

Seed germination and seedling emergence of three *Artemisia* species (*Asteraceae*) inhabiting desert sand dunes in China

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Abstract

Artemisia ordosica, *A. arenaria* and *A. sphaerocephala* are semi-shrubs inhabiting desert sand dunes in China and often used to rehabilitate desertified areas. Improvement of dune rehabilitation success by sowing requires better understanding of the processes involved in the control of seed germination and seedling emergence in these species. Thus, (1) effects of temperature, light and osmotica (polyethylene glycol-6000) on seed germination, and (2) effects of seed burial depth in sand and irrigation regime on seedling emergence, were studied under controlled conditions. Seeds of the three species required light for germination, and the light fluence needed for germination was dependent on temperature. Seedling emergence of the three species was maximal (70–94%) for seeds sown at a depth of 2.5mm, and decreased with increasing seed burial depth when the pots were initially and subsequently treated with 16mm and 3mm irrigation at 1-d intervals. However, when the pots were initially and subsequently treated with 8mm and 3mm irrigation at 2-d intervals, seedling emergence was almost completely suppressed due to water deficiency in sand. It is suggested that the probability of seed germination and seedling emergence of the three species in the field is very limited, because the light requirement restricts seed germination to shallow sand layers where water is lost rapidly due to evaporation. Temperature appeared to have secondary effects on seed germination, by modifying the light sensitivity of seeds.

Keywords: *Artemisia arenaria*, *Artemisia ordosica*, *Artemisia sphaerocephala*, irrigation, light, seed burial depth, temperature

Introduction

Artemisia species (*Asteraceae*) are widely distributed in the northern hemisphere. Some *Artemisia* species are dominant in the deserts of Israel and North America, and their seed germination characteristics have been reported (Koller *et al.*, 1964a, b; Friedman and Orshan, 1975; Young and Evans, 1975, 1989a, b; Meyer *et al.*, 1990; Huang and Gutterman, 1998). Fifty-one species of *Artemisia* (17 semi-shrubs and 34 herbs) are reported to occur in the deserts of China (Lanzhou Institute of Desert Research, 1992). Among them, the deciduous semi-shrubs, *A. ordosica* Krasch., *A. arenaria* DC. and *A. sphaerocephala* Krasch., inhabit sand dunes and are often used to rehabilitate desertified dunes in China. *A. arenaria* occurs in the central and western Chinese deserts, while *A. ordosica* and *A. sphaerocephala* occur in the central and eastern deserts. *A. sphaerocephala* is found on mobile dunes, whereas *A. ordosica* and *A. arenaria* are found on semi-stable or stable dunes (Kobayashi *et al.*, 1995; Hirobe *et al.*, 2001; Qiu *et al.*, 2001a, b). For the rehabilitation of desertified dunes in China, seeds (achenes) of these species are often dispersed by aircraft. Improvement of dune rehabilitation success by seed dispersal requires a better understanding of the processes involved in the control of seed germination and seedling emergence in these species.

Huang and Gutterman (1999, 2000) reported that seeds of *A. ordosica* and *A. sphaerocephala* required light for germination and did not germinate when buried deeply in sand. Seeds of both species have a

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mucilaginous coat and accumulate a substantial amount of water in the mucilage after watering. Positive photoblastism and the presence of a mucilaginous seed coat have been reported for other species of *Artemisia*. In *A. monosperma*, a dominant shrub in sandy deserts of Israel, germination was stimulated by very weak light (Koller *et al.*, 1964b), independently of wavelength (Koller *et al.*, 1964a), thus suggesting that germination was induced by the very low fluence response (VLFR) of phytochromes (Dixit and Amritphale, 1996; Casal *et al.*, 1998).

In psammophytic species requiring light for germination, seed germination is limited to the shallow sand layers where light penetrates (Woolley and Stoller, 1978; Bliss and Smith, 1985; Tester and Morris, 1987; Mandoli *et al.*, 1990). However, seed germination in shallow sand layers may be constrained by additional critical factors, such as higher temperature and lower water availability due to rapid evaporation after rainfall events (Mott, 1972, 1974; Tobe *et al.*, 2005a), thus hindering water uptake by seeds in this layer. Water availability is an important limiting factor for seed germination in desert environments (Tevis, 1958; Ackerman, 1979; Loria and Noy-Meir, 1979/1980; Gutterman and Evenari, 1994). Thus, restriction of seed germination to the shallow sand layers may considerably reduce seedling establishment of the *Artemisia* species used for dune rehabilitation. Although seedling emergence in *A. ordosica* and *A. sphaerocephala* was examined under sufficiently irrigated conditions (Huang and Gutterman, 1999, 2000), their response to limited water availability is not known.

