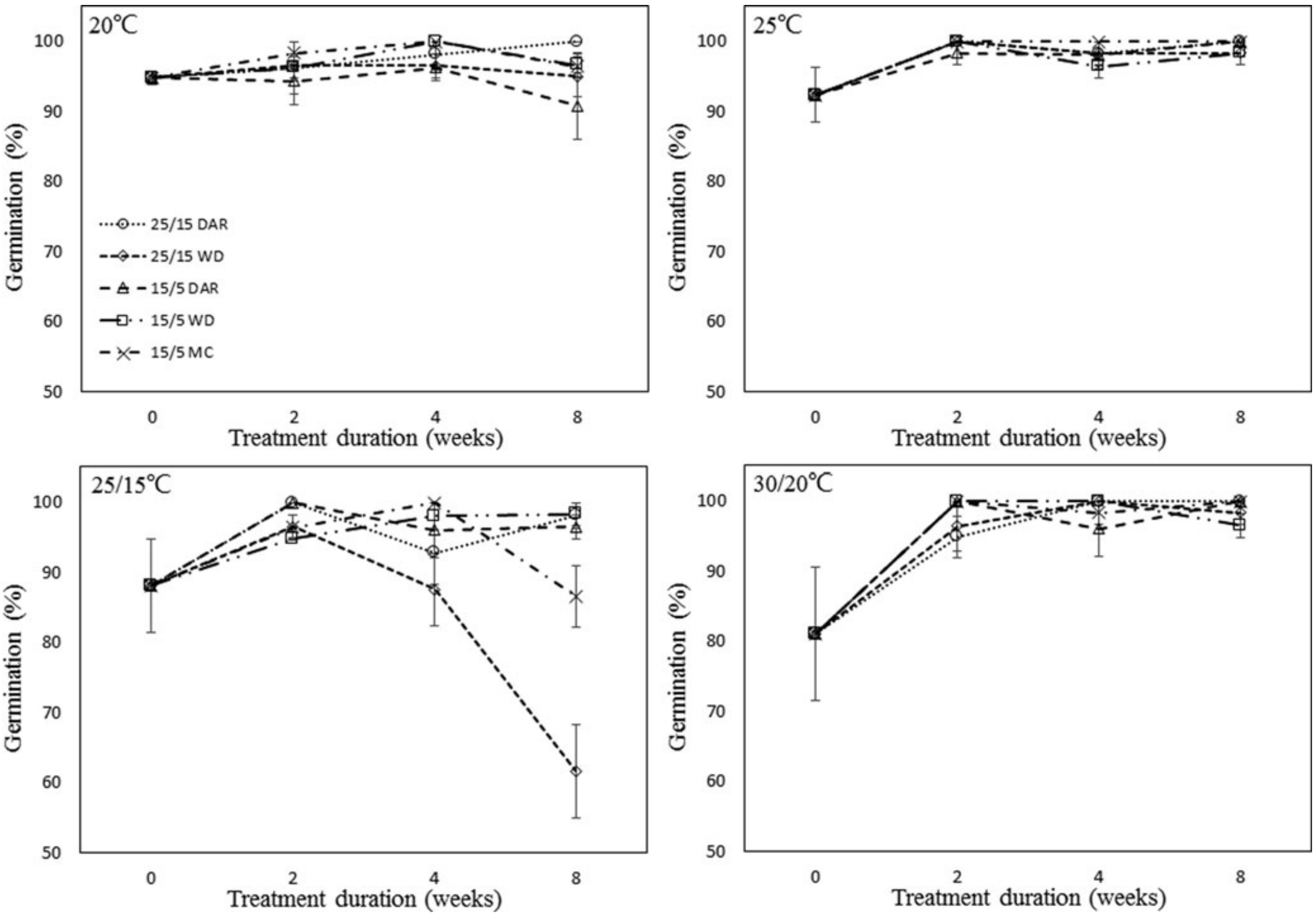

Fig. 2. Final germination (mean ± s.e.) of *B. guishanensis* seeds on agar with KNO_3 in light at 20, 25, 25/15 and 30/20°C after different durations of DAR, WD and MC treatment under 25/15 and 15/5°C.

