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Ecological role of physical dormancy in seeds of *Oxytropis racemosa* in a semiarid sandland with unpredictable rainfall

Dandan Hu[1,2], *Jerry M. Baskin*[3], *Carol C. Baskin*[3,4], *Xuejun Yang*[1] *and Zhenying Huang*[1,*]

[1] *State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, No. 20 Nanxincun, Xiangshan, Haidian District, Beijing 100093, P.R. China*
[2] *University of Chinese Academy of Sciences, No. 19(A) Yuquan Road, Shijingshan District, Beijing 100039, P.R. China*
[3] *Department of Biology, University of Kentucky, Lexington, KY 40506, USA*
[4] *Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA*
*Correspondence address. State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, No. 20 Nanxincun, Xiangshan, Haidian District, Beijing 100093, P.R. China. Fax: +86-10-62836276;
E-mail: zhenying@ibcas.ac.cn

Abstract

Aims
Seed dormancy and the soil seed bank are crucial to plant regeneration strategy, especially in semiarid ecosystems with unpredictable precipitation. The aim of this study was to investigate how seed dormancy is controlled by environmental factors and how it is correlated with the soil seed bank and regeneration of the perennial legume *Oxytropis racemosa*, a dominant perennial herb in Mu Us Sandland of semiarid China.

Methods
Germination and imbibition experiments on fresh intact and scarified seeds of *O. racemosa* were used to identify physical dormancy (PY) in seeds of this species. Soil seed bank dynamics, timing of seedling emergence and the fate of buried seeds in the natural habitat were investigated.

Important Findings
PY was broken by mechanical scarification or wet heat/ice water cycles but not solely by dry heat or wet heat treatment. The soil seed bank exhibited seasonal changes in the number of seeds, which was highest in September and lowest in July. Seeds buried at different sand depths gradually lost dormancy; 20–42% of the seeds remained dormant after 20 months of burial. Dormancy break occurs gradually throughout the year. Our results indicate that *O. racemosa* exhibits hardcoatedness heterogeneity that spreads germination of a seed cohort between seasons and years in the semiarid environment, where the amount of precipitation during the growing season is highly variable.

Keywords: *Oxytropis racemosa*, physical dormancy, seedling emergence, soil moisture, soil seed bank, soil temperature, unpredictable rainfall

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INTRODUCTION

Seed dormancy is a characteristic of the seed that prevents it from germinating when environmental conditions are not suitable for seedling establishment and survival (Baskin and Baskin 2014; Fenner and Thompson 2005; Vleeshouwers *et al.* 1995). It optimizes germination of seeds at the right time and in a suitable place. Seed dormancy is important in modulating the distribution and abundance of a plant species, and it is also critical to population persistence (Baskin and Baskin 2014; Cabin *et al.* 2000; Handley and Davy 2005; Huang *et al.* 2016; Vleeshouwers *et al.* 1995). Diverse mechanisms and processes are involved in seed dormancy, which has been divided into five classes: physiological, morphological, morphophysiological, physical and combinational dormancy (Baskin and Baskin 2004).

Physical dormancy (PY), the subject of this investigation, is caused by (a) water-impermeable palisade cell layer(s) in the seed (or fruit) coat that inhibits water absorption, and thus seeds are unable to germinate (Baskin and Baskin 2004;

Smýkal *et al.* 2014). PY is the second most common type of dormancy world-wide and is present in herbs, shrubs, liana/vines and trees in arid to tropical regions (Baskin and Baskin 2014). PY is known to occur in 18 angiosperm families (no gymnosperms), and it is very common in the Fabaceae, Cistaceae, Geraniaceae, Malvaceae, Rhamnaceae and Sapindaceae (Baskin and Baskin 2014; Baskin *et al.* 2006). The lens ('water gap'), sometimes erroneously called the strophiole, in seeds of Fabaceae with PY serves as the environmental signal detector for germination by opening at the right time and place, thus ensuring the best chances for seedling survival and plant establishment (Baskin *et al.* 2000). PY seeds can remain non-germinated and viable in soil for many years, thus allowing them to form a soil seed bank (Baskin and Baskin 2014). In addition, soil seed bank persistence depends on the maintenance of PY in fire-prone regions (Ooi *et al.* 2012).

A wide range of environmental factors that potentially may disrupt seed-coat or endocarp-imposed dormancy under natural conditions have been identified. Temperature and water are the two important factors in breaking PY in seeds of many species (Baskin and Baskin 2014). Dormancy breaking occurs when a physically dormant seed receives an environmental signal, such as temperature fluctuations (Van Assche *et al.* 2003), wet heat (Martin *et al.* 1975; van Klinken and Flack 2005) dry heat (Jayasuriya *et al.* 2008, 2009; Morrison *et al.* 1998) or alternate wet–dry conditions (Gama-Arachchige *et al.* 2012). In Mediterranean ecosystems, dry heat from fires is a strong environmental cue for many species (Morrison *et al.* 1998). For the tropical tree *Parkinsonia aculeata*, wet heat is considered to be the principle dormancy release mechanism regulated by temperatures typically encountered in tropical regions (van Klinken and Flack 2005). Erickson *et al.* (2016) showed that both wet heat and dry heat have an effect on dormancy release in seven Fabaceae species in the arid zone of northwestern Western Australia (the Pilbara), but the optimal heat treatment for the release of PY was species-specific.

Several studies focused on how germination is regulated in seeds with PY in natural conditions. By comparing the actual proportion of the remaining dormant seeds in seed burial experiments, it has been shown that PY exhibits a marked seasonal pattern in germination, with seeds of most species germinating only in spring (Roberts and Boddrell 1985; Van Assche *et al.* 2003) or only in summer or autumn (Cook *et al.* 2008; Jaganathan *et al.* 2014; Turner *et al.* 2009; Van Assche *et al.* 2003). In *Geranium carolinianum*, low temperature in late summer/early autumn is the environmental signal that promotes PY breaking in sensitive seeds, and seeds germinate in early autumn. However, PY breaking of *G. dissectum* occurs in early summer, and germination of imbibed seeds is delayed by high temperatures until autumn. Depending on these two dormancy release strategies, seedling establishment of the two *Geranium* species occurs under favorable environmental conditions for seedling survival and plant establishment in autumn (Gama-Arachchige *et al.* 2012). However, not all species with PY show a marked seasonal pattern. Only six of 14 species studied by Van Assche *et al.* (2003) showed a marked seasonal pattern in germination; the other eight species germinated erratically in all seasons. These authors concluded that it is possible that these seeds gradually lose dormancy during storage in the soil and germinate in different seasons.

Oxytropis racemosa Turcz. (Fabaceae, subfamily Papilionoideae) is an herbaceous perennial widely distributed in Mu Us Sandland in semiarid northern China. This species is a pioneer in sand land and has high value in stabilizing this semiarid ecosystem (Lu *et al.* 2007). However, the dormancy characteristics of the seeds and the regeneration strategies of this species that are adaptive to inland semiarid areas characterized by cold, dry winters and hot summers with variable rainfall heretofore have not been investigated. Thus, the aims of our study were to: (i) identify the seed dormancy characteristics of this species; (ii) determine how seed dormancy release responds to moisture and temperature; (iii) examine the dynamics of the soil seed bank and seedling emergence in the natural habitat; and (iv) document seed dormancy loss via burial experiments in the natural habitat.

MATERIALS AND METHODS

Study species

Oxytropis racemosa is a low growing, sprawling polycarpic perennial herb with up to many stems, pinnately compound leaves and racemose inflorescences. The species is widely distributed in northern China, Korea and Mongolia. Its natural habitats include sandy soil on hillsides, valleys, grasslands, damp places, sandy or gravelly floodplains and riverbanks (Editorial Board of the Flora of China of Chinese Academy of Sciences 1998). The rose-purple flowers are papilionaceous. Fruits are ovoid, inflated and have a 0.5 mm-wide septum. Flowering occurs from May to July, and the fruits ripen from July to September, during which time the seeds are dispersed. Each individual plant can produce hundreds of seeds.

Study area and seed collection

Experiments were carried out at the Ordos Sandland Ecological Research Station of Chinese Academy of Science (39°29′N, 110°11′E, 1296 m a.s.l.) located in the north-eastern Mu Us Sandland in Inner Mongolia, China. Mu Us Sandland is a semiarid area with a typical continental semiarid climate characterized by cold and dry winters and hot summers with significant rainfall. Mean annual precipitation in the Sandland is 345.8 mm, and rainfall mostly occur during the growing season (April to September). Mean annual soil temperature is 11.8°C with a minimum of −20.4°C in December and maximum of 41.7°C in July. Data for temperature and precipitation during the study period were obtained from Ordos Sandland Ecological Research Station of Chinese Academy of Science.

In August 2014, ripe fruits were collected from a total of 50 individuals of *O. racemosa* growing in a natural population

near the Station. Seeds were removed from the fruits by hand, mixed thoroughly and stored dry in paper bags until used.

Seed size, mass and moisture content

Length and width of 20 haphazardly chosen seeds were measured using a binocular microscope equipped with an ocular micrometer. For determination of seed mass, 10 replicates of 1000 seeds were weighed to the nearest 0.0001 g using a digital analytical balance (Sartorius CPA324S, Sartorius AG, Gottingen, Germany). Seed moisture content (SMC) was determined by weighing 20 replicates of 100 seeds before and after drying in an oven at 105°C for 12 h. SMC was then calculated as follows: SMC = [(fresh seed mass − oven-dry seed mass)/fresh seed mass] × 100%.

Seed coat structure

Freshly matured seeds were manually scarified and then fixed in FAA solution (formaldehyde: glacial acetic acid: ethyl alcohol: water; 2:1: 10: 7, v/v/v/v). Then, seed coats were removed from the fixed seeds and dehydrated in 50% and then in 70% ethanol for 40 min each, sequentially transferred to 25, 50, 75 and 100% tertiary-butanol, embedded in paraffin (paraplast plus) and kept under ambient laboratory condition until used. Thin (16 µm) longitudinal sections of the seed coat were cut transversely using a hand rotary microtome (Leica RM 2135, Leica Microsystems, Wetzlar, Germany). Paraffin was removed using the Hemo-De ethanol gradient, and tissue was stained with 3% safranin in water and then with 1% fast green in 95% ethanol. Seed coat sections were observed using an Olympus light microscope (Olympus Corporation, Tokyo, Japan), and photographs were taken with a digital camera (Canon EOS 30 D; Canon, Tokyo, Japan).

Germination test of freshly matured seeds

Twenty-five fresh seeds were placed in each of four 5-cm-diameter Petri dishes on filter paper moistened with distilled water. The seeds were incubated at a 12 h daily photoperiod or in continuous darkness (Petri dishes in black opaque bags) at 12 h daily alternating temperature regimes approximating the average daily maximum and minimum temperatures in the natural habitat of *O. racemosa* in Ordos in April and October (5/15°C), May and September (10/20°C), June and August (15/25°C) and July (20/30°C). Light (12 h daily photoperiod, ~100 µmol m^{-2} s^{-1}) was provided by fluorescent tubes in the incubator. The Petri dishes in light were checked daily for 30 days for germinated seeds, which if present were recorded and removed from the dishes at each counting. However, to avoid exposing seeds to light, the dishes in darkness were examined only when the test was ended (30 days). The criterion for germination was a protruded radicle ≥1 mm.

Effect of mechanical scarification on seed imbibition and germination

To determine whether the seed coat is water-impermeable, an imbibition experiment was conducted for manually scarified and non-scarified (intact) seeds. To get rid of all permeable seeds, seeds were pre-incubated at 10/20°C on wet filter papers for 2 days before heat treatments, and germinated or swollen (imbibed) seeds were discarded. Four replicates of 25 impermeable seeds were individually scarified with a single-edge razor blade, and another four replicates of 25 intact seeds were used as control. Each sample of prepared seeds was weighed (time 0) and then placed on filter paper moistened with distilled water in 5-cm-diameter Petri dishes and kept in the laboratory at room temperature (20–25°C). After 1, 2, 4, 6, 8, 10, 12, 16, 18 and 24 h, the seeds were removed from each dish, blotted dry, weighed to the nearest 0.0001 g and, except for those weighed at 24 h, returned to the moist filter paper in Petri dishes. The amount of water imbibed was determined by increase in seed mass which was converted to a percentage.

$$\text{Percentage increase in mass} = \left[\left(W_i - W_d\right) / W_d\right] \times 100\%$$

where W_i is mass of seeds after a given interval of time of imbibition, and W_d is fresh dry seed mass.

Manually scarified and intact hard seeds were tested for germination in light at 10/20°C for 30 days and checked daily as described above.

Effects of dry heat and wet heat on germination

Different heat treatments were carried out to determine the effect of dry heat and wet heat on seed dormancy break. To ensure that all seeds in this experiment were impermeable, seeds were pre-incubated at 10/20°C on wet filter papers for 2 days before heat treatments, and germinated and swollen seeds were discarded.

Four replicates of 25 impermeable seeds were placed in nylon-mesh bags and dry-heated in a laboratory oven at 65, 80 and 95°C for 10, 30, 60, 120 or 180 min. For wet-heat treatments, 25 impermeable each were placed in each of four nylon-mesh bags (replicates) and dipped in a electric-heated, thermostat-controlled water bath at 65, 80 and 95°C for 5, 15, 30, 45 or 60 min. Following the treatments, seeds were incubated in light at 10/20°C for 30 days and checked daily as described above.

Effects of wet heat/ice water cycles on seed germination

Different cycles of wet heat/ice water treatments were carried out to determine the effect of hot–cold treatments on dormancy breaking. To ensure that all seeds used in this experiment were impermeable, seeds were pre-incubated at 10/20°C on wet filter papers for 2 days, and germinated and swollen seeds were discarded.

Four replicates of nylon bags (5 cm × 10 cm), each containing 100 seeds, were used in the wet heat/ice water treatments. Seeds were subjected to hot water (90°C) for 15 s and then immediately transferred to ice water for 2 min; seeds were given 0, 1, 4, 7, 10 and 20 cycles of hot–cold treatments. Following a treatment, seeds were incubated in light at 10/20°C for 30 days and checked daily as described above.

Seasonal dynamics of seed bank at different sand depths

To investigate the dynamics of the soil seed bank of *O. racemosa*, we collected soil samples in May, July, September and November from the habitat of *O. racemosa* near the Station, starting on 7 July 2014 and ending on 1 July 2016. For each sampling date, 30 1 m × 1 m plots were set along each of two transects that were 4 m apart. Each transect was 60 m in length, and quadrats in a transect were 10 m apart. In each quadrat, soil samples at depths of 0–2, 2–5 and 5–10 cm were collected using a soil sampler (10 cm ×10 cm ×10 cm = 1000 cm^3). The soil samples were taken to the laboratory, air dried for 2 days and sieved through a 0.8 mm mesh. Seeds of *O. racemosa* were separated from the sand, and the viable seeds were counted as being in the soil seed bank. Viability was tested using 1% triphenyl tetrazolium chloride (TTC) (Baskin and Baskin 2014). Finally, the density of seeds in the seed bank was calculated as the number of seeds per square meter.

Seed burial experiment

One-hundred and fifty freshly collected seeds were placed in each of 240 nylon bags, and 80 bags each were buried at soil depths of 0, 2 and 5 cm on 1 September 2014. The burial site was an open area near the Station, and it was fenced by wire mesh to prevent disturbance by animals. Four bags from each depth were exhumed and brought to the laboratory each month for 20 months beginning on 30 September 2014 and ending on 30 April 2016. The retrieved seeds were divided into three groups: (i) germinated in field, the percentage of germinated seeds (seedlings) in the retrieved bags; (ii) germination percentages in the laboratory of the remaining intact viable seeds in the bags; and (iii) dormant, percentage of remaining hard seeds after the laboratory germination test. Germination tests were conducted in light at 10/20°C for 30 days. Dead seeds in the field are rare, and thus we did not include them in calculations of seed fate. Viability of the non-germinated seeds was tested using 1% TTC (Baskin and Baskin 2014). A button-type recorder (Maxim IC, MAXIM INTEGRATED, USA) was placed at each of the three sand depths adjacent to the burial site for monitoring temperatures at 30-min intervals.

Seedling emergence in field-sown experiment

To monitor the dynamics of seedling emergence, 10 plastic pots (10 cm diameter × 20 cm depth) were nearly filled with sand and then sunk into the sand in the field near the Station until the rim of the pot was level with the sand surface. On 7 September 2014, after seed dispersal in the natural habitat, 200 seeds were sown at a depth of 2 cm in each pot. At 2-week intervals until 1 September 2016, the number of emerged seedlings was counted, after which they were removed with minimal disturbance of the soil. Seedling emergence percentage in each month and cumulative seedling emergence percentage were calculated.

A button-type recorder (Maxim IC, MAXIM INTEGRATED) was placed 2 cm below the sand surface adjacent to the plastic pots for monitoring temperatures at 30-min intervals. Monthly total rainfall data from September 2014 to August 2016 were obtained from the Ordos Sandland Ecological Research Station of the Chinese Academy of Science.

Statistical analyses

Values were expressed as means ± standard error. Germination data were $\log_{10}$, arcsine or square-root transformed to ensure normality and homogeneity of variance before statistical analysis. One-way analyses of variance (ANOVA) were used to test the effects of wet heat/ice water cycles on germination. Two-way ANOVA were carried out to analyze the effects of temperature, light and their interaction on germination percentage; the effects of temperature, scarification and their interaction on germination percentage; the effects of temperature, dry/wet heat and their interaction on germination; and the effects of burial time, soil depth and their interaction on seed fates. If ANOVA indicated a significant difference, Tukey's HSD post-hoc test was used to determine differences between treatments ($P < 0.05$). Data were analyzed using SPSS version 18.0 (SPSS, Chicago, IL, USA).

RESULTS

Seed characteristics and seed coat structure

Mature seeds of *O. racemosa* are dark brown and heart-shaped. The 1000-seed mass of fresh seeds was 1.35 ± 0.09 g. Length and width of seeds were 2.10 ± 0.21 and 1.79 ± 0.17 mm, respectively. Moisture content of fresh seeds was 7.98 ± 2.10%.

The seed coat is covered by a thin cuticle. Beneath the cuticle, tightly packed cells form the palisade layer, which is responsible for the water impermeability of the seeds. A light line appears to run through the upper part of the palisade cells. Osteosclereids occurred under the palisade layer and parenchyma cells below the osteosclereids.

Germination test of fresh seeds

Germination of fresh non-scarified seeds was 4–11% in light and 5–14% in darkness across the four temperature regimes. The highest germination was 14 ± 2%, at 5/15°C in darkness. Two-way ANOVA showed that light ($F = 33.375$, $P < 0.05$) and temperature ($F = 3.818$, $P < 0.05$) had significant effects on seed germination percentages, while the effect of their interaction ($F = 1.137$, $P = 0.354$) was not significant.

Germination percentage in darkness was higher than it was in light at 5/15°C, while light had no effect on germination at the other three temperature regimes. Germination percentage at 20/30°C was lower than it was at the other three temperature regimes in both light and darkness.

Effect of mechanical scarification on seed imbibition and germination

After 2 h, scarified seeds had imbibed significantly more water than non-scarified ones (Fig. 1B). The mass of scarified seeds increased by 87 ± 5% in 1 h and 156 ± 5% (fully imbibed)

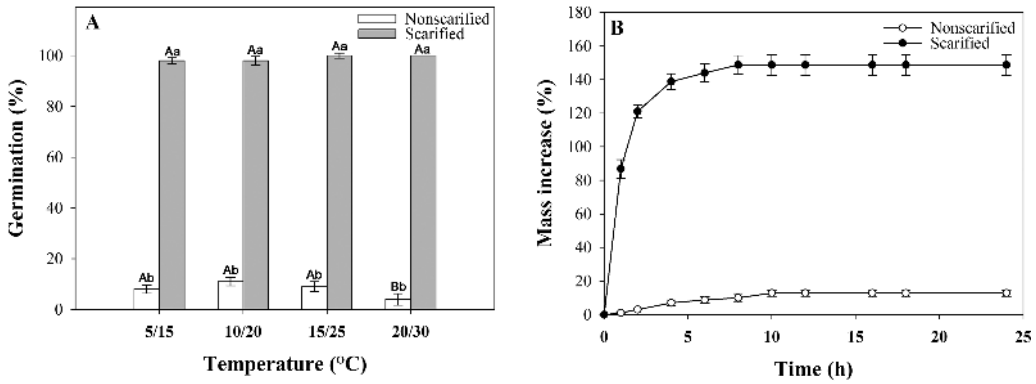

Figure 1: (**A**) germination percentages (mean ± SE) of scarified and non-scarified *Oxytropis racemosa* seeds in light at four temperature regimes after 30 days of incubation. Different uppercase letters indicate significant difference between temperatures in the same scarified or non-scarified treatment and different lowercase letters significant difference between scarified vs non-scarified treatments at the same temperature ($P < 0.05$). (**B**) Increase in mass of scarified and non-scarified *O. racemosa* seeds. Data are mean ± SE. Abbreviation: SE = standard error.

in 24 h, whereas the mass of non-scarified seeds increased by only 1 ± 0.6% in 1 h and 12 ± 2% in 24 h (Fig. 1B). None of the scarified seeds had begun to germinate after 24 h. Increase in mass in non-scarified seed reached 12% after 10 h and remained at 12% increase throughout the experiment. None of the non-scarified seeds germinated. The increase in mass was due to imbibition of a few seeds; most of the seeds did not imbibe any water.

Two-way ANOVA showed that scarification (F = 960.775, $P < 0.05$) and the interaction of scarification and temperature ($F = 9.739$, $P < 0.05$) had significant effects on germination, while the effect of temperature was not significant. After 30 days of incubation, mechanically scarified seeds had germinated to 96–100% in light at all temperature regimes, while the non-scarified seeds had germinated to only 7–10% (Fig. 1A).

Effects of dry heat and wet heat on germination

Neither dry heat nor wet heat was effective in breaking dormancy. For the dry heat treatments, the highest germination was for seeds heated 80°C for 120 min, but only 18 ± 3.42 % of them germinated (Fig. 2A).

For wet heat treatments, the highest germination was for seeds heated at 95°C for 5 min, but only 22 ± 3.83 % of them germinated (Fig. 2B). With increase in time and temperature of wet heat, the percentage of non-viable seeds increased. Most seeds were killed at 95°C, reaching 85 ± 6.19 % at 60 min (Fig. 2D).

Effects of wet heat/ice water cycles on germination

One-way ANOVA showed that wet heat/ice water treatment ($F = 11.782$, $P < 0.05$) had a significant effect on germination. Germination percentage of seeds increased with increase in number of wet heat/ice water cycles. After 30 days of incubation, germination of seeds that had been subjected to 1, 4, 7, 10 and 20 cycles of wet heat/ice water treatment were 23.75 ± 3.14, 38.75 ± 4.26, 58.75 ± 7.18, 87.50 ± 4.33 and 90 ± 4.08%, respectively.

Seasonal dynamics of soil seed bank at different sand depths

Most of the soil seed bank of *O. racemosa* was in the 0–2 cm soil depth layer, and it exhibited an annual cycle in number of seeds. The highest density of seeds in the 0–2 cm and in the 2–5 cm layer was in September 2014 and 2015, and the lowest density of seeds in these two layers were in July of all 3 years of the experiment. The highest density of seeds in the 0–2 cm soil layer was 2042 ± 201 m^{-2}, in September 2014 following seed dispersal. Then, the density of the soil seed bank declined with time and reached the lowest density (439 ± 110 m^{-2}) in July 2015. After that, the soil seed bank increased again in September 2015 following seed dispersal and then declined again with time to 1178 ± 153 m^{-2} in July 2016. A small portion of the seed bank was in the 2–5 cm soil depth layer, but there were no seeds in 5–10 cm soil depth layer. The density of seeds in the 2–5 cm layer also was highest in September 2014 (326 ± 55 m^{-2}) and September 2015 (363 ± 56 m^{-2}), and then declined with time to its lowest number in July 2015 (30 ± 15 m^{-2}) and July 2016 (48 ± 20 m^{-2}) (Fig. 3).

Burial experiment

Monthly mean maximum and minimum soil temperatures at the 0, 2 and 5 cm depths exhibited seasonal changes, and they differed greatly. The maximum–minimum temperature differences at 0 cm was greater than those at 2 and 5 cm (Fig. 4A).

Two-way ANOVA showed that burial time ($F = 315.756$, $P < 0.001$), burial depth ($F = 5198$, $P < 0.001$) and their interaction ($F = 16.540$, $P < 0.001$) had a significant effect on germination (emergence) in the field (Table 1). With an increase in burial time, seeds gradually lost dormancy, and germination (emergence) percentage of seeds in the field increased. More seeds buried at 2 cm (Fig. 4C) and 5 cm (Fig. 4D) lost dormancy than those on the soil surface (Fig. 4B).

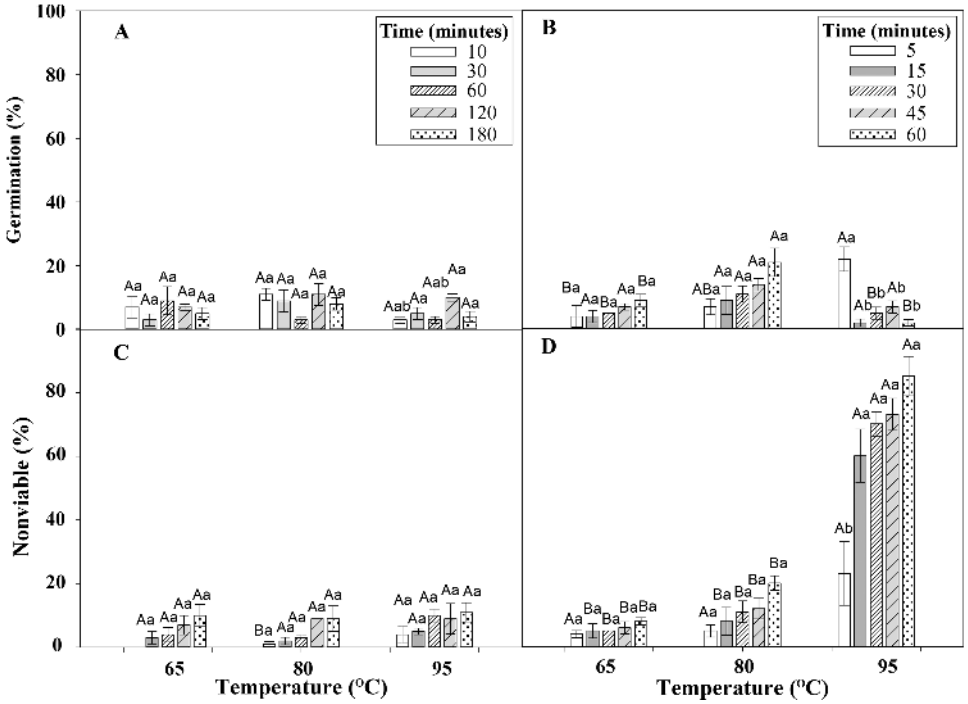

Figure 2: germination at 10/20°C and loss of viability (mean % ± SE) of *Oxytropis racemosa* seeds after exposure to dry-heat (**A**, **C**) and wet-heat (**B**, **D**) at three temperatures for different periods of time. No control seeds germinated. Different uppercase letters indicate significant differences between temperatures in the same treatment time and different lowercase letters significant differences between treatment times at the same temperature ($P < 0.05$). Abbreviation: SE = standard error.

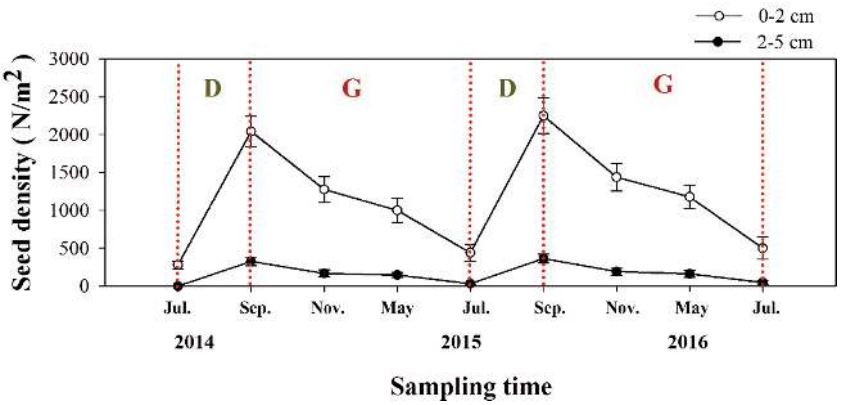

Figure 3: density (mean ± SE) of *Oxytropis racemosa* seeds at soil depths of 0–2 and 2–5 cm in soil seed bank from July 2014 to July 2016. Seeds are dispersed from July to September. D: dispersal period; G: Germination period. Abbreviation: SE = standard error.

Two-way ANOVA showed that burial time ($F = 2.492$, $P < 0.05$) and burial depth ($F = 3.798$, $P < 0.05$) had a significant effect on germination in the lab, while their interaction ($F = 1.161$, $P = 0.256$) was not significant (Table 1). With an increase in burial time, germination percentage in the lab gradually increased from September 2014 to July 2015 and then decreased from August 2015 to April 2016 at 2 and 5 cm burial depths (Fig. 4C and D).

Two-way ANOVA showed that burial time ($F = 40.155$, $P < 0.001$), burial depth ($F = 641.983$, $P < 0.001$) and their interaction ($F = 2.430$, $P < 0.001$) had significant effects on seed dormancy (Table 1). With an increase in burial time, seeds gradually lost dormancy, and thus the percentage of dormant seeds decreased. After 20 months, 42 ± 2.16% of seeds on the soil surface remained dormant, while only 30 ± 3.03% and 20 ± 2.65% of those buried at 2 and 5 cm depth were still dormant, respectively (Fig. 4B–D).

Seedling emergence in field-sown experiment

Monthly mean maximum and minimum soil temperatures at the 2-cm depth changed seasonally. During the experimental period, monthly soil temperature reached a high of 34.9°C, in

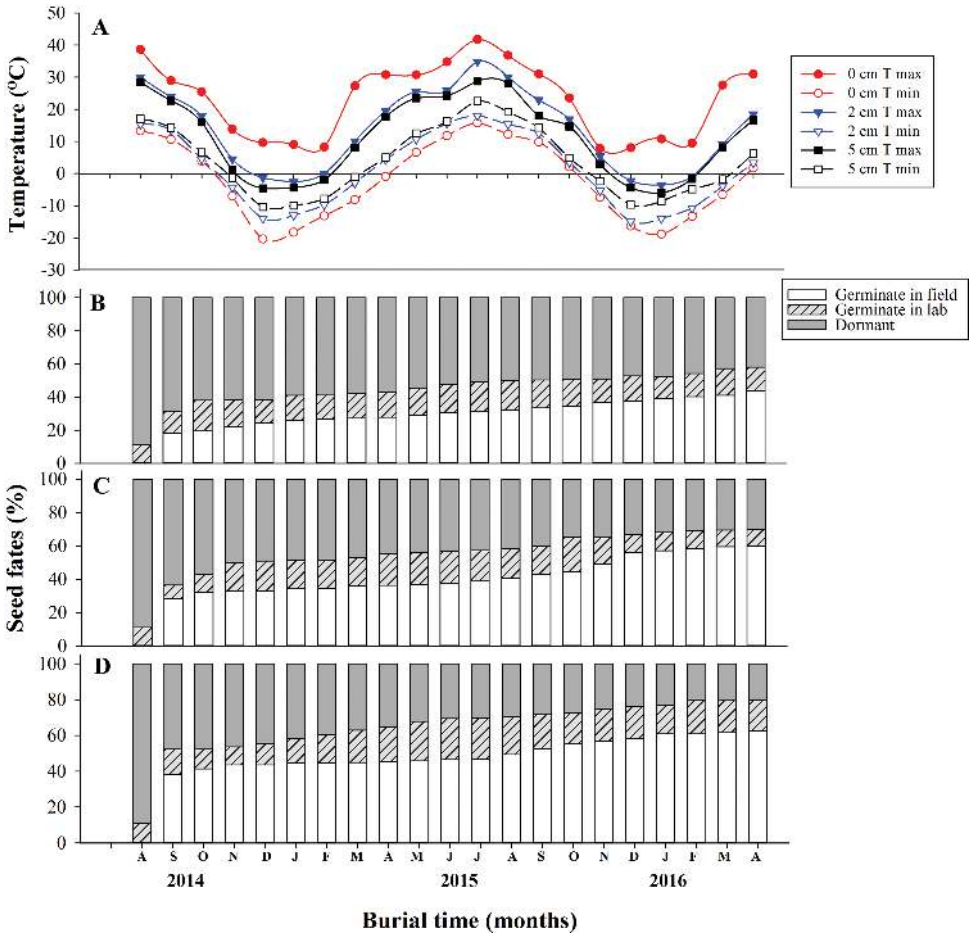

Figure 4: monthly mean maximum and minimum soil temperatures at 0, 2 and 5 cm soil depths from August 2014 to August 2016 (**A**) and fates of seeds at soil depths of 0 cm (**B**), 2 cm (**C**) and 5 cm (**D**).

Table 1: two-way ANOVA of the effects of burial time, burial depth and their interaction on seed germination in the field, germination in the lab and dormancy of *Oxytropis racemosa*

Dependent variable	Source	Type III sum of squares	*df*	*F* value	*P* value
Germination in field	Time	3.443	19	315.756	<0.001
	Depth	5.966	2	5198	<0.001
	Time × Depth	0.361	38	16.540	<0.001
Germination in lab	Time	0.128	19	2.492	0.001
	Depth	0.020	2	3.798	0.024
	Time × Depth	0.119	38	1.161	0.256
Dormancy	Time	2.744	19	40.155	<0.001
	Depth	4.618	2	641.983	<0.001
	Time × Depth	0.332	38	2.430	<0.001

July, and a low of −15°C, in December 2015 (Fig. 5A). Rainfall events occurred mainly from April to September in the growing season, but the amount of precipitation differed greatly among months and years (Fig. 5A). The total rainfall was 197.6 mm from April to September in 2015, whereas it was 445 mm in 2016.

Generally, seedling emergence was coordinated with temperature and rainfall in the growing season. Cumulative seedling emergence increased from April to October in 2015 and from April to August in 2016 (end of experiment). The highest seedling emergence percentage was in September 2015 (9 ± 2.0%) and August 2016 (10 ± 2.0%) (Fig. 5B). After 2 years, cumulative seedling emergence had reached 81 ± 0.9% (1629 of 2000 seeds), and there were 19 ± 1.4% impermeable viable seeds remaining in

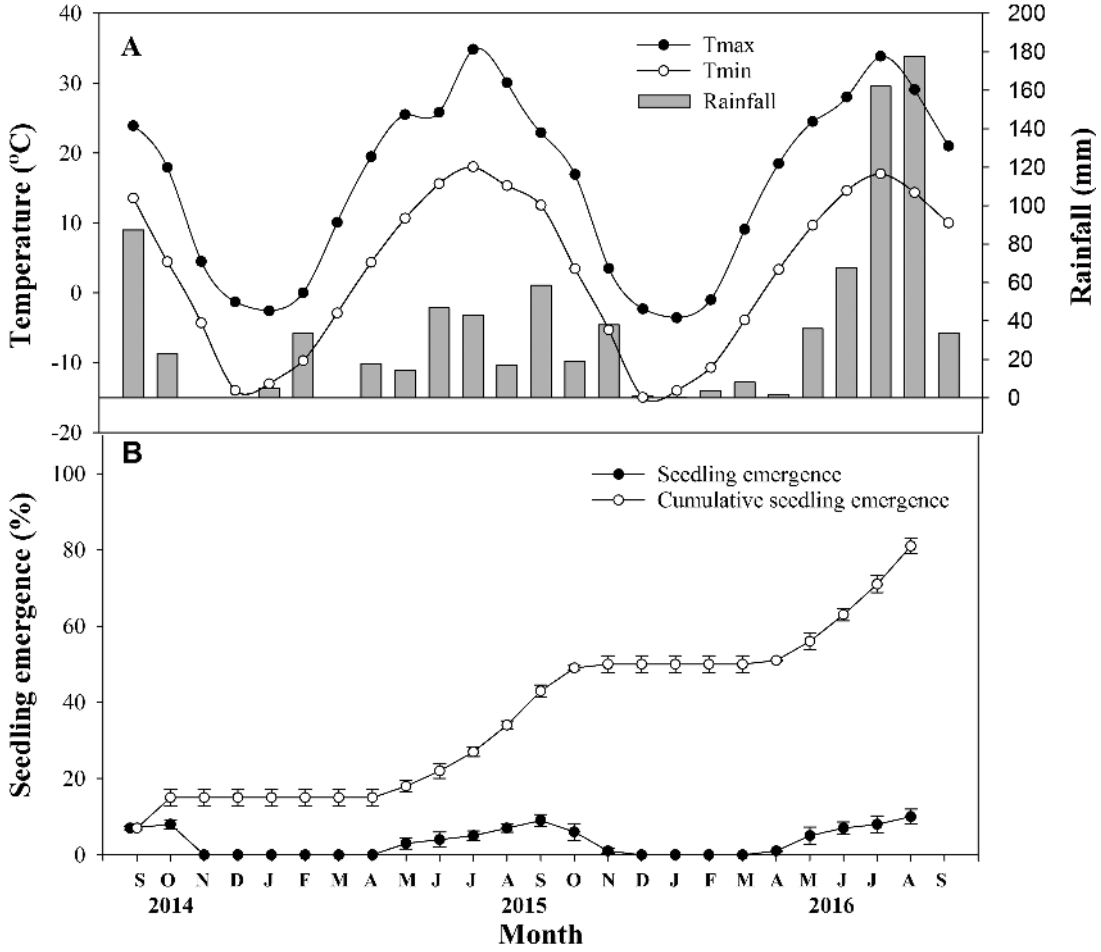

Figure 5: (**A**) monthly mean maximum and minimum soil temperature at 2 cm soil depth from September 2014 to August 2016 and monthly total rainfall recorded at Ordos Sandland Ecological Research Station of Chinese Academy of Science. (**B**) Seedling emergence percentage and cumulative seedling emergence (mean ± SE) of *Oxytropis racemosa* seeds buried at 2 cm soil depth in the field. Abbreviation: SE = standard error.

the soil. Thus, no seeds lost viability during the 2-year burial period.

DISCUSSION

As a contribution to understanding the adaptive strategy of *O. racemosa* to its unpredictable habitat, we investigated seed dormancy and germination of this species. A high percentage of freshly matured intact seeds did not imbibe water, and only a low percentage of them germinated. In contrast, all mechanically scarified seeds imbibed water rapidly (Fig. 1B), and 96–100% of them germinated over a wide range of temperature regimes (Fig. 1A). Thus, the seeds have PY and the embryo is non-dormant, i.e. they do not have combinational dormancy (physical + physiological dormancy). In addition, the seed coat of *O. racemosa* consists of a palisade layer that prevents imbibition of water by seeds. Our results are consistent with the occurrence of PY in almost all temperate and arctic zone species of Fabaceae (Baskin and Baskin 2004, 2014).

Timing of release of PY is important for the seedling establishment under variable natural conditions (Baskin and Baskin 2014). Water and temperature are the two principal environmental factors involved in dormancy breaking under natural conditions. Several natural mechanisms of PY breaking have been proposed, such as high temperatures in summer, fire, temperature fluctuation and wet heat (van Klinken and Flack 2005). High temperature has been shown to be critical in breaking PY, but species vary in temperature requirements. For example, dry heat treatment at 80°C is required to break dormancy of *Acacia* species from fire-prone environments, but it kills seeds of *Trifolium* species (Auld and O'Connell 1991). However, a temperature of 95°C for 180 min did not break dormancy of *O. racemosa* seeds (Fig. 2A), suggesting that high temperatures in the natural sandland habitat is not effective in releasing dormancy of seeds of this legume. High temperatures and dry heat also have been shown not to be effective in breaking PY in some other legumes (Martin *et al.* 1975; Morrison *et al.* 1998). Low temperature is also an

environmental cue that can release PY in some Fabaceae species (Van Assche *et al.* 2003). Seeds of the central Asian sand dune endemic species *Eremosparton songoricum* (Fabaceae, subfam. Papilionoideae) exposed to natural temperature and soil moisture regimes in the field in autumn and winter germinated to higher percentages than those exposed only to autumn conditions, indicating that low temperatures in winter make the seeds sensitive to the following high temperatures in spring (Liu *et al.* 2017). Therefore, high spring temperatures following exposure to low winter temperatures may be effective in dormancy release of *O. racemosa*.