In this study, we investigated seed germination and seedling emergence of *A. ordosica*, *A. arenaria* and *A. sphaerocephala* that are used for dune rehabilitation, particularly with regard to water availability in sand. We examined the effects of temperature, light and polyethylene glycol (PEG)-6000 on seed germination and the effects of seed burial depth in sand and irrigation regime on seedling emergence.

Materials and methods

Materials

Seeds of *A. ordosica* were collected in December 1997 and October 2000 from a sand dune in Shapotou (37°26'N, 104°57'E; annual precipitation, 188 mm; annual mean temperature, 10.5°C); *A. arenaria* seeds in October 2000 from a field botanical garden in Minqin (38°34'N, 102°58'E; annual precipitation, 120 mm; annual mean temperature, 7.8°C); and *A. sphaerocephala* seeds in February 2000 from a sand dune in Dengkuo (40°23'N, 106°56'E; annual precipitation, 149 mm; annual mean temperature, 7.4°C). In

the deserts in China, seeds of these *Artemisia* species ripen in October. In these regions, winter temperatures fall below 0°C, inhibiting seed germination in winter. In the middle and eastern parts of the deserts in China, which are the main habitats of these species, precipitation predominates in summer due to a continental monsoon (Fullen and Mitchell, 1994). In Shapotou, where *A. ordosica* and *A. sphaerocephala* occur, 87% of the annual precipitation fell between April and September and 55% in July and August (1990–1995). In Shapotou, daily precipitation was usually less than 8 mm between October and early June, but often exceeded 24 mm between late June and September; in 1996, the number of days per month in which daily precipitation exceeded 1 mm was 1, 2, 5, 7 and 5 d in April, May, June, July and August, respectively. In Shapotou, in 1996, the means of the daily lowest and highest temperatures in April were 4.2°C and 17.4°C, respectively, and both the daily lowest and highest mean temperatures were highest in July (18.0°C and 29.0°C, respectively).

Except for seeds used for the dormancy test, the collected seeds of *A. ordosica* and *A. arenaria* were initially stored dry at room temperature (c. 23°C) for 12 months (*A. ordosica*) or 6 months (*A. arenaria*) to break seed dormancy, and thereafter, stored dry in a refrigerator (c. 0°C) until they were used for experiments. *A. sphaerocephala* seeds were stored dry in a refrigerator (c. 0°C) until they were used for experiments. Seed weights of *A. ordosica*, *A. arenaria* and *A. sphaerocephala* were 0.27 ± 0.10 mg, 0.76 ± 0.11 mg and 0.70 ± 0.12 mg (mean ± SD, *n* = 20), respectively. Experiments were carried out from January 2000 to September 2002, except for experiments with *A. ordosica* seeds collected in 2000, which were carried out in July and August 2005.

Methods

Experiment 1: Effects of temperature on seed germination in interrupted dark and light/dark photoperiods

Replicates of 25 seeds were sown on three layers of filter paper (Toyo, No. 1) in a 90-mm glass or plastic Petri dish with about 10 ml of deionized water. The Petri dishes were covered with lids and maintained in an incubator at a constant temperature (10–30°C) or a diurnally alternating temperature regime (12-h thermoperiods of 10°C/20°C, 15°C/25°C or 20°C/30°C) in interrupted dark or 12-h light/12-h dark. In the interrupted dark treatment, seeds were maintained in darkness, except for 1–2 min daily exposure to dim light [photon flux density (PFD) c. 2 µmol m⁻² s⁻¹ at wavelengths 400–700 nm], to monitor germination. In light/dark treatment, seeds were illuminated with fluorescent lamps (PFD 80–90 µmol m⁻² s⁻¹) during

the 12-h photoperiods. In the treatments in which photoperiods were combined with alternating temperature regimes, seeds were exposed to the lower temperatures during the 12-h dark period. During the period of exposure to light, the temperature in the Petri dishes was *c.* 2 °C higher than that in the incubator. In all the treatments, germination was monitored daily, and seeds with radicles longer than 5 mm were regarded as germinated and discarded; about two-thirds of the volume of the fluid in each Petri dish was replaced daily. Final germination percentage (G_F), the number of days until seed germination was initially observed (T_{init}) and the number of days until 0.5 × G_F % of seeds germinated ($T_{1/2}$) were determined after 14 d of incubation.

Experiment 2: Effects of water potential on seed germination in interrupted dark and light/dark photoperiods

The effects of water potential on seed germination were examined by incubating seeds, as described in Experiment 1, at 20 °C either in interrupted dark or 12-h light/12-h dark with deionized water or PEG-6000 solutions [water potential (Ψ_w) 0 to -1.2 MPa, determined using an isopiestic psychrometer at 20 °C; Boyer and Knippling, 1965]. To maintain the temperature of the seeds at 20 °C, the temperature in the incubator was set at 18 °C during the 12-h photoperiods. G_F , $T_{1/2}$ and T_{init} were determined after 20 d of incubation.

Experiment 3: Seed germination in complete dark and effects of low-fluence light on seed germination

To examine seed germination in complete darkness, seeds were incubated in the dark, as described in Experiment 1, at different temperature regimes (20 °C, 12-h 10 °C/12-h 20 °C, 12-h 15 °C/12-h 25 °C or 12-h 20 °C/12-h 30 °C) without daily observation. Additionally, to examine the sensitivity of seed germination to low-fluence light exposure, the effects of one-time illumination with dim light (PFD *c.* 2.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 5 min, 5 d after irrigation, on G_F were examined. G_F was determined after 10 d of incubation.

Experiment 4: Effects of seed burial depth and irrigation regimes on seedling emergence

Replicates of 16 seeds were sown on the surface or at a depth of 2.5, 5.0, 10 or 15 mm in dry sand in drained cylindrical plastic pots (inner diameter, 80 mm; height, 50 or 100 mm; sand depth, 45 or 90 mm). Sand used in this experiment was described in Tobe *et al.* (2005a), and is similar in particle-size distribution to that of the sand in the desert sand dunes in China. The pots were placed in an incubator and maintained at 12-h 25C

(light)/12-h 15C (dark), 40–70%/90–95% relative humidity (RH), and PFD of 80–130 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the sand level from fluorescent lamps during the photoperiod. Six irrigation regimes were applied: initial irrigation with water of x mmP ($x = 8$ or 16; x mmP denotes irrigation equivalent to x mm precipitation) and subsequent irrigation with water of 3 mmP at 1-d intervals ([8 + 1/1] and [16 + 1/1] treatments) or 2-d intervals ([8 + 1/2] and [16 + 1/2] treatments), and initial irrigation with water of x mmP ($x = 16$ or 24) and no subsequent irrigation ([16 + 0] and [24 + 0] treatments). The pots were examined daily, and the number of emerging (seedling height > 3 mm) and surviving seedlings was counted. Seeds that germinated but failed to emerge were retrieved in the [16 + 1/1] treatment by sieving the sand. At seed burial depths of 10 mm and 15 mm, where very low emergence was observed, viability of the ungerminated seeds was examined by spreading the sand of each pot in a flat plastic container (mean sand depth *c.* 2 mm), further irrigating for 16 d and monitoring germination under interrupted dark (PFD *c.* 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) daily for 2–5 min. For each irrigation regime, sand water content at different depth ranges (0–5 mm, 5–10 mm and 10–20 mm) in the pots was determined as described in Tobe *et al.* (2005a), 1, 2, 3, 4, 5 or 10 d after the irrigation was begun.

Statistical analysis

Student's *t*-test was used to test statistically the difference between two means. Analysis of variance (ANOVA), followed by Tukey's test, was used to compare more than three means. Percentage values were arcsine transformed prior to statistical analysis.

Results

Seed dormancy

In *A. ordosica*, at 0, 2.5 and 8.7 months after storage at 23 °C, G_F (20 °C, interrupted darkness) was 24 ± 5%, 37 ± 5% and 87 ± 4% (mean ± SE), respectively, while G_F of the seeds stored at *c.* 0 °C for 8.7 months remained low (19 ± 3%). In *A. arenaria*, 13 d after seed collection, G_F was 20 ± 2%, as compared to 87 ± 3% and 61 ± 3% for seeds stored for 6 months at 23 °C and 0 °C, respectively. Thus, in *A. ordosica* and *A. arenaria*, a large proportion of seeds possess primary dormancy, which is gradually relaxed during storage at 23 °C for several months. In contrast, it appeared that *A. sphaerocephala* either lacked or possessed a very short period of primary dormancy, since germination was high ($G_F = 92 \pm 3\%$) 2 months after seed collection.