the results for *B. guishanensis* seeds and partly by those for *P. mileense* seeds. This is the first report of dormancy induction by WD cycles at relatively high temperatures, which are also suitable for seed germination. However, in the case of *B. guishanensis*, DAR at both low and high temperatures alleviated seed dormancy, although DAR at 25/15°C was more effective than at 15/5°C (Figs 1 and 2). These results are consistent with the thermal afterripening time model for other species such as *Bromus tectorum* (Bauer et al., 1998), *Elymus elymoides* (Meyer et al., 2000) and *Helianthus annuus* (Bazin et al., 2010), in which dormancy loss increased with the temperature above a threshold.

MC was effective in breaking dormancy of *B. guishanensis* seeds. MC for 2 and 4 weeks was usually better than, or as effective as, DAR at 25/15°C, while 8 weeks of MC was less effective than DAR at 25/15°C (Figs 1 and 2). The reduced effectiveness of prolonged MC also was reported for other species such as *Pedicularis rex* and *P. rhinanthoides* (Ren and Guan, 2008). WD cycles at 15/5°C had a similar effect as MC at 15/5°C on promoting germination at 20 and 25°C (Figs 1 and 2). WD cycles at 15/5°C can be considered as an MC treatment interrupted by DAR since the effects of DAR at 15/5°C were usually not significant. The effect of WD cycles at 15/5°C was also less effective compared to MC at 15/5°C (Figs 1 and 2). However, it is clear that seed dormancy of *B. guishanensis* can be released by WD cycles, MC and DAR at low temperatures in the field during the early cool and dry season. Unlike 15/5°C, 25/15°C is a temperature at which seeds of *B. guishanensis* can germinate. Thus, WD cycles at 25/15°C can be considered as incubation interrupted by DAR. Although DAR at 25/15°C was more effective than at 15/5°C on breaking dormancy, the effect of WD cycles at 25/15°C generally was suppressive (Figs 1 and 2), implying that inadequate rainfall during the latter (warmer) part of the dry season in the field could induce seeds of *B. guishanensis* into a deeper state of conditional dormancy.

Some, but not all, species with non-deep PD exhibit dormancy cycles if seeds are exposed to natural seasonal environmental changes, especially temperature (Baskin and Baskin, 2014). Depending on the species, non-deep PD can be broken by high summer temperatures or by low winter temperatures (Baskin and Baskin, 2014). If seeds with non-deep PD exhibit dormancy cycling in response to changes in the seasonal environmental conditions, the time of dormancy varies with the species. If high summer temperatures break PD, then low temperatures of winter induce dormancy or conditional dormancy, which is broken the following summer (Baskin and Baskin, 1983). If low winter temperatures break PD, then high temperatures of summer induce dormancy or conditional dormancy, which is broken by following winter (Baskin and Baskin, 1980).

In the field experiment, seeds of *B. guishanensis* exhibited a dormancy cycle and were less dormant in February, which is the middle of the dry season, and they were mostly dormant in June, which is the beginning of the wet season (Fig. 4). These

