Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species★

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A greenhouse study was conducted to determine the effects of sand burial on seed germination and seedling emergence of ten *Calligonum* L. species. The results suggested that the deeper the seeds in sand, the lower and slower their germination and seedling emergence. Seedling emergence occurred from a maximum depth of 12 cm with most seedlings emerging from 2 and 4 cm depths. The percent seedling emergence, number of days of first emergence, percent seed germination and percent of dormant seeds were significantly affected by different species and burial depth. There was a significant positive correlation between the number of days to first emergence and burial depth. Both mean percent germination and percent seedling emergence were negatively correlated with depth significantly for each species.

Introduction

Burial in sand is an important factor controlling the distribution and composition of vegetation in desert ecosystem (Freas & Kemp, 1983; Gutterman, 1993; Baskin & Baskin, 1993, 1998; Maun, 1996; Wang *et al.*, 1997, 1998; Chen & Kuo, 1999). Seeds, seedlings and adult plants are regularly buried to varying depths depending on the habitat of their occurrence, the prevailing wind velocities and the characteristics of sand deposited. In fact, sand deposition has been recognized as a major selective force in the evolution of seed size, seed germination, seedling emergence, and survivorship of seedling and adult plants (Freas & Kemp, 1983; Maun & Lapierre, 1986; Meyer & Monsen, 1991; Zhang, 1992; Gutterman, 1993; Maun, 1994; Huang & Gutterman, 2000).

The germination of seeds was directly related to the depth at which seeds were buried (Harper & Obeid, 1967; Maun & Riach, 1981; Freas & Kemp, 1983; Maun &

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Lapierre, 1986; Zhang & Maun, 1990; Gutterman, 1993; Baskin & Baskin, 1993, 1998; Maun, 1996; Yangful & Maun, 1996; Wang *et al.*, 1997, 1998; Huang & Gutterman, 1998; Chen & Kuo, 1999; Chen & Maun, 1999; Benvenuti *et al.*, 2001; Zhang, 2001). Burial at shallow depths stimulated more germination than surface lying seeds because it maintains a moist environment around seeds and prevented seeds and seedlings from drying out (Meidan, 1990; Maun, 1996; Zaady *et al.*, 1997; Huang & Gutterman, 1998; Morgan, 1998; Wang *et al.*, 1998; Benvenuti *et al.*, 2001). However, excessive burial may affect seed germination and prevent the seedling emergence above the sand. Generally, as the depth of burial increased there was a significant decrease in both seed germination and seedling emergence.

Calligonum L. (Polygonaceae) species are dominant perennial shrub in active sand dunes and stabilized sand field in the northern extreme drought desert of China (Mao & Pan, 1986; Tao, 2000). They can exist in mobile sand dunes in conditions of intensive and quick cover over or exposure (Liu, 1985–1990; Mao & Pan, 1986). They appear to be suitable for revegetating of desert. Although little is known about their uses, they have great potential in providing different products and services such as forage, traditional medicine, halting desert encroachment and stabilizing sand dune (Liu, 1985–1990; Tao, 2000). They have attracted some attention in terms of their great importance in providing many uses and services. The available information about these species is their botany, cultivated method, taxonomy, genetic diversity, brief descriptions of their habitat condition and the range of their geographical distribution (Mao *et al.*, 1983; Mao, 1984; Liu, 1985–1990; Mao & Pan, 1986; Zhang, 1992; Yu & Wang, 1998; Tao, 2000; Tao *et al.*, 2000). However, there has been little experimental research dealing with their seed germination and seedling emergence reposes in relation to the sand burial.

The impacts of sand burial depth on seed germination and seedling emergence of ten *Calligonum* species were investigated experimentally; the different responses of different sand burial treatments were examined among *Calligonum* species, as an understanding of these factors is crucial for the successful management of these long-lived desert plant species.