Table 1. The effects of temperature and light on the final germination percentages (G_F), the number of days until germination was initially observed (T_{init}) and the number of days until $0.5 \times G_F\%$ ($T_{1/2}$), for seeds of three *Artemisia* species. D, interrupted dark; L, light/dark. Dashes denote that the values were not calculated because G_F of one or more replications was zero. Values are the means of four replications. For each parameter and species, the values having the same letters are not significantly different ($P < 0.05$; Tukey's test)

Temperature	G_F (%)		T_{init} (d)		$T_{1/2}$ (d)	
	D	L	D	L	D	L
<i>A. ordosica</i>						
10°C	43 c	8 d	6.7 c	11.2 d	7.7 e	12.2 f
15°C	94 ab	90 ab	3.7 ab	3.7 ab	3.8 abc	5.4 bcd
20°C	94 ab	90 ab	2.2 a	2.5 ab	2.8 a	3.7 abc
25°C	81 ab	77 b	2.0 a	2.0 a	2.8 a	3.4 ab
30°C	20 cd	26 cd	2.7 ab	3.5 ab	3.8 abc	6.7 de
10°C/20°C	88 ab	95 ab	3.5 ab	4.2 b	4.4 abc	5.7 cde
15°C/25°C	94 ab	97 a	2.5 ab	3.2 ab	2.7 a	4.1 abc
20°C/30°C	86 ab	84 ab	2.5 ab	3.0 ab	2.9 a	4.0 abc
<i>A. arenaria</i>						
10°C	63 bc	22 de	7.0 d	9.5 e	7.5 ef	10.6 g
15°C	90 a	84 ab	3.5 abc	4.5 bc	3.9 abc	5.9 bcdef
20°C	89 a	80 ab	2.5 ab	3.2 abc	2.6 a	4.3 abcd
25°C	47 c	43 cd	2.0 a	3.2 abc	2.8 a	6.7 cdef
30°C	1 f	8 ef	–	4.5 bc	–	5.7 bcdef
10°C/20°C	90 a	82 ab	4.0 abc	5.2 cd	4.1 abc	7.9 fg
15°C/25°C	85 a	88 a	3.0 abc	3.5 abc	2.9 a	5.0 abcde
20°C/30°C	81 ab	82 ab	3.0 abc	2.7 ab	3.3 ab	7.2 def
<i>A. sphaerocephala</i>						
10°C	10 def	0 f	8.0 c	–	8.2 cde	–
15°C	79 ab	40 cde	3.7 ab	7.0 bc	4.3 ab	9.0 de
20°C	96 a	85 ab	2.2 a	3.7 ab	3.2 a	5.6 abc
25°C	85 ab	45 cd	2.0 a	2.7 a	3.1 a	5.0 ab
30°C	6 ef	14 def	4.0 abc	–	3.7 a	–
10°C/20°C	55 bc	35 cde	4.2 abc	8.0 c	7.3 bcd	10.8 e
15°C/25°C	86 ab	86 ab	3.0 ab	4.0 abc	3.6 a	7.2 bcd
20°C/30°C	93 a	94 a	2.5 a	3.0 ab	3.1 a	4.9 ab

Effects of temperature on seed germination in interrupted dark and light/dark photoperiods

In both the interrupted dark and light/dark treatments, G_F in the three species was significantly lower at 10°C and 30°C than at the other temperature regimes (Table 1). At 10°C and a few other temperature regimes, G_F was lower and germination was retarded more in light/dark than in interrupted dark. As compared to *A. ordosica*, *A. arenaria* and *A. sphaerocephala* showed somewhat lower G_F at higher and lower temperatures, respectively. G_F was considerably higher at 10°C/20°C and 20°C/30°C than at 10°C and 30°C, indicating that the inhibitory effects of high/low temperature during the day/night on seed germination can be mitigated by lower/higher temperature during the night/day. On comparing the results with temperature data in the field, we found that seeds of these three species are germinable, at least in interrupted dark, at most temperatures recorded between mid-spring and mid-autumn in many desert regions in China (data not shown).

Effects of water potential on seed germination in interrupted dark and light/dark

A decrease in Ψ_W decreased G_F and retarded seed germination in both the interrupted dark and light/dark treatments in the three species (Table 2). G_F at -1.2 MPa was zero or almost zero, except in the interrupted dark treatment of *A. ordosica* (13%). As compared to interrupted dark, the light/dark treatment resulted in the reduction of G_F , particularly at lower Ψ_W ; and the inhibitory effect was stronger and over a wider Ψ_W range for *A. sphaerocephala*.

Seed germination in complete dark and effects of low-fluence light on seed germination under different temperature regimes

For some temperature regimes, G_F in complete darkness (Table 3) was considerably lower than that in interrupted dark (Table 1), indicating that the high G_F values in interrupted dark resulted from the

Table 2. The effects of water potential and light on final germination percentages (G_F), the number of days until seed germination was initially observed (T_{init}) and the number of days until $0.5 \times G_F\%$ ($T_{1/2}$) at 20°C for seeds of three *Artemisia* species. D, interrupted dark; L, light/dark. Dashes denote that the values were not calculated because G_F of one or more replications was zero. Values are the means of four replications. For each parameter and species, the values having the same letters are not significantly different ($P < 0.05$; Tukey's test)

Water potential (MPa)	G_F (%)		T_{init} (d)		$T_{1/2}$ (d)	
	D	L	D	L	D	L
<i>A. ordosica</i>						
0	92 a	90 ab	2.0 a	3.0 a	2.7 a	3.5 a
-0.3	91 ab	89 ab	2.5 a	3.0 a	2.9 a	4.8 ab
-0.6	78 b	81 bc	3.0 a	5.5 a	4.6 ab	9.7 c
-0.9	45 c	18 e	4.5 a	–	7.7 bc	–
-1.2	13 d	2 f	12.2 b	–	14.9 d	–
<i>A. arenaria</i>						
0	84 a	82 a	2.0 a	3.2 a	2.5 a	4.8 a
-0.3	83 a	74 a	3.0 a	3.5 a	3.0 a	5.5 ab
-0.6	70 a	60 a	4.0 ab	8.0 bc	5.6 ab	9.4 bc
-0.9	23 b	2 c	8.2 c	–	11.2 c	–
-1.2	0 c	0 c	–	–	–	–
<i>A. sphaerocephala</i>						
0	94 a	85 ab	2.7 a	4.2 ab	3.0 a	8.3 bc
-0.3	89 ab	52 cd	3.0 a	5.2 bc	4.1 a	9.3 c
-0.6	70 bc	14 ef	4.0 ab	–	5.7 ab	–
-0.9	32 de	2 f	6.7 c	–	9.0 bc	–
-1.2	3 f	0 f	–	–	–	–

stimulation of seed germination by the dim light used for daily observation. G_F in complete darkness differed among species and among temperature regimes. Generally, G_F in complete darkness was greater in the order of 20°C, 15°C/25°C, 10°C/20°C and 20°C/30°C treatments.

One-time illumination with low-fluence light resulted in a significant increase in G_F in 20°C and 15°C/25°C treatments in all the three species (Table 3), but was generally insufficient for increasing G_F to the corresponding values observed in the interrupted dark treatment (Table 1). In the

10°C/20°C and 20°C/30°C treatments, although mean G_F was increased by the low-fluence light in most cases, none of the differences were significant ($P > 0.05$). In the one-time illumination treatment, G_F was significantly higher only at 20°C and 15°C/25°C.

Seedling emergence from seeds sown at different depths in sand and irrigated under different regimes

In all the irrigation regimes, sand water content increased with increasing sand depth (data not shown). For all the depth ranges, both an increase in the amount of initial irrigation and an increase in the frequency of irrigation resulted in persistence of water in sand for longer periods.

When seeds in pots were irrigated under the [16 + 1/1] regime (0–16 d in Fig. 1), final percentage seedling emergence (E_F) in the three species was the highest for seeds buried at 2.5 mm, followed by seeds sown on the sand surface. E_F from 10 mm and 15 mm was almost zero for *A. arenaria* and *A. sphaerocephala*, as compared to 31% and 16% for *A. ordosica*. When the seeds were dug out from the sand on day 16, the percentages of seeds that germinated, but did not emerge, were very small (0–4%), except for *A. ordosica* seeds that were buried at 15 mm (18%). The percentage germination of

Table 3. Final percent germination (G_F) of *Artemisia* seeds that were maintained under different temperature regimes, illuminated with dim light (+) (PFD $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 5 min 5 d after the onset of imbibition, or in complete darkness (–). Values are the means of four replications. In each column, the values having the same letters are not significantly different ($P < 0.05$; Tukey's test)

Temperature	Light	<i>A. ordosica</i>	<i>A. arenaria</i>	<i>A. sphaerocephala</i>
10°C/20°C	–	19 bcd	7 cd	1 b
	+	38 b	19 bc	8 b
15°C/25°C	–	27 bc	4 d	1 b
	+	73 a	31 b	35 a
20°C/30°C	–	5 d	1 d	1 b
	+	11 cd	0 d	8 b
20°C	–	30 bc	21 bc	4 b
	+	84 a	68 a	39 a

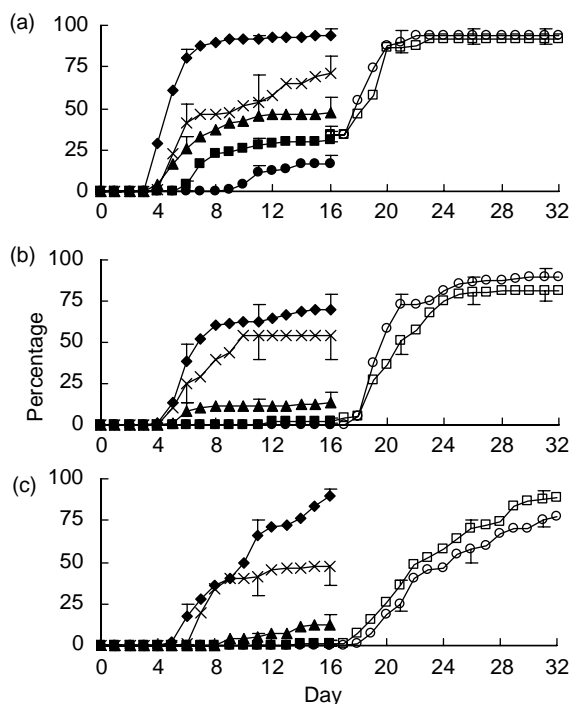


Figure 1. Time course of percent seedling emergence or percentage germination of *A. ordosica* (a), *A. arenaria* (b) and *A. sphaerocephala* (c). Seeds were sown on sand surface (\times) or buried at 2.5 mm (\blacklozenge), 5.0 mm (\blacktriangle), 10 mm (\blacksquare) or 15 mm (\circ) in sand in pots (height 50 mm) placed at 25°C (12 h, light)/15°C (12 h, dark). The pots were irrigated initially with 16 mmP and subsequently with 3 mmP at 1-d intervals ([16 + 1/1] treatment; 0–16 d). Seeds buried at 10 mm or 15 mm were moved to shallow depths in containers on day 16, and percentage germination was examined for another 16 d (16–32 d) (initial seed burial depth: \square , 10 mm; \circ , 15 mm). Each value represents the mean of five replications; error bars indicating SE are shown only for some representative values.

A. ordosica seeds buried at 10 or 15 mm was similar to G_F of the seeds maintained in complete darkness at the same temperature regime (15°C/25°C in Table 3). When the sand with non-germinated seeds buried at 10 mm or 15 mm in the [16 + 1/1] treatment was spread into a thin layer and illuminated with low-fluence light, most of the seeds germinated within 16 d (16–32 d in Fig. 1).

In the [8 + 1/2] treatment, seedling emergence was almost completely suppressed for the three species at all seed burial depths, except *A. ordosica* seeds at 5.0 mm (0–14 d in Fig. 2; $E_F = 24\%$). When these pots were subsequently treated with [8 + 1/1] (14–24 d in Fig. 2), higher percentages of seedlings emerged at all seed burial depths in the three species. E_F was significantly lower ($P < 0.001$) in the [8 + 1/1] than in the [16 + 1/1] treatment (Fig. 1) at burial depths of 0 and 2.5 mm, whereas at a seed burial depth of 5.0 mm,

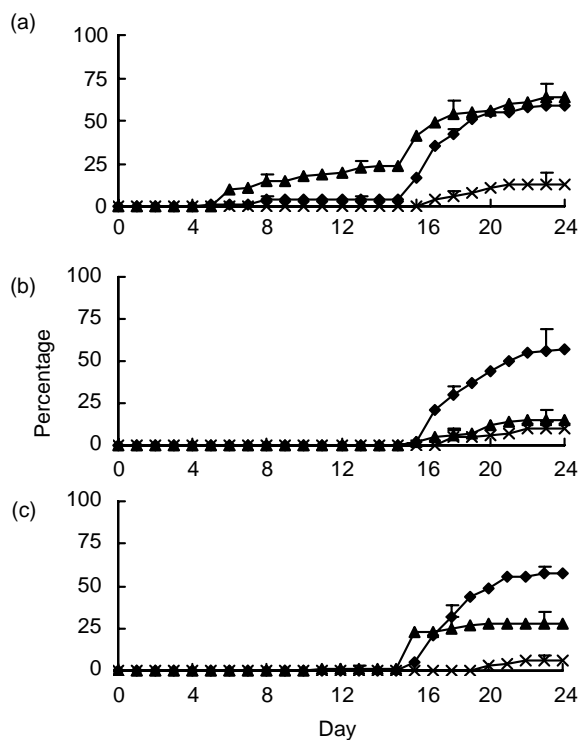


Figure 2. Time course of percent seedling emergence of *A. ordosica* (a), *A. arenaria* (b) and *A. sphaerocephala* (c) when seeds were sown on sand surface (\times) or at a depth of 2.5 mm (\blacklozenge) or 5.0 mm (\blacktriangle) in sand in pots (height 50 mm) placed at 25°C (12 h, light)/15°C (12 h, dark). The pots were irrigated initially with 8 mmP and subsequently with 3 mmP at 2-d intervals ([8 + 1/2] treatment; 0–13 d), and thereafter, the pots were irrigated initially with 8 mmP and subsequently with 3 mmP at 1-d intervals ([8 + 1/1] treatment; 14–24 d). Each value represents the mean of five replications; error bars indicating SE are shown only for some representative values.

E_F was significantly higher ($P < 0.05$) in the [8 + 1/1], followed by [8 + 1/2], than in the [16 + 1/1] treatment, which is presumably because the moist seeds received light for a longer period (24 d rather than 16 d). After the [8 + 1/1] treatment, when the ungerminated seeds sown on the sand surface were irrigated in Petri dishes, they germinated rapidly and G_F was 83% (*A. ordosica*), 81% (*A. arenaria*) and 99% (*A. sphaerocephala*).

The effects of different irrigation regimes on seedling emergence were examined with *A. ordosica* seeds collected in 2000 (Table 4). G_F at 15°C/25°C in complete dark was lower in these seeds ($9 \pm 4\%$; mean \pm SE, $n = 4$) than in *A. ordosica* seeds collected in 1997 and used in the previous experiments ($27 \pm 1\%$; Table 3). In all the irrigation regimes, mean E_F was the highest at 2.5 mm depth (Table 4). While there were not significant differences in E_F between the [16 + 1/1] and the [8 + 1/1] treatments at

Table 4. The effects of irrigation regime on the final percent seedling emergence (E_F ; values 20 d after the irrigation was begun) for *A. ordosica* seeds (collected in 2000) buried in sand in pots (height 100 mm) placed at 25°C (12 h, light)/15°C (12 h, dark). Dashes denote not examined. Values are the means of five replications. The values having the same letters are not significantly different ($P < 0.05$; Tukey's test)

Seed depth (mm)	E_F (%)				
	[16 + 1/1] ^v	[8 + 1/1] ^w	[16 + 1/2] ^x	[24 + 0] ^y	[16 + 0] ^z
0	91 a	75 ab	21 cde	15 def	0 f
2.5	96 a	94 a	54 bc	44 bcd	24 cde
5.0	55 bc	72 ab	39 bcde	29 cde	0 f
10.0	7 ef	–	–	0 f	–

^v Initial irrigation with water equivalent to 16 mm precipitation, with subsequent irrigation equivalent to 3 mm precipitation at 1 d intervals.

^w Initial irrigation with water equivalent to 8 mm precipitation, with subsequent irrigation equivalent to 3 mm precipitation at 1 d intervals.

^x Initial irrigation with water equivalent to 16 mm precipitation, with subsequent irrigation equivalent to 3 mm precipitation at 2 d intervals.

^y Initial irrigation with water equivalent to 24 mm precipitation, with no subsequent irrigation.

^z Initial irrigation with water equivalent to 16 mm precipitation, with no subsequent irrigation.

either 0 or 2.5 mm seed depths, E_F was significantly lower in the [16 + 1/2] than in the [16 + 1/1] treatment at the depths of 0 and 2.5 mm. E_F values in one-time irrigation treatments ([16 + 0] and [24 + 0]) were generally lower than those in the other three treatments. In the [16 + 0] treatment, seedlings began to die 16 d after irrigation and only 39% of seedlings survived 20 d after irrigation; in the other four irrigation regimes, all the seedlings survived during the 20 d of the experimental period.

Discussion

Seed burial depth and the frequency/amount of irrigation were important determinants of seed germination in the three *Artemisia* species. The light requirement for seed germination in these species limited their germination to shallow sand layers, where water is rapidly lost due to evaporation. Nevertheless, water potentials preventing seed germination in these species ($c. -1.2$ MPa) were similar to, or rather higher than, the water potentials that prevent germination of non-photoblastic seeds of other species inhabiting deserts (Tobe *et al.*, 2005a, b) or more mesic habitats (Evans and Etherington, 1990).

When the seeds were irrigated in/on sand, [8 + 1/2] irrigation was insufficient for appreciable seedling emergence in the three *Artemisia* species (Fig. 2), while for several other species inhabiting desert dunes in China and producing non-photoblastic seeds, [8 + 1/2] irrigation was sufficient to yield high percentages of seedling emergence from seeds buried at 10 mm depth (Tobe *et al.*, 2005a, b;

unpublished results). Additionally, while one-time irrigation of 8–16 mmP was sufficient for yielding high percentages of seedling emergence in those species with non-photoblastic seeds (Tobe *et al.*, 2005a, b; unpublished results), 24 mmP irrigation did not yield a high percentage of seedling emergence in *A. ordosica* (Table 4). These results suggest that the opportunity for seed germination of the three *Artemisia* species in the field is more limited, compared to other species growing in the same habitats and producing non-photoblastic seeds.

Precipitation in the field (see Materials and methods) is considerably less frequent than the irrigation in our experiments. It is expected that, in Shapotou and many other desert regions in China, seed germination of the three *Artemisia* species is almost completely suppressed from mid-autumn to mid-spring, when rainfall events are usually less than 8 mm and occur at longer intervals. Seed germination of these species is expected only in the summer rainy season, when rainfall events often exceed 24 mm and occur at a higher frequency. Some proportion of seeds buried at relatively shallow depths will germinate in this season, but seeds buried deeply or located near the surface will remain ungerminated, but viable, and will act as a seed reservoir for the future years. However, the longevity of *A. ordosica* seeds was rather short (<6.7 years) when they were stored dry at $c. 23^\circ\text{C}$ (unpublished data). Although seed longevity in laboratory conditions cannot be extended to field conditions, *Artemisia* seeds may not persist for very long in seed banks in the field. A substantial proportion of seeds may die before they have an opportunity to germinate.

It should be stressed that the light intensity on sand in our experiments was 10–20 times lower than in sunny field conditions. In sunny places where light penetrates deeper into the sand (Huang and Gutterman, 1998), seed germination will be enabled in deeper sand layers where the water content is higher. On the other hand, direct sunlight will cause a temperature rise near the sand surface, and evaporation will be enhanced to a greater extent, compared to our experimental conditions. Thus, in sunny places in the field, seedling emergence may be disfavoured more at shallower depths and favoured more in deeper sand than expected from our study. In addition, as was suggested by higher seedling emergence percentages at 5 mm depth in the [8 + 1/1], followed by [8 + 1/2], than in the [16 + 1/1] treatment (Figs 1, 2), if imbibed seeds buried at relatively deep sand levels receive very weak light for an extended period, they may be enabled to germinate at deeper sand levels than observed in our experiments.

In the Negev Desert, Huang and Gutterman (1998) found that the highest percentage of *A. monosperma* seedlings emerged from seeds buried at 8–10 mm, and that emergence from depths shallower than 4 mm and deeper than 12 mm was very limited. Since light sensitivity of the seeds of the three *Artemisia* species examined in this study (Table 3) was considerably lower than that of *A. monosperma* seeds [for which irradiation with 0.1 lux ($\approx 0.002 \mu\text{mol m}^{-2} \text{s}^{-1}$) of light for 4 min was sufficient to saturate germination (Koller *et al.*, 1964b)], seed germination of these three *Artemisia* species would be limited to a shallower sand depth range where water content is lower. Hence, the opportunity for seedling establishment would be more limited for the three *Artemisia* species studied here, compared to *A. monosperma*. For seeds of *A. sphaerocephala*, higher photoinhibition and lower light sensitivity than the other two species may limit its germination to a narrower depth range in sand. On the other hand, for *A. ordosica* and *A. arenaria*, primary seed dormancy would prevent seed germination in some proportion of the seeds in the initial year after maturation.

At optimal light conditions for germination (interrupted dark), seeds of the three *Artemisia* species germinated at a wide range of temperature conditions, overlapping those occurring in the field between mid-spring and mid-autumn, during which a high proportion of annual precipitation occurs. However, under non-optimal light conditions (complete dark and one-time illumination with low-fluence light; Table 3), seed germination in these three species showed stronger dependence on temperature. They required lower-fluence light for germination, and a higher proportion of seeds germinated without light in 15 °C/25 °C than in 10 °C/20 °C and in 20 °C/30 °C

(Table 3). The light sensitivity of seeds would have a crucial effect on seedling emergence because higher sensitivity to light would enable seed germination at sand depths where the water content is higher. The temperature conditions in the summer rainy season will correspond to the 15 °C/25 °C and 20 °C/30 °C regimes in our experiments. Since seed germination in limiting light conditions considerably differed between these two temperature regimes (Table 3), the temperature after precipitation can affect seedling emergence of these species. In the field, sand temperatures near the surface in the daytime can be considerably higher than air temperatures (Mott, 1972; Zhang and Maun, 1990); however, when the sand near the surface is moist, the only condition in which seeds germinate, evaporative cooling will prevent an extreme rise in sand temperature.

Our study suggested that the opportunity for seed germination of the three *Artemisia* species in the field is limited because the light requirement for germination restricts their germination to dry, shallow sand depths. Nevertheless, our experimental study under laboratory conditions did not always simulate the environmental conditions of the species' habitats. In particular, light intensity, which depends on locations (sun or shade) and weather (sunny or cloudy), will influence evaporation rate from sand and sand temperature, and thus will affect the features of germination in these species. Field studies would be needed for further understanding of the germination behaviour of these species on dunes.

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