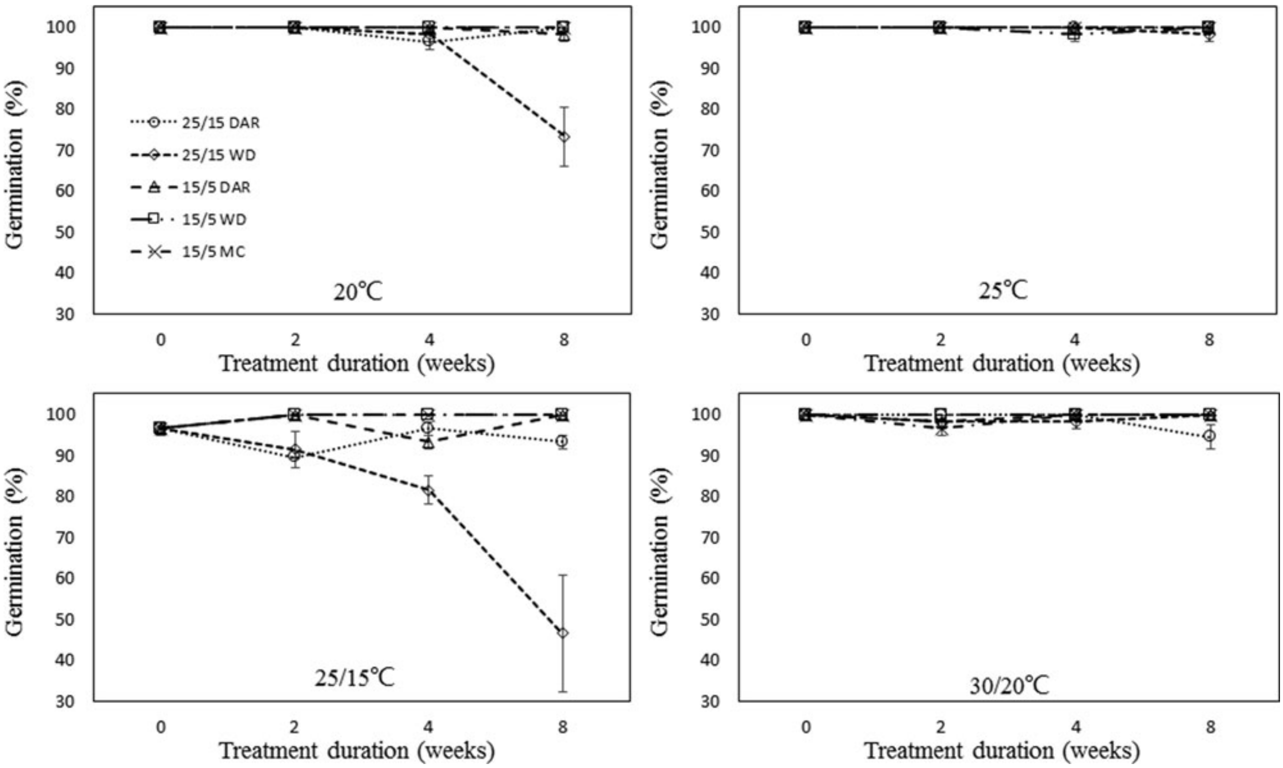

Fig. 3. Final germination (mean ± s.e.) of *P. mileense* seeds on plain agar in light at 20, 25, 25/15 and 30/20°C after different durations of DAR, WD and MC treatment under 25/15 or 15/5°C.

changes in dormancy correspond to changes in the environment (Fig. 4). According to the meteorological record during the experiment, before day 120 (14 March 2014), precipitation was sporadic, and the average temperature ranged roughly from 5 to 15°C (Table 1 and Fig. 4). Thus, the seeds in the field may have experienced DAR, WD cycles or stratification at relatively low temperatures, which usually have a dormancy-breaking effect for *B. guishanensis*. However, although the germination of *B. guishanensis* at 20°C on plain agar was promoted significantly during the field experiment at this early dry season period, germination at other temperatures on both medium did not show a very positive effect (Fig. 4).

We speculated that WD cycles under the relatively low temperatures that seeds experienced in the field might be different from those we gave in the laboratory experiment. For example, the dry period in the field may be longer than 5 d because rainfall is very sporadic during this period (Table 1 and Fig. 4). The effect of DAR at 15/5°C was less effective than MC at 15/5°C, thus the prolonged dry phase may be one of the reasons for the poor dormancy alleviation in the field experiment. From late April to early June, the average temperature ranged roughly from 15 to 25°C, but the precipitation was not abundant (Table 1 and Fig. 4). Thus, seeds in the field may experience DAR and WD cycles at high temperatures, the former has an enhancing effect while the latter has a suppressing effect on germination. The results of the field experiment suggest that the effect of WD cycles at high temperatures may have been prominent during this period especially between days 150 (13 April) and 180 (13 May) (Fig. 4) and that WD cycles at higher temperatures induced seeds of *B. guishanensis* into a deeper conditional dormancy. From early June to early September, temperatures roughly ranged from 15 to 25°C, and precipitation was abundant (Table 1 and Fig. 4). Thus, during this period, seeds may experience a suitable temperature and moisture condition for germination in the field, but none of the *B. guishanensis* seeds in bags in the field germinated. Also, the germination of retrieved seeds on plain agar was decreased at all temperatures except 25°C (Fig. 4). However, their induced dormancy was released during the rainy period (Fig. 4), suggesting that secondary dormancy can be alleviated by warm stratification during the wet season when the temperature is around 20°C.