Materials and methods

The species and source of seeds

Seeds of ten *Calligonum* species (*C. junceum* (Fisch. et Mey.) Litv., *C. leucocladum* (Schrenk) Bge., *C. rubicundum* Bge., *C. densum* A. Los., *C. mongolicum* Turcz., *C. chinese* A. Los., *C. caput-medusae* Schrenk, *C. arborescens* Litv., *C. alaschanicum* A. Los. and *C. potaninii* A. Los.) were collected from at least ten plants per species in August, 1998 at Shapotou Desert Research and Experimental Station of the Chinese Academy of Sciences (37°32′N, 105°02′E, 1339 m a.s.l.), Ningxia province, China. The aborted and predated seeds were discarded. Intact plump seeds were surface sterilized with Na-hypochlorite prior to experimental usage. Seed viability is variable, but is generally between 30% and 50% (Yu & Wang 1998; Tao 2000).

Experimental procedure

All sand burial treatments consisted of six replicates of 40 seeds of each species. For each replicate, counted seeds (40 each) were planted at 2, 4, 6, 8, 10, 12, and 14 cm depths in plastic pots (17·5 cm in diameter) filled with unsterilized sifted sand. The drainage outlet at the bottom of pots was covered with strips of nylon mesh to prevent the loss of sand while allowing drainage of excess water. Sand was poured into each

pot up to the lower mark and moistened. Seeds ($n = 40$) were then placed on the sand surface and the pots were filled up to the upper mark with additional sand. There were six pots per treatment for each species.

The pots were placed outside in the garden at the Gansu Agricultural University for stratification of seeds from 15 October, 1998 to 29 March, 1999, after which they were allowed to germinate in the greenhouse. Pots were watered daily with tap water. Temperature in the greenhouse was maintained at 24°C during the day (13–15 h photoperiod) and 14°C at night. Emerged seedlings (cotyledons visible at the sand surface) were counted daily and removed.

After 4 weeks of germination (radicle emerged) and seedling emergence (cotyledons visible at the sand surface), the experiment was terminated. The contents of pots were removed and washed over a sieve with 3 mm openings through which the sand escaped but the ungerminated seeds and unemerged seedlings (seeds germinated but the etiolated seedling below sand surface) were retained. Ungerminated seeds were soaked in water at 30°C for 24 h. Seed coats were cut and the embryo was soaked in 1% tetrazolium chloride for 24 h at 30°C. Pink embryos were scored as alive. Seeds which tested as viable were considered to be dormant. Germination and seedling were expressed as the percentage of viable seeds.

The experiments were conducted during March–April 1999. All percentage data on seedling emergence, seed germination and dormancy were arcsin square root transformed and the numbers of days to the first emergence were log transformed before analysis using one- and two-way ANOVA at 95%. According to our observation, no seedling emerged after 28 days, so the number of days to the first emergence was considered as 28 days if the seedlings did not emerge but the seeds were viable or germinated in one pot. If ANOVA showed significant differences, Duncan's multiple comparison test was used to determine differences between treatments. All data transform and analyses were carried out using STATISTICA software (Statsoft, 1993).

Results

Seedling emergence

The two-way ANOVA showed that seedling emergence was significantly affected by different species ($F_9 = 32 \cdot 4163$, $p < 0 \cdot 0001$), burial depth ($F_5 = 320 \cdot 4671$, $p < 0 \cdot 0001$) and the interaction between depth and species ($F_{54} = 2 \cdot 6332$, $p < 0 \cdot 0001$; Table 1). For all ten species, even though seed germination occurred at all burial depths, a large percentage of the seedlings germinated only from 2, 4, 6 and 8 cm burial depths (Fig. 1). Very few seedlings of only six species (*C. junceum*, *C. rubicundum*, *C. densum*, *C. chinese*, *C. alaschanicum* and *C. potaninii*) emerged in the 10 cm burial treatment. Only 1–2 seedlings emerged from two pots of *C. junceum* and *C. alaschanicum*, from three pots of *C. densum* in the 12 cm burial treatment. No seedlings emerged from 14 cm depth. For all species, