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Seed dormancy and germination characteristics of *Astragalus arpilobus* (Fabaceae, subfamily Papilionoideae), a central Asian desert annual ephemeral

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Abstract

Although *Astragalus* is the largest genus of seed plants, it contains only a relatively few annual species, about which little is known with regard to seed dormancy and germination. Thus, seed dormancy-break and germination were investigated in the cold-desert annual *Astragalus arpilobus* Kar. et Kir. Most of the seeds had a water-impermeable seed coat that became permeable after mechanical or acid scarification, i.e. physical dormancy. Both wet heat and alternate wet heat and cold (ice water) made only a small portion of the seeds permeable, and neither exposure to high nor to low temperatures was effective in breaking dormancy. Scarified seeds germinated at temperatures ranging from 5/2 to 30/15 °C, with 20/10 °C and 25/15 °C being optimal. Non-dormant seeds germinated to high percentages in light and in darkness across the range of temperatures. Germination percentages did not increase with duration of dry storage at room temperature. Scarified seeds germinated to nearly 100% at water potentials between 0 and −0.30 MPa but to 0% at −0.77 MPa. Seedling emergence was higher for seeds buried at soil depths of 1 and 2 cm than at 0 or >2 cm. Forty-three percent of the scarified seeds sown in July in an experimental garden under natural conditions germinated in autumn, and an additional 5% germinated in spring. In contrast, no non-scarified seeds germinated in autumn, and only 5.3% of them germinated in spring. Most of the non-scarified seeds that did not germinate became part of the persistent seed bank. Our results indicate that the high intensity of hard-seed dormancy in the annual *A. arpilobus* is similar to that reported for the perennial species of *Astragalus*.

Keywords: *Astragalus*; Burial depth; Physical dormancy; Seed dormancy; Seed germination; Water potential

1. Introduction

Astragalus (Fabaceae, subfamily Papilionoideae) is a taxon-rich plant genus with about 2500 species, and it is the largest genus of seed plants. Species may be found growing in steppes, prairies and other arid or semiarid habitats in North America, South America, Europe and Asia and on tropical African mountains (Mabberley, 2008; Scherson et al., 2008). Typically, the species are low-growing shrubs or herbaceous perennials, and relatively few of them are annuals. For example, about 120 species of *Astragalus* occur in Xinjiang Province, China, but only 10 of them are annuals (Fu et al., 1993).

For long-term persistence of a sexually-reproducing annual plant species at a particular site, seed germination, seedling establishment and seed production by mature plants must occur every year if a persistent seed bank is not present and at least occasionally if a persistent seed bank is present. Information on

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seed dormancy and the control of timing of germination is an important part of understanding how a species is adapted to its habitat. That is, seed dormancy-break and timing of germination are important components of plant life history strategies (Rees, 1997), and they may help modulate the distribution and abundance of a plant species (Handley and Davy, 2005).

Considering the huge size of the genus, seed dormancy has been studied in relatively few species of *Astragalus*, most of which are perennials. We found information in the literature on seed dormancy in 33 perennial taxa (species, subspecies, varieties), and all of them were reported to have water-impermeable seeds and thus physical dormancy (PY). In contrast, our search yielded information on seed dormancy in only two annual *Astragalus* species, *Astragalus hamosus* (Hammouda and Bakr, 1969; Patanè and Gresta, 2006) and *Astragalus sinicus* (Kim et al., 2008). Dormancy was broken in 100% of *A. hamosus* seeds scarified with sandpaper, 63% of seeds exposed to 15 min of wet heat at 70 °C and 70% of those exposed to 10 min of wet heat at 80 °C (Patanè and Gresta, 2006). Dormancy of *A. sinicus* seeds was effectively broken by concentrated sulfuric acid and heat treatments (Kim et al., 2008). These results suggest that both annual species of *Astragalus* have PY.

PY, which is due to a water-impermeable seed coat, occurs in seeds of 18 plant families of angiosperms (no gymnosperms) including the Fabaceae (Baskin et al., 2000, 2006; Koutsovoulou et al., 2005; Morrison et al., 1992, 1998). In addition to PY, seeds of a very small percentage of species with a water-impermeable seed coat also have physiological dormancy (PD), which is caused by low growth potential of the embryo, i.e. combinational dormancy (PY + PD). PY + PD is known to occur only in seeds of Fabaceae and seven other angiosperm families. In contrast to PY, PY + PD is uncommon in all species and vegetation zones on earth (Baskin and Baskin, 1998, 2003). The proportion of seeds in a given seed crop that develops impermeable coats can vary with the environmental conditions of the mother plant during seed development, degree of drying after the seed is fully developed and genetics (Baskin and Baskin, 1998). Different aspects of the biology of water-impermeable seeds have been reviewed by Rolston (1978).

Although legume seeds are known for their hard water-impermeable seed coats ("hardseededness"), seeds of some tropical legumes have relatively soft, water-permeable seed coats. Seeds of these tropical species are either non-dormant or have PD (Baskin and Baskin, 1998), and some of them are desiccation intolerant (recalcitrant) (Baskin and Baskin, 1998; Dickie and Pritchard, 2002). Since our literature review revealed that at least 33 perennial and two annual taxa of *Astragalus* have been reported to have seeds with PY, we hypothesized that annual species of *Astragalus* growing in the temperate desert of Xinjiang Province, China, also would have seeds with this kind of dormancy. More specifically, we wanted to know if the kind and intensity of seed dormancy in the annual ephemeral *Astragalus arpilobus* Kar. et Kir. are the same as, or similar to, those reported for perennial species of the genus. That is, are the dormancy-breaking and germination requirements the same or similar to those in annual and perennial species of *Astragalus*? Thus, the primary objective of this study was to investigate the seed germination biology of *A. arpilobus*. The following questions were addressed. (1) Are fresh seeds dormant, and if so what class of seed dormancy do they have? (2) What are the optimum conditions for germination of seeds after dormancy is broken? (3) What is the best artificial method to break dormancy? (4) Do seeds form a persistent seed bank in situ?

2. Material and methods

2.1. Study species, field site description and seed collection

A. arpilobus grows in disturbed desert habitats of Central Asia, Afghanistan, Eastern Europe and China (Fu et al., 1993). Of the 10 annual species of *Astragalus* in Xinjiang, *A. arpilobus* is the most widely distributed, and it is an important component of the desert vegetation in early spring. The species has important ecological value in stabilizing sandy soil (Liu et al., 2011a). In China, *A. arpilobus* is found only in Xinjiang, where it occurs only in the Junggar Basin in areas in which the vegetation cover is typically sparse. Flowering of this species occurs from May to June, and seeds mature in late June to early July with fruits dehiscing as the seeds mature. During 2 years of field observations, only a few newly-germinated seedlings were seen in autumn, but many were seen in spring.

The study site is located on the western edge of the Junggar Basin of Xinjiang Province (45°04′15″ N, 86°01′21″ E, 334 m asl), and it has typical desert vegetation, gravelly sandy soil and a continental climate. The mean annual temperature for the Junggar Desert is 8 °C, the highest recorded summer temperature is >40 °C, the lowest recorded winter temperature is −40.5 °C, the mean temperature of the warmest month (July) is 27 °C and the mean temperature of the coldest month (January) is −16.3 °C. Annual precipitation (rain and snow) is <150 mm, annual potential evaporation is >2000 mm and the frost-free period is 140 days (Zhang and Chen, 2002).

Mature fruits containing seeds were collected on July 5, 2010 from plants of *A. arpilobus* growing on a sand dune at the study site. Undamaged and fully-formed seeds were collected from the fruits and stored in paper bags under ambient laboratory conditions (18–30 °C, 20–30% relative humidity) until used. Unless otherwise stated, the period of storage did not exceed 7 days.

2.2. Imbibition of water

To determine if the seed coat is permeable or impermeable to water, imbibition of water was compared in scarified and non-scarified seeds. Seeds were scarified individually with a razor blade (mechanical scarification), and four replicates of 25 scarified and non-scarified seeds each were used. Each replicate of treated and non-treated seeds was weighed to the nearest 0.0001 g using a Sartorius electronic balance (Sartorius Co., Goettingen, Germany) and placed on a filter paper moistened with distilled water in Petri dishes in the laboratory. At time zero and at the time intervals shown in Fig. 1, seeds were removed from the filter paper, blotted dry and weighed. Percentage

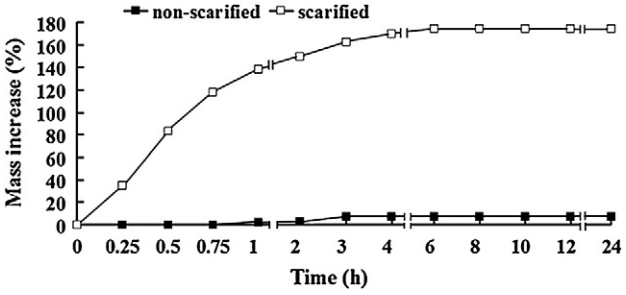

Fig. 1. Time-course for water absorption by scarified (□) and non-scarified (■) seeds of *A. arpilobus* at ambient laboratory temperature.

increase in seed mass was calculated using the following equation (Baskin et al., 2004): % increase in mass = $[(W_i - W_d)/W_d] \times 100$, where W_i = mass of imbibed and W_d = mass of dry seeds.

2.3. General methods for all germination tests and germination requirements

Seeds were incubated on two layers of Whatman No. 1 filter paper moistened with 2.5 ml distilled water in 9-cm-diameter plastic Petri dishes, and four replicates of 25 seeds each were used for each test condition, unless otherwise stated. Seeds were incubated in light (12-h daily photoperiod, about 100 μmol m^{-2} s^{-1}, 400–700 nm, cool white fluorescent light; hereafter light) at 30/15, 25/15, 20/10, 15/2 and 5/2 °C (12/12 h) temperature regimes, unless otherwise stated. In some tests, seeds were also incubated in constant darkness, which was provided by enclosing the dishes in black bags. Seeds incubated in light were monitored daily during the incubation period, and each day seeds that had germinated were removed from the Petri dishes; distilled water was added as needed. Seeds incubated in darkness were checked for germination only at the end of the experiment, and thus they were not exposed to any light during the incubation period.

To determine the optimum temperatures and light:dark conditions for germination, scarified and non-scarified freshly-matured seeds were tested over the range of temperatures in light and in darkness for 28 days. A modified Timson (1965) germination index (GI), $GI = (\sum G_t)/D_t$, where G_t is the cumulative germination percentage of seeds at 1-day intervals and D_t is the total number of days of incubation (Khan and Ungar, 1997), was calculated for germination of scarified seeds in light at the five incubation temperatures. The single figure calculated using this index gives an indication of the speed and extent of germination. Index values can range from 0, when no seeds germinate, to 100, when all seeds germinate on day one.

2.4. Effect of water stress on germination

To determine the effect of water stress on germination, we used PEG-6000 (Walter Technology Co., Shang Hai, China) to create six levels of water stress (see Michel, 1983): −0.05, −0.15, −0.30, −0.51, −0.77 and −1.09 MPa; distilled water was used in the control (0.00 MPa). Seeds were incubated in light at a constant temperature of 20 °C. Petri dishes were sealed with plastic film to prevent evaporation. Germination in light was monitored daily for 14 days, after which seeds were washed with distilled water and placed on a filter paper moistened with distilled water. Then, germination was monitored daily for an additional 14 days (germination recovery), making a total of 28 days for the experiment. Finally, non-germinated seeds were tested for viability with 2,3,5-triphenyl-2H-tetrazolium chloride (TTC). Each seed was cut open and placed in a 0.1% aqueous TTC solution at 20 °C for 24 h. Embryos that stained red or pink were considered to be viable and those that did not stain nonviable (Baskin and Baskin, 1998).

2.5. Dormancy-breaking requirements

Our purpose here was to evaluate the effectiveness of some well-known dormancy-breaking treatments in overcoming the water impermeability of the seeds (Baskin and Baskin, 1998; Bewley and Black, 1994; Crocker and Barton, 1957). At the conclusion of each germination test, viability of the non-germinated seeds was determined by scarifying the seeds with a razor blade and then incubating them in light at 20/10 °C for 28 days. Germinated seeds were considered to be viable and the non-germinated ones nonviable.

2.5.1. Dry heat

Fresh (intact water-impermeable) seeds were exposed to 60, 70 and 80 °C in a drying oven for 0 (the control) 3, 6, 12, 24 and 48 h. Seeds were incubated at 25/15 and at 20/10 °C in light and in constant darkness for 28 days.

2.5.2. Wet heat

Fresh seeds were placed in a mesh-cloth bag and dipped in (1) boiling water for 5, 10, 15 or 20 min; (2) hot water (90 °C) for 15, 30, 60 and 120 min; (3) water at 80 °C for 60, 120 and 240 min; and (4) water at 70 °C for 120 and 240 min. Following treatment, seeds were incubated at 25/15 and at 20/10 °C in light for 28 days.

2.5.3. Acid scarification

Fresh seeds were soaked in concentrated H_2SO_4 (98%) for 0, 5, 10, 15, 20, 25, 30, 35, 40 and 50 min with stirring. Seeds were removed from the acid and washed with distilled water for 3 min. Control seeds were soaked in distilled water for equal amounts of time as those soaked in the acid. After treatment, seeds were tested for germination at 25/15 and at 20/10 °C in light for 28 days.

2.5.4. Cold treatment

Fresh seeds were placed on moist sand in 12-cm-diameter Petri dishes and kept at 4 °C for 4, 8, 12 and 16 weeks in darkness. After each period of cold treatment, seeds were incubated in light and in constant darkness at the five temperature regimes for 28 days. Seeds that germinated during the cold treatments (3%) were removed from the Petri dishes before incubation.

2.5.5. Dry storage

Fresh seeds were stored dry in a closed cotton bag at room conditions (18–30 °C, 20–30% relative humidity). At time 0 and after 1, 2, 3, 6, 9 and 12 months of storage, scarified and non-scarified seeds were tested for germination at the five alternating temperatures in light and in darkness for 28 days.

2.5.6. Alternate wet heat and cold

Following the procedure of Baskin et al. (2007), fresh seeds were placed in a cloth-mesh bag and dipped in boiling water for 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 90 or 120 s and then quickly placed into ice water for 2 min. The cycle of wet heat and cold water was repeated seven times for each period of wet-heat treatment. Twelve replicates of 25 seeds each were used for each treatment and control (soaked in distilled water at room temperature). Following treatments, seeds were incubated at 20/10 °C in light for 28 days.

2.6. Effect of seed burial depth and duration on dormancy-break

Seed germination was monitored under natural conditions to determine if dormancy is broken during burial. Five hundred seeds were placed in each of 60 nylon mesh bags that allowed water to penetrate. Thirty bags were buried at a depth of 3 cm in pots (21 cm diameter × 18 cm depth) filled with sand from the natural habitat on July 12, 2010. The other 30 bags were placed on the surface of the sand. The pots were buried (with top even with soil surface) in the experimental garden located near the Xinjiang Agricultural University (Urümqi) weather station. Seeds were exhumed or taken from the soil surface on the same day of each month for 12 months, except for months with a snow cover on the ground (December 2010 to February 2011), when the soil was frozen and seeds could not be exhumed. Exhumed seeds were incubated at the five temperature regimes in light and in constant darkness for 28 days.

2.7. Effect of burial depth on seedling emergence

To test the effect of burial depth on the emergence of *A. arpilobus* seedlings under natural conditions, five replicates of 25 scarified seeds each were placed on the soil surface or buried at depths of 1, 2, 3, 4, 5, 6 and 7 cm in sand in plastic pots that are 21 cm in diameter and 18 cm in height and placed in a greenhouse on the campus of Xinjiang Agricultural University on April 1, 2011. Sand in the pots was watered every 2 days to keep it moist. A seedling was considered to be emerged when the cotyledons were visible, and the number of emerged seedlings was counted 30 days after planting.

2.8. Germination phenology

The purpose of this experiment was to monitor the germination phenology of scarified and of non-scarified seeds of *A. arpilobus* under natural temperature and precipitation. Seeds collected on July 5, 2010 were sown on bare soil in 1 × 1 m plots on 30 July 2010. Each of the two treatments (scarified and non-scarified seeds) consisted of three replications of 200 seeds, for a total of six plots.

The study was carried out in the experimental garden on the campus of Xinjiang Agricultural University. Seedling emergence was monitored at 7-day intervals from August 2010 to May 2011. Information on temperature and rainfall at the study site was obtained from data collected at the Xinjiang Agricultural University weather station near the study plots.

2.9. Soil seed bank

To determine if seeds could form a persistent seed bank, three replicates of 400 non-scarified seeds collected on July 5, 2010 were sown on bare soil in 1 × 1 m plots on August 20, 2010 in the experimental garden on the campus of Xinjiang Agricultural University. The soil received water only via precipitation and snowmelt. We monitored the plots for germinated seeds (seedlings) from August 2010 to May 2011 at 7-day intervals, and all plants were removed from the plots before any seeds were dispersed. In June 2011, we collected all the soil in the top 5 cm in each of the three plots in which the non-scarified seeds had been sown and used sieves to separate seeds from the soil. The number of seeds was counted, and seeds were tested for germination in light at 20/10 °C for 28 days, as previously described.

2.10. Data analysis

All data were transformed as necessary to meet normality and homogeneity of variance assumptions. If the ANOVA assumptions were violated after data transformation, treatment differences were assessed by using the more conservative Kruskal–Wallis non-parametric test. Analysis was done on transformed data, but non-transformed data are presented in the figures and tables.

All data analyses were conducted using SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA). One-way ANOVA tested for the significance of the main effects of cold stratification, dry heat, wet heat, alternate wet heat and cold, concentrated sulfuric acid, duration and depth of burial on dormancy-break and germination/seedling emergence. Two-way ANOVA was used to test for the significance of the main effects (light and temperature) and their interaction on the germination percentage of fresh seeds. Three-way ANOVA was used to test the significance of the main effects (temperature, light condition, storage time) and their interaction on germination percentages. Tukey's HSD was performed for multiple comparisons to determine the significant ($P<0.05$) differences between individual treatments. Statistical tests were conducted at $P=0.05$, and all data were expressed as mean ± SE (Sokal and Rohlf, 1995).

3. Results

3.1. Imbibition of water

Mass of the manually-scarified seeds had increased >140% after 2 h and >170% after 6 h, while mass of the non-scarified

seeds had increased only about 5% after 24 h, due to one (4%) of the 25 non-treated seeds imbibing in each of the four replicates. Thus, scarified seeds imbibed water, whereas the non-scarified ones did not (Fig. 1).

3.2. Germination requirements

Germination percentage was significantly affected by temperature ($P<0.001$) and the interaction between light and temperature ($P<0.001$) (Table 1). Only 4% of fresh non-scarified seeds imbibed water, and they germinated (Fig. 2). Manually-scarified seeds germinated to ≥95% at all temperature regimes within 28 days in light except at 5/2 °C, where germination was 78% after 28 days (Fig. 2, $P<0.05$) and 95% after 40 days (data not shown). In darkness, scarified seeds germinated to >90% at all temperature regimes within 28 days. The germination index for scarified seeds in light was 20.6±0.50 at 30/15 °C, 24.4±1.7 at 25/15 °C, 24.5±0.5 at 20/10 °C, 18.3±1.7 at 15/2 °C and 5.1±0.2 at 5/2 °C.

3.3. Effect of water stress on germination

Germination of scarified seeds was 99% or 100% at water potentials of 0, −0.05, −0.15 and −0.30 MPa, but it declined to 86% at −0.51 MPa, where the radicles of the germinated seeds died. Little or no germination occurred at −0.77 or at −1.09 MPa. The recovery percentage (seeds germinated after transfer to distilled water) was only 43% at −0.77 MPa and 32% at −1.09 MPa (Fig. 3); the other seeds had lost viability.

3.4. Dormancy-breaking requirements

3.4.1. Dry heat

Regardless of the dry heat temperature and length of exposure, only ≤5% of the seeds became water-permeable, and all of them germinated at 25/15 and at 20/10 °C in light and in constant darkness within 28 days (data not shown). At the end of the experiment, the non-germinated seeds were mechanically scarified, and 99.0±1.0% of them germinated quickly.

3.4.2. Wet heat

The highest germination (31.0±1.2%) was obtained for seeds exposed to boiling water (100 °C) for 10 min (Table 2). When the time in boiling water exceeded 10 min, germination percentages decreased. Regardless of the exposure time to 70, 80 or 90 °C, only 1.3% to 8% of the seeds germinated. Generally, more seeds imbibed after a particular treatment than germinated, and the imbibed non-germinated seeds were dead.

Table 1
Two-way ANOVA of the effects of light, temperature and their interactions on the germination of *Astragalus arpilobus* seeds stored dry at laboratory conditions.

Source	*d.f.*	SS	MS	*F*-value	*P*-value
Light (L)	1	3.613	3.613	1.897	0.174
Temperature (T)	4	570.550	142.638	74.908	<0.001
L×T	4	299.950	74.988	39.381	<0.001

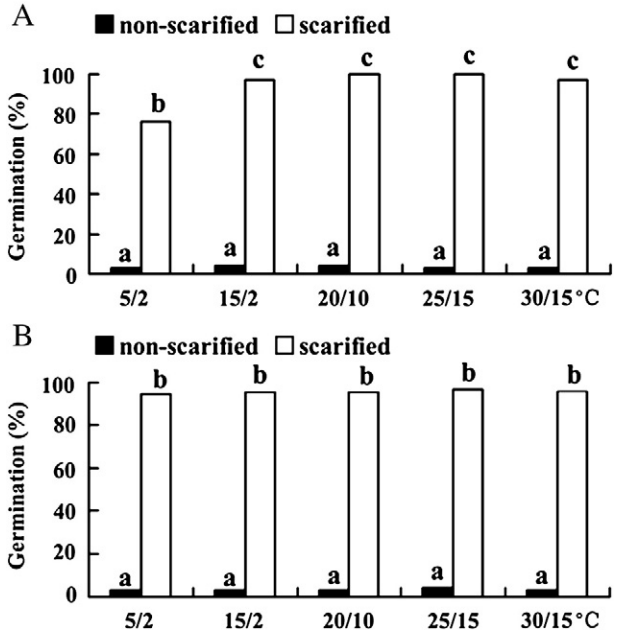

Fig. 2. Mean germination percentages of fresh non-scarified (■) and scarified (□) seeds of *A. arpilobus* in light (A) and in darkness (B) at five temperature regimes. Bars in A and in B with different lowercase letters are significantly different ($P<0.05$). Standard errors are ≤1.9% in all cases.

3.4.3. Acid scarification

Treatment with concentrated H_2SO_4 was a very effective way to break seed dormancy, and 30 and 40 min of acid scarification resulted in the highest (94–96%) germination at 20/10 °C (Fig. 4). Fifty minutes of acid-scarification caused a significant decrease in germination percentage. The non-germinated seeds were not viable. Similar results were obtained for seeds incubated at 25/15 °C (data not shown).

3.4.4. Cold treatment

Up to 16 weeks of cold treatment on moist sand was almost completely ineffective in breaking dormancy. Germination of non-scarified seeds was only 2–5% in light and in darkness at the five temperatures (data not shown). The results are similar to those of the water control in Fig. 4. At the end of the experiment, the seeds were mechanically scarified, and they germinated quickly and to high percentages.

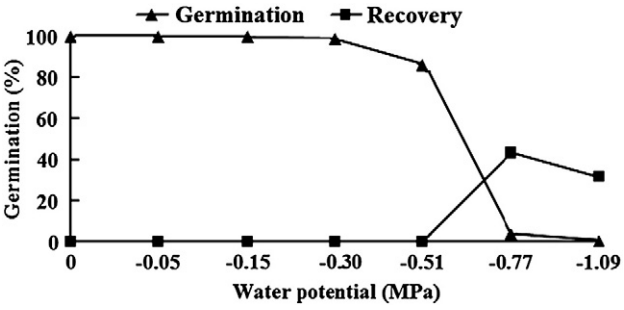

Fig. 3. Effect of water potential on germination (▲) and recovery (■) of *A. arpilobus* seeds at 20 °C in light. Standard errors are ≤3.27% in all cases.

Table 2
Mean (±SE) final germination percentages (GP) and swelling percentages (SP) of non-scarified seeds of *A. arpilobus* incubated in light at 20/10 °C after receiving wet heat treatments for various periods of time. Different uppercase letters within a row and different lowercase letters within a column indicate significant differences (Tukey's HSD, $P=0.05$).

Temperature (°C)	Time (min)	GP	SP
control	0	4.0 ± 0.6^{Aabc}	4.0 ± 0.6^{Aa}
100	5	23.0 ± 1.8^{Ae}	24.3 ± 1.2^{Aab}
100	10	31.0 ± 1.2^{Af}	52.3 ± 2.0^{Bc}
100	15	15.0 ± 0.6^{Ad}	75.0 ± 2.9^{Be}
100	20	8.7 ± 1.2^{Acd}	85.0 ± 2.6^{Bf}
90	15	3.3 ± 1.5^{Aabc}	5.3 ± 2.0^{Aa}
90	30	6.3 ± 1.9^{Aabc}	19.3 ± 1.5^{Bb}
90	60	4.7 ± 0.9^{Aabc}	50.0 ± 2.2^{Bc}
90	120	1.3 ± 0.9^{Aa}	80.0 ± 1.2^{Bf}
80	60	4.0 ± 1.2^{Aabc}	4.0 ± 1.2^{Aa}
80	120	8.0 ± 2.3^{Abc}	27.0 ± 2.7^{Bb}
80	240	2.3 ± 0.9^{Aabc}	60 ± 3.2^{Bd}
70	120	2.0 ± 0.6^{Aab}	4.3 ± 0.9^{Aa}
70	240	1.3 ± 0.3^{Aa}	6.0 ± 0.6^{Ba}

3.4.5. Dry storage

The length of time of dry storage did not affect germination percentage ($P=0.517$). After 12 months of dry storage, germination percentages of non-scarified seeds were equal to those of fresh intact seeds in both light and darkness (Fig. 5A, B). The main factors and their interactions significantly affected germination except for the interactions including time of storage (Table 3). After 12 months of storage, germination of non-scarified seeds was ≤4% at all temperature regimes in light and in darkness. However, the manually-scarified 12-month-old seeds germinated to ≥95% at four of the five temperature regimes tested within 28 days in light and in darkness; 78% of the seeds germinated at 5/2 °C (Fig. 5C, D).

3.4.6. Alternate wet heat and cold

The highest germination (29.6±3.5%) was obtained for seeds exposed to 7 cycles of boiling water for 60 s/ice water for 2 min (21 min total) (Table 4). With an increase in exposure time to boiling water from 10 to 60 s (time in ice water was always 2 min), the percentage of water-permeable seeds increased. At first, the percentage of germination increased, but with 90 or 120 s of exposure to boiling water germination percentages decreased. The imbibed, non-germinated seeds were dead (Table 4).

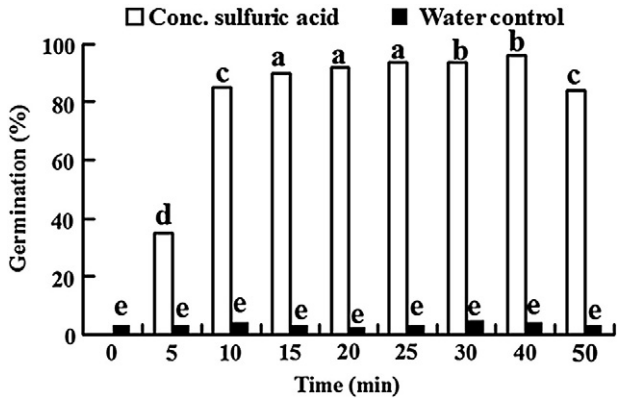

Fig. 4. Mean germination percentages of seeds at 20/10 °C in light after soaking in concentrated sulfuric acid for different periods of time. Bars with different lowercase letters are significantly different ($P<0.05$). Standard errors are ≤1.7% in all cases.

3.5. Effect of seed burial depth and duration on dormancy break

After 12 months, 93% of seeds on the soil surface and 91% of those buried at a depth of 3 cm remained ungerminated and not imbibed; a few seeds had germinated in the bags. When seeds at soil depths of 0 and 3 cm were exhumed after 12 months of burial (and on the surface), only 2–4% of them germinated in light and in darkness at the five temperature regimes (date not shown). All non-germinated seeds were viable.

3.6. Effect of burial depth on seedling emergence

Seedling emergence from scarified seeds of *A. arpilobus* was influenced by burial depth and occurred from seeds buried 1 to 5 cm deep (Fig. 6). Optimum soil depths for seedling emergence were 1 and 2 cm, and 0% to 48% of the seedlings emerged from depths ≥3 cm.

3.7. Germination phenology

Under natural temperature and rainfall conditions (Fig. 7), none of the non-scarified seeds germinated in autumn, but 43% of the scarified seeds did so, mostly between November 8 and 15, 2010 (Fig. 8), when the mean daily maximum air temperature was 8.7 °C and the mean daily minimum temperature was 0.5 °C. The first snow fell on 4 November. In spring 2011, 5.3% of the non-scarified seeds and an additional 5% of the scarified seeds germinated between March, 22 and April 5, 2011, when the mean daily maximum temperature was 13.8 °C and the mean daily minimum temperature was 2.2 °C.

3.8. Soil seed bank

Of the 1200 non-scarified seeds sown in the three 1×1 m plots in August 2010, we could account for 73.7±5.6% of them in June 2011; 5.0±0.8% had germinated and 68.7±4.2% of the non-germinated seeds were retrieved. When the intact non-germinated seeds were tested in light at 20/10 °C for 28 days in June 2011, about 1% of them germinated, but after mechanical scarification all of them did so quickly.

4. Discussion

Seeds of *A. arpilobus* clearly have a water-impermeable seed coat, as has been reported for many legumes (Baskin and Baskin, 1998), including at least 35 species of *Astragalus* (see statement about this in the Introduction). Only 4% of the fresh non-treated *A. arpilobus* seeds took up water, whereas all fresh manually-scarified seeds did so within 6 h and germinated within 6 days, indicating that the embryo was not dormant.

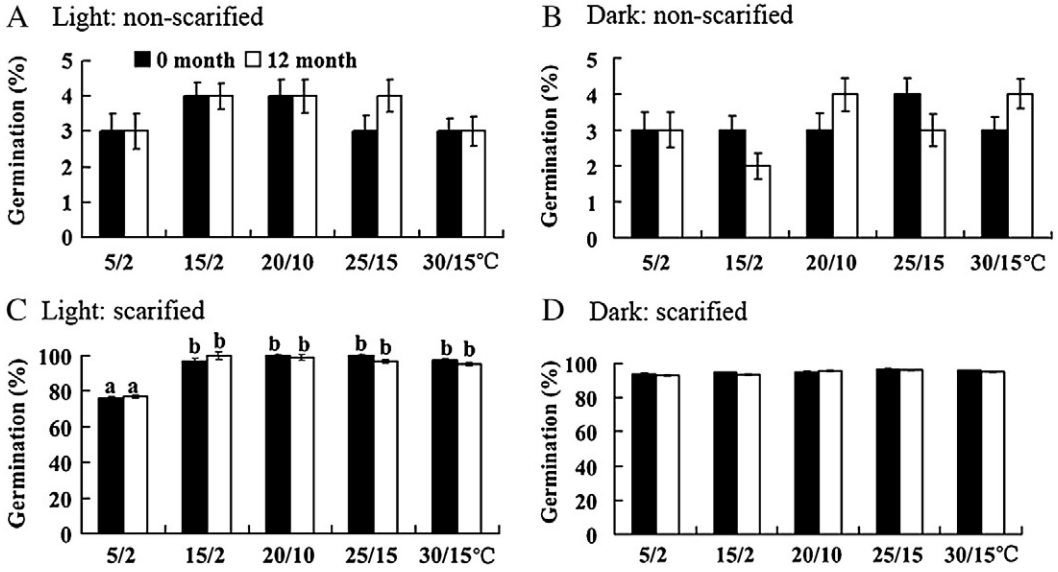

Fig. 5. Germination percentages of fresh (0-month-old) and of 12-month dry-stored non-scarified (A, B) and scarified (C, D) seeds incubated in light (A, C) and in darkness (B, D) at five temperature regimes. Bars with different letters in C are significantly different ($P<0.05$) within all temperature regimes. There were no significant differences in A, B or D. Error bars are ±1 SE of the mean. Note the different scales on the y-axis for the non-scarified (A, B) and scarified seeds (C, D).

Thus, a high percentage (96%) of the fresh seeds of *A. arpilobus* used in this study had PY imposed by a water-impermeable seed coat, and only 4% were nondormant. The embryo did not exhibit any PD, i.e. scarified seeds germinated over a wide range of temperature regimes in both light and darkness. Thus, seeds did not have PY+PD.

Although scarification broke PY in a very high percentage of *A. arpilobus* seeds, other techniques were not very successful. Dry heat (60, 70 and 80 °C) did not make seeds water permeable, suggesting that the hot-dry summer conditions in the desert in northwestern China, where the sand-surface temperature can reach >60 °C (Zhang et al., 2012), are not the cause (at least not the direct cause) of dormancy break. However, dry heat is a useful method for breaking PY in seeds of some legumes (Martin et al., 1975; Norman et al., 2002). Further, 1 year of dry storage at room temperature did not significantly reduce the number of seeds with PY. Seeds of some legumes, especially those belonging to the subfamily Papilionoideae, became permeable during dry storage (Cavanagh, 1987; Morrison et al., 1992). Laboratory studies indicated that although wet heat released dormancy of *A. arpilobus* seeds more effectively than dry heat, it broke dormancy in only a low percentage of them.

Seeds that became permeable during burial imbibed and either germinated or died (rotted) within the burial packet. Dormancy release was not greatly affected by depth or duration of seed burial. However, a high percentage of the seeds survived 1 year of burial in soil, and they germinated quickly and to high percentages when mechanically scarified. Seeds with PY generally are considered to be long-lived, and some species have very specific germination seasons (Baskin and Baskin, 1998). Various other legumes such as *Medicago lupulina*, *Trifolium pratense* and *Vicia cracca* show very marked seasonal phases in germination (Van Assche et al., 2003). Seeds of these three species were made sensitive to dormancy-breaking conditions (sensu Jayasuriya et al., 2009) by cold stratification, and if cold stratification was followed by alternating low spring temperatures PY was broken. In contrast to many seeds with non-deep physiological dormancy,

Table 3
Three-way ANOVA of the effects of light, time of storage, temperature and their interactions on the germination of *Astragalus arpilobus* seeds stored dry at laboratory conditions.

Source	*d.f.*	SS	MS	*F*-value	*P*-value
Light (L)	1	28.017	28.017	12.255	0.020
Time of storage (T)	2	23.408	11.704	6.089	0.517
Temperature (T′)	4	1774.208	443.552	136.594	<0.001
L×T	2	2.758	1.379	0.852	0.437
L×T′	4	1028.025	257.006	112.421	<0.001
T×T′	8	16.092	2.011	1.046	0.425
L×T′×T′	8	12.825	1.603	0.990	0.463

Table 4
Mean (±SE) final germination percentages (GP) and swelling percentages (SP) of seeds of *A. arpilobus* incubated in light at 20/10 °C after receiving 7 cycles of wet heat (different periods of time in boiling water) and cold (2 min in ice water). Different uppercase letters within a row and different lowercase letters within a column indicate significant differences (Tukey's HSD, $P=0.05$).

Time (s) (in boiling water)	GP	SP
0	4.0 ± 0.6^{Aa}	4.0 ± 0.6^{Aa}
10	5.4 ± 0.3^{Aa}	8.3 ± 1.5^{Ba}
15	7.7 ± 1.2^{Aa}	11.7 ± 3.7^{Bab}
20	8.3 ± 0.9^{Aa}	13.3 ± 0.9^{Bab}
25	8.7 ± 3.1^{Aa}	15.0 ± 7.5^{Bab}
30	9.0 ± 0.6^{Aab}	15.0 ± 0.6^{Bab}
35	11.0 ± 1.2^{Aab}	15.3 ± 1.5^{Bab}
40	10.5 ± 0.8^{Aab}	16.1 ± 0.5^{Bab}
45	12.0 ± 1.2^{Aab}	16.3 ± 0.7^{Bab}
50	20.0 ± 2.1^{Ac}	26.3 ± 6.4^{Bbc}
60	29.6 ± 3.5^{Ad}	34.3 ± 1.5^{Bcd}
90	23.3 ± 0.9^{Acd}	38.3 ± 2.2^{Bcd}
120	15.7 ± 2.2^{Ab}	47.7 ± 1.8^{Bd}

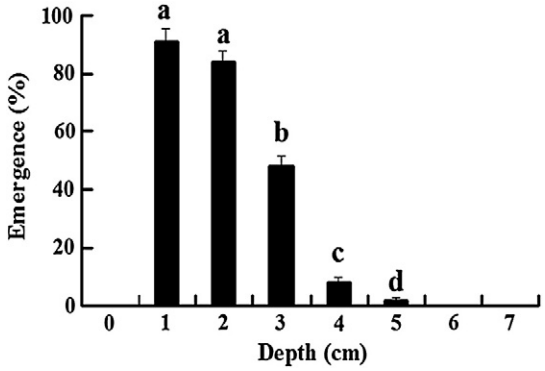

Fig. 6. Effect of burial depth of scarified seeds in sand on percent emergence of *A. arpilobus* seedlings after 30 days. Bars with different lowercase letters are significantly different ($P<0.05$). Error bars are ±1 SE of the mean.

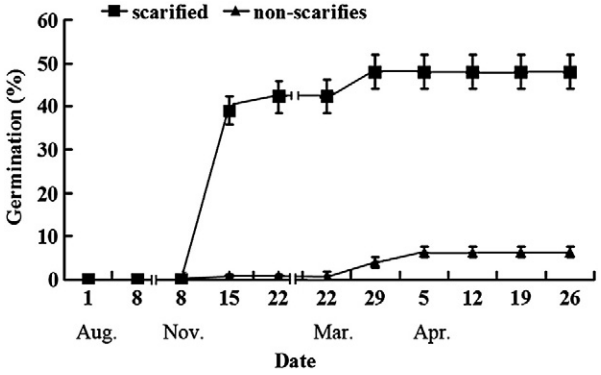

Fig. 8. Germination percentages of scarified (■) and non-scarified (▲) seeds of *A. arpilobus* under natural temperature and rainfall in the experimental garden during autumn 2010 and spring 2011. Error bars are ±1 SE of the mean.

those with PY do not exhibit annual dormancy/non-dormancy cycles. However, if a winter cold stratification period is not followed by appropriate alternating spring temperatures, seeds lose their sensitivity to dormancy breaking conditions (Van Assche et al., 2003). Thus, seeds exhibit an annual cycle with respect to their ability to respond to dormancy-breaking conditions, i.e. the alternating low spring temperatures. Other species of legumes whose seeds have PY germinate a few at a time throughout the growing season. For example, *Trifolium dubium* seeds germinated throughout the growing season to a low percentage, generally less than 5%, after burial (Van Assche et al., 2003).

In the present study, PY was broken in only a small percentage of seeds both on the surface and at a depth of 3 cm in the soil, but more in the latter than in the former position. Dormancy was broken in a small percent of the seeds by spring, when temperature and soil moisture (from rainfall and snowmelt) are likely to be optimal for seed germination and seedling establishment. Since only a small percentage of the seeds on the soil surface or buried 3 cm deep for 1 year germinated without scarification and a high proportion of them were still viable, it appears that *A. arpilobus* forms a long-lived persistent seed bank in its native habitat.

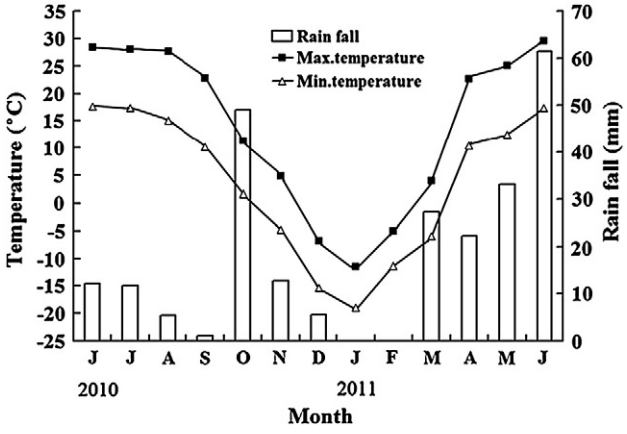

Fig. 7. Monthly total rainfall and mean minimum and mean maximum monthly temperatures recorded at the Xinjiang Agricultural University weather station from June 2010 to June 2011. No rainfall occurred in January or February, only snowfall.