For seeds of *B. guishanensis*, KNO_3 mitigated the suppressive effect of both artificial and natural WD cycles at high temperatures at all the germination test temperatures (Figs 1, 2 and 4). Thus, KNO_3 may play an important role in the field for seed germination of this species. Physiologically, KNO_3 is considered to be a germination cue or a dormancy releasing factor (Finch-Savage and Leubner-Metzger, 2006). Ecologically, KNO_3 is considered to be an indication of disturbance and gap formation, and it usually is affected by other environmental factors such as temperature fluctuation and light conditions (Fenner and Thompson, 2005). However, in the case of *B. guishanensis*, seeds have a negative response to temperature fluctuation, as germination on plain agar at 25/15 and 30/20°C is lower than at the constant temperatures 20 and 25°C (Figs 1 and 2). The natural habitat of *B. guishanensis* is under the canopy where the temperature is relatively stable, thus the combination of a constant temperature and KNO_3 may indicate low competition under a canopy coverage (Hu et al., 2012).

The results of the field experiment suggest that the suppressive effect on the germination of WD cycles at high temperatures (e.g. 25/15°C) may play a role in the rarity of *B. guishanensis* seedlings

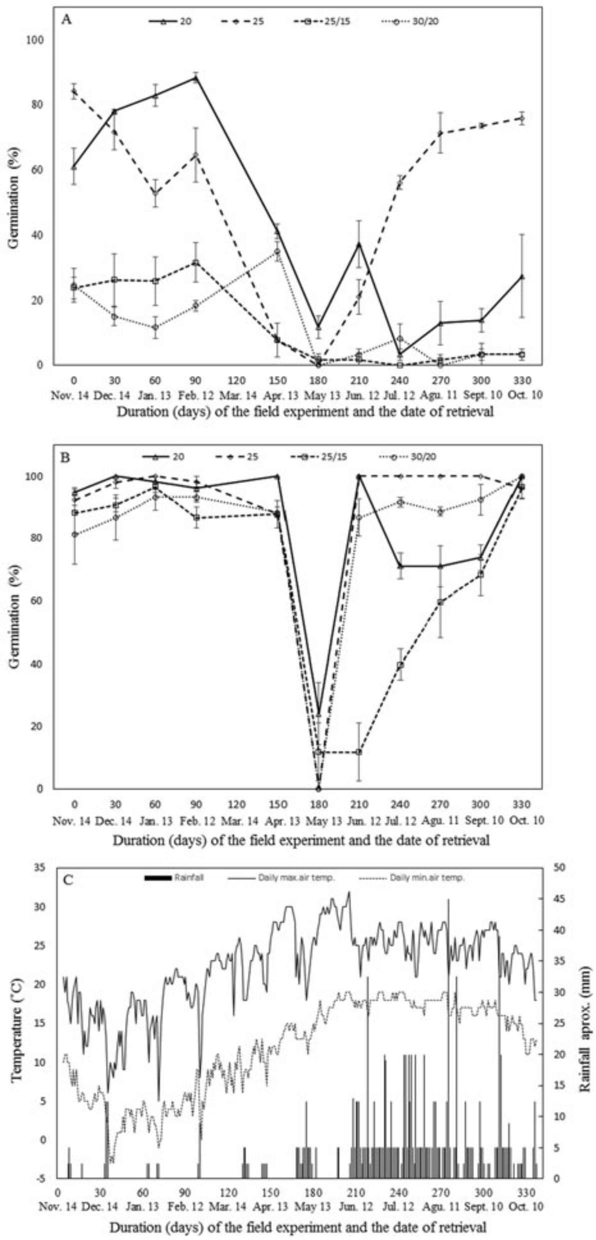

Fig. 4. Final germination (mean ± s.e.) of *B. guishanensis* seeds on plain agar (A) and agar with KNO_3 (B) at 20, 25, 25/15 and 30/20°C after different durations in the field (C). Daily maximum and minimum air temperatures and daily rainfall of Kunming during the field experiment.

in the field. Seeds were conditionally dormant (CD) at the beginning of the rainy season and could not germinate. Although CD was released gradually during the rainy season, the lack of micro-environmental conditions that can provide a constant temperature (e.g. 25°C) and/or nitrate may result the poor seedling emergence in the field for *B. guishanensis*. We speculate that the usually intermittent rainfall during the transition from the dry to the wet season induces secondary dormancy and thus is detrimental for the seedling emergence of *B. guishanensis*. Consequently, the successful seedling establishment of *B. guishanensis* probably requires a microenvironment that could prevent desiccation during the transition period and provide a canopy coverage with reduced competition for nitrate.

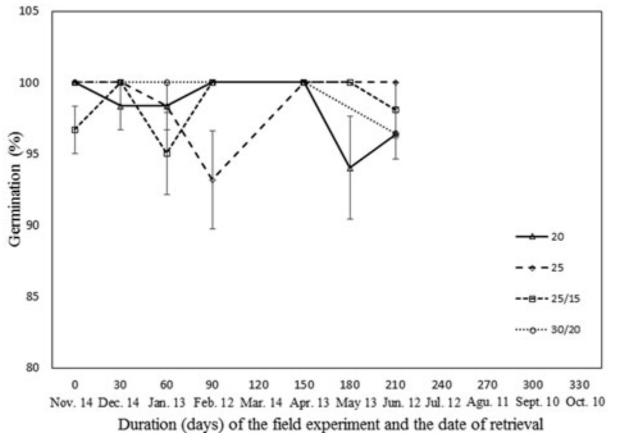

Fig. 5. Final germination (mean ± s.e.) of *P. mileense* seeds on plain agar at 20, 25, 25/15 and 30/20°C after different durations in the field.

GP of *P. mileense* did not change much during the field experiment. Although some significant differences were detected, the reduced germination was still above 90% (Fig. 5). Further, germination percentages of *P. mileense* did not show any significant response to most of the dormancy-breaking treatments (Fig. 3), which is consistent with the former report that seed dormancy in this species is very shallow and can be detected only below 20°C (Liu et al., 2015). However, WD cycles at 25/15°C had a significant depressing effect on the germination of seeds tested at 20 and 25/15°C (Fig. 3). In both the laboratory and field, WD cycles at relatively high temperatures induced a deeper CD in seeds of *P. mileense*. Some seeds of *P. mileense* had germinated in the field on day 210, indicating that a portion of the seeds started to germinate immediately after the onset of the rainy season, implying that the induced CD was very superficial.

Although on day 240 most seeds of *P. mileense* had germinated inside the bags in the field, none of the *B. guishanensis* seeds germinated during burial. It has been reported that seeds of *B. guishanensis* need light to germinate (Ma et al., 2005), so the light requirement may also have contributed to the lack of germination of *B. guishanensis* seeds in the cloth bags during the field experiment. Apparently, the germination requirements for seeds of *B. guishanensis* are more rigorous than those of *P. mileense*. Based on these results, we conclude that seeds of *B. guishanensis* may form a persistent seed bank, while those of *P. mileense* do not because they all germinated at the beginning of the wet season. Since *P. mileense* is an evergreen perennial with a high desiccation tolerance (Li et al., 2014) and the above-ground parts of *B. guishanensis* plants cannot survive the dry season, we assume that seedlings of *P. mileense* are probably more tolerant to drought and/or cold than those *of B. guishanensis*.

With respect to conservation, it is important to preserve plants of *P. mileense* in the field because if most of the individuals of a population are harvested or die, it will be difficult for the population to recover due to the potential lack of a persistent seed bank for seedling recruitment. *Ex situ* conservation such as seed banking is also necessary for *P. mileense*. For *B. guishanensis*, the maintenance of a suitable habitat and the avoidance of intensive competition (e.g. for nitrate) seem essential for seedling establishment. If those two species need to be re-introduced to the field by seeds, different timing of seed sowing should be considered. For *B. guishanensis*, it is preferable to sow the seeds after the beginning of the wet season using seeds that have afterripened, while for *P. mileense* sowing at the beginning of the wet season would give seedlings the maximum period of time for growth before the dry season begins.

In summary, seeds of *B. guishanensis* showed the evidence of dormancy cycling in the field experiment, while those of *P. mileense* did not show clear evidence for dormancy cycling. The induction of dormancy was correlated with the occurrence of natural WD cycles under relatively high temperatures during the transition from the cool, dry to the warm, wet season. WD cycles at 25/15°C induced dormancy in seeds of both species, while WD cycles at 15/5°C broke dormancy of *B. guishanensis* but not *P. mileense* seeds. KNO_3 mitigated the depressing effect of both artificial and natural WD cycles under relatively high temperatures for *B. guishanensis*. Most freshly matured seeds of *P. mileense* were nondormant. DAR at 25/15 and 15/5°C broke dormancy for *B. guishanensis*, with DAR at 25/15°C being more effective than at 15/5°C. MC at 15/5°C also has a positive effect on dormancy break for *B. guishanensis*. Seeds of *B. guishanensis* may be able to form a persistent soil seed bank, while these of *P. mileense* germinate with the onset of the wet season.

Acknowledgements. The authors would like to thank the two anonymous reviewers and Dr Peng De-Li for their advice on the revision of the manuscript. The first author thanks Mr Guo Yun-Gang and Ms Li Mei-Juan for their assistance in the experiments.

Financial support. This study was supported by the Science & Technology Basic Resources Investigation Program of China (Grant No. 2017FY100100) and was conducted using the facilities of Large Research Infrastructures User Service Platform, Chinese Academy of Sciences.

References

Allen P, White D and Markhart A (1993) Germination of perennial ryegrass and annual bluegrass seeds subjected to hydration–dehydration cycles. *Crop Science* **33**, 1020–1025.

Baskin J and Baskin C (1980) Ecophysiology of secondary dormancy in seeds of *Ambrosia artemisiifolia*. *Ecology* **61**, 475–480.

Baskin J and Baskin C (1983) Seasonal changes in the germination responses of buried seeds of *Arabidopsis thaliana* and ecological interpretation. *Botanical Gazette* **144**, 540–543.

Baskin C and Baskin J (2014) *Seeds: ecology, biogeography, and evolution of dormancy and germination* (2nd ed). San Diego, CA, Elsevier/Academic Press.

Bauer MC, Meyer SE and Allen PS (1998) A simulation model to predict seed dormancy loss in the field for *Bromus tectorum* L. *Journal of Experimental Botany* **49**, 1235–1244.

Bazin J, Batlla D, Dussert S, El-Maarouf-Bouteau H and Bailly C (2010) Role of relative humidity, temperature, and water status in dormancy alleviation of sunflower seeds during dry after-ripening. *Journal of Experimental Botany* **62**, 627–640.

Bradford KJ, Steiner JJ and Trawatha SE (1990) Seed priming influence on germination and emergence of pepper seed lots. *Crop Science* **30**, 718–721.

Cao D, Baskin CC, Baskin JM, Yang F and Huang Z (2013) Dormancy cycling and persistence of seeds in soil of a cold desert halophyte shrub. *Annals of Botany* **113**, 171–179.

Chen W-H, Shui Y-M, Yang J-B, Wang H, Nishii K, Wen F, Zhang Z-R and Möller M (2014) Taxonomic status, phylogenetic affinities and genetic diversity of a presumed extinct genus, *Paraisometrum* WT Wang (Gesneriaceae) from the karst regions of Southwest China. *PLoS One* **9**, e107967.

Chin SC (1977) Limestone Hill flora of Malaya. I. Gardens' *Bulletin Singapore* **30**, 165–219.

Fenner M and Thompson K (2005) *The ecology of seeds*. Cambridge, Cambridge University Press.

Finch-Savage WE and Leubner-Metzger G (2006) Seed dormancy and the control of germination. *New Phytologist* **171**, 501–523.

Hoyle GL, Daws MI, Steadman KJ and Adkins SW (2008) Mimicking a semi-arid tropical environment achieves dormancy alleviation for seeds of Australian native Goodeniaceae and Asteraceae. *Annals of Botany* **101**, 701–708.

Hu XJ, Yang LH and Guan KY (2012) Physiological dormancy in seeds of two endemic species of *Begonia* from Yunnan Province, China: diagnosis and classification. *Plant Species Biology* **27**, 201–209.

Huang S and Shui Y (1994) New taxa of *Begonia* from Yunnan. *Acta Botanica Yunnanica* **16**, 333–342.

Imaichi R, Nagumo S and Kato M (2000) Ontogenetic anatomy of *Streptocarpus grandis* (Gesneriaceae) with implications for evolution of monophylly. *Annals of Botany* **86**, 37–46.

Kastner WW Jr, Goebel CJ and Maguire JD (1981) Effects of a wet-dry seed treatment on the germination and root elongation of "Whitmar" beardless wheatgrass under various water potentials. *Journal of Range Management* **34**, 305–307.

Li A, Wang D, Yu B, Yu X and Li W (2014) Maintenance or collapse: responses of extraplastidic membrane lipid composition to desiccation in the resurrection plant *Paraisometrum mileense*. *PLoS One* **9**, e103430.

Lima A and Meiado M (2018) Effect of hydration and dehydration cycles on *Mimosa tenuiflora* seeds during germination and initial development. *South African Journal of Botany* **116**, 164–167.

Liu C, Qin SF and Hu XJ (2015) Dormancy and germination of *Paraisometrum mileense* and their ecological implications. *Plant Diversity and Resources* **37**, 278–282.

Ma H, Li HZ and Guan KY (2005) Study on characters of seed germination of four species of *Begonia* from China. *Seed* **24**, 10–13.

Ma Y, Chen G, Grumbine RE, Dao Z, Sun W and Guo H (2013) Conserving plant species with extremely small populations (PSESP) in China. *Biodiversity and Conservation* **22**, 803–809.

McDonald MB and Kwong FY (2005) *Flower seeds: biology and technology*. Oxfordshire, UK, CABI Publishing.

Meyer SE, Debaene-Gill SB and Allen PS (2000) Using hydrothermal time concepts to model seed germination response to temperature, dormancy loss, and priming effects in *Elymus elymoides*. *Seed Science Research* **10**, 213–223.

Ren YQ and Guan KY (2008) Effects of moist-chilling and GA_3 applications on seed germination of three *Pedicularis* species from Yunnan, China. *Seed Science and Technology* **36**, 225–229.

Ren J and Tao L (2003) Effect of hydration–dehydration cycles on germination of seven *Calligonum* species. *Journal of Arid Environments* **55**, 111–122.

Santini BA, Rojas-Aréchiga M and Morales EG (2017) Priming effect on seed germination: Is it always positive for cacti species? *Journal of Arid Environments* **147**, 155–158.

Shimizu T (1963) Studies on the limestone flora of Japan and Taiwan II. *Journal of the Faculty of Textile Science and Technology Shinshu University* **12**, 1–85.

Tebbitt MC and Garden BB (2005) *Begonias: cultivation, identification, and natural history*. Porland, Timber Press.

Tong X, Brandt M, Yue Y, Horion S, Wang K, Keersmaecker WD, Feng T, Schurgers G, Xiao X and Luo Y (2018) Increased vegetation growth and carbon stock in China karst via ecological engineering. *Nature Sustainability* **1**, 44–50.

Weitzmann AL, Skog LE, Wang WT, Pan KY and Li ZY (1997) New taxa, new combinations, and notes on Chinese Gesneriaceae. *Novon* **7**, 423–435.

Yang Z, Chen W-H and Shui Y-M (2015) Seed micromorphology and its taxonomic significance of Begonia (Begoniaceae) in China and Vietnam. *Plant Diversity and Resources* **37**, 376–388.

Zhu H (2007) The karst ecosystem of southern China and its biodiversity. *Tropical Forestry* **35**, 44–47.

Zhu H, Wang H, Li B and Sirirugsa P (2003) Biogeography and floristic affinities of the limestone flora in southern Yunnan, China. *Annals of the Missouri Botanical Garden* **90**, 444–465.