Soil moisture also could be an important environmental cue for breaking PY. When immersed in water for 18 weeks, seeds of *Acacia nilotica* plants growing on the riverside in Sudan and Egypt imbibed and germinated best, corresponding to the average time of annual flooding duration (Warrag and Eltigani 2005). For the leguminous tree *Parkinsonia aculeata*, wet heat is an important dormancy-release mechanism (van Klinken and Flack 2005) that maximizes the probability of seedling establishment (van Klinken *et al.* 2008). Erickson *et al.* (2016) showed that wet heat was more effective than dry heat for release of PY in seeds of Fabaceae (seven species of *Acacia* and one of *Senna*). Germination of *O. racemosa* seeds increased slightly after wet heat at 60 and 80°C, but germination percentage was relatively low over all wet heat treatments (Fig. 2B). However, a high percentage of *O. racemosa* seeds lost viability when incubated at 95°C for >15 min in the wet heat treatment (Fig. 2D). These results suggest that a single exposure of *O. racemosa* seeds to wet heat in its habitat will break PY in a small percentage of the seeds. Liu *et al.* (2017) suggested that *Eremosparton songoricum* seeds in a cohort may have different requirements for heat and/or cold to break PY. Thanos and Georghiou (1988) showed that a certain heat treatment breaks PY of a portion of the seeds of *Cistus* spp. while killing another fraction of them. They suggested that the seeds form a hardseededness continuum. In addition, heterogeneity of seed coat hardness also has been reported in several species of Fabaceae (Doussi and Thanos 1994). Thus, we suggest that seeds of *O. racemosa* may exhibit a continuum of hardseededness. In which case, in the field one exposure to wet heat may break PY in 10% of the seeds, the second exposure 10% of the remaining seeds, etc.

Previous studies have reported that fluctuating temperature is an important factor in releasing PY (Baskin and Baskin 2006, 2014). Wet heat/ice water cycles may be an effective way to break PY when neither wet nor dry heat treatments make seeds water-permeable. PY in *Sophora moorcroftiana* seeds from the Qinghai-Tibet Plateau of China did not germinate after dry heat, wet heat or chilling in ice water, but they germinated to 90–100% when subjected to wet heat (90 or 100°C)/ice water (0°C) cycles (Baskin *et al.* 2007). Consistent with this, germination of *O. racemosa* seeds reached 90% after treating them with 20 wet heat/ice water cycles. Although seeds of this species would never be exposed to such temperature fluctuations in nature, the method could be useful in germinating seeds for restoration purposes.

Studies have reported that temperature and soil moisture at different burial depths are two key factors that influence PY release in the natural environment (Cox *et al.* 1993; Hu *et al.* 2009; Liu *et al.* 2017). Liu *et al.* (2017) found that seeds of *Eremosparton songoricum* buried at a depth of 3 cm in sand in the field germinated to higher percentages than those on the soil surface and of those buried at 8 cm. The authors indicated that higher soil moisture might be responsible for the higher germination percentage of seeds buried at 3 cm compared to those on the soil surface. They further suggested that the higher dormancy break at 3 cm than at 8 cm is related to the the higher temperature fluctuation at 3 cm and not to differences in soil moisture. In the papilionaceous legume *Sophora alopecuroides*, dormancy was released from a higher percentage of seeds buried at 2 and 7 cm soil depth than it was for those on the soil surface (Hu *et al.* 2009). The possible explanation for the difference in dormancy breaking percentage between seeds on the soil surface and those buried at 2 and 7 cm is that buried seeds were exposed to more suitable temperature and moisture (wet heat) for dormancy release than those on the surface (Hu *et al.* 2009).

In our study, the percentages of seeds of *O. racemosa* placed on the soil surface that broke dormancy was lower than those for seeds buried at 2 or 5 cm (Fig. 4B–D). This difference may be due to higher water availability for a longer period of time at 2 and 5 cm depths than on the soil surface. Although temperature fluctuation on the soil surface was higher than it was at 2 and 5 cm depths (Fig. 4A), the soil surface would have experienced a shorter period of moisture (due to higher evaporation) than the soil atmosphere at depths of 2 and 5 cm. High water availability was an important environmental cue promoting dormancy release of seeds of *Vicia villosa* subsp. *villosa* in semi-arid environments (Juan *et al.* 2016). Thus, the variation in temperature and moisture among burial depth of 0, 2 and 5 cm resulted in various amounts of dormancy release of *O. racemosa* seeds during burial experiments. Further, seedlings of *O. racemosa* gradually emerged from burial at 2 cm soil depth from May to October in 2015 (Fig. 5B), indicating that dormancy of *O. racemosa* seeds was gradually broken (Fig. 4B–D and 5B). These data show that difference in the PY-breaking requirement spreads germination of *O. racemosa* seeds over time in its harsh unpredictable habitat, Thus, heterogeneity of the hardness of the *O. racemosa* seed coat may be responsible for the different requirements for PY-breaking and for gradual PY release.

There were some dormant seeds of *O. racemosa* in the 0–2 and 2–5 cm soil layers in the natural habitat after 2 years of investigation of the soil seed bank (Fig. 3). Thus, this species can form at least a short-lived persistent seed bank. The soil seed bank has been shown to contribute to population maintenance in variable environments (Auld *et al.* 2000; Fenner and Thompson 2005; Gao *et al.* 2014; Pake and Venable 1996). PY seeds are known to be long-lived and to form a persistent seed bank (Baskin and Baskin 2014; Ooi 2012; Ooi *et al.* 2012; Peguero and Espelta 2014). For example, some seeds of

Astragalus distortus (Fabaceae) germinated 24 years after sowing them on the soil surface under near-natural conditions in a non-heated greenhouse in Lexington, Kentucky (USA) (Baskin and Baskin 2014).

We conclude that a persistent seed bank and the spreading germination of a seed cohort between seasons and years are important components of the adaptive life history strategy of *O. racemosa* to successfully cope with the unpredictable environment in its semiarid habitat.

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