At water potentials of 0 to −0.30 MPa, 99–100% of the seeds germinated, showing that seeds can germinate when subjected to some water stress; the seedlings were healthy. However, although 86% of the seeds germinated at −0.51 MPa, the radicles on the young seedlings died. About 30% of the scarified seeds of *A. australis* var. *olympicus* germinated at −1.5 MPa (Kaye, 1999). Seedling emergence of *A. arpilobus* was influenced by seed burial depth. The optimal depth for seedling emergence was 1–2 cm, and no seedlings emerged from a depth of 6 cm. Also, no seedlings emerged from seeds sown on the surface of the sandy soil. On the surface, non-dormant seeds may not have enough water available to germinate, while at a depth of 1–2 cm soil moisture potentially would be higher than it is on the surface, thus promoting germination. Since non-dormant seeds germinated equally well over a wide range of temperatures in both light and darkness, we can conclude that germination of non-dormant seeds in the field is controlled by temperature and moisture and not by light/dark conditions. Although soil water at depths of >3 cm might be more favorable for germination than at shallower depths, the small (0.0014±0.0001 g per seed) seeds of *A. arpilobus* apparently do not have enough stored food in the cotyledons (no endosperm) for the seedling to grow to the surface from these depths (Fig. 6). In seeds of the sand dune papilionoid legume *Eremosparton songoricum* the optimal depth for seedling emergence was 1 cm and the maximum depth was 6 cm (Liu et al., 2011a,b).

On average, water is much less likely to be a limiting factor for growth of annuals in spring than in summer or autumn in the Junggar Desert. Thus, germination of *A. arpilobus* seeds in early spring would result in successful seedling establishment. However, to germinate in early spring PY must be broken by this time. We suggest that one way to ensure germination in early spring is for the physically-dormant seeds to become sensitive to dormancy break during winter. Then, the sensitive seeds could respond to early spring alternating temperature regimes and PY will be broken. In the experimental garden germination phenology study, non-scarified seeds germinated in March and early April. However, after early April no additional germination occurred, suggesting that unless seeds germinate in

early spring they lose their sensitivity and PY is not broken. Thus, seeds would remain dormant in the soil, and the next opportunity for them to germinate would be the following spring after they experience another winter. Further, seeds may remain in the persistent seed bank for many years. Seeds of *Astragalus tennesseensis* remained viable in the seed bank, and a few seeds germinated each year for 14 consecutive years (Baskin and Baskin, 1989, 1998, unpublished data). Seeds of *Astragalus distortus* sown on soil in a non-heated greenhouse in Lexington, Kentucky (USA) germinated over a period of 20 years (Baskin and Baskin, unpublished data). A long-term persistent seed bank also occurs in *Astragalus arenarius* (Symonides, 1986), *Astragalus bibullatus* (Morris et al., 2002), *Astragalus danicus* (Partzsch, 2009), *Astragalus lentiginosus* var. *salinas* (Ralphs and Cronin, 1987) and var. *wahweapensis* (Ralphs and Bagley, 1988) and *Astragalus neglectus* (Catling and Sinclair, 2002). Thus, it seems reasonable that some of the seeds of *A. arpilobus* produced in any given year (a seed cohort) would remain viable in the habitat for many years, with a few germinating each spring.

We suggest the following scenario for seed dormancy break and germination of *A. arpilobus* in the field. A low percentage of the fresh seeds are non-dormant (Figs. 2, 4) and germinate in autumn if soil moisture is not limiting. Two percent of the 600 non-scarified seeds sown in three plots in the experimental garden in July 2010 and provided with supplemental water germinated in autumn 2010, and the plants survived the winter; an additional 3% of them germinated in spring 2011 (data not shown). On the other hand, none of the non-scarified seeds not provided with supplemental water germinated in autumn (Fig. 8). Thus, under natural conditions (i.e. without supplemental water) all of the seeds that germinated did so in spring. These results support our field observations that most *A. arpilobus* seeds germinate in the field in spring and only a few in autumn, strongly suggesting that winter/early spring is the dormancy-breaking season for this species. Seeds capable of germinating in autumn following seed dispersal in early summer were non-dormant at maturity. Seed coat impermeability did not develop in this low percentage of seeds. Further, PY is not broken by the hot, dry summer/autumn conditions in the habitat. Thus, seeds in the persistent seed bank probably germinate only in spring. Plants from autumn-germinating seeds that survive and reproduce would be winter annuals and those from spring-germinating seeds spring ephemerals.

Seeds of *A. arpilobus* have been collected and placed into long-term storage in the China Germplasm bank in Kunming, Yunnan Province. At some point, seeds will be removed from storage, and the question will be how to germinate them and propagate the species. The easiest and most economical way to propagate legumes usually is through seed germination. Water-impermeable seeds can be made permeable by treatments such as mechanical or acid scarification (Addis, 2003); exposure to dry heat, wet heat or alternate wet and cold (ice water) (Baskin et al., 2007); cooling at low (winter) temperatures followed by warm spring temperatures (Van Assche et al., 2003); or warm, dry followed by warm, wet conditions (Jayasuriya et al., 2009). However, except for scarification, the best method to break PY in legumes varies with the species (Baskin and Baskin, 1998; Morrison et al., 1992, 1998) and must be determined experimentally.

Based on the germination of a relatively small sample of perennial species of *Astragalus* (33, see statement in Introduction) reported in the literature, and on an even much smaller one of the annual species, including *A. arpilobus*, we can preliminarily conclude that: (1) PY is the characteristic kind of seed dormancy in the genus *Astragalus*; (2) PY in this genus is not readily broken in nature or by simulated natural factors such as high or fluctuating temperatures, freezing/thawing, etc. — i.e. only a small percentage of the seeds at a population site germinate in any given year and thus a long-lived seed bank is formed; (3) the best way to break PY artificially is by mechanical or sulfuric acid scarification, (4) non-dormant (e.g. scarified) seeds germinate to high percentages over a wide range of temperatures in both light and darkness; and (5) PY in seeds of annuals is of similar high intensity as that found in perennials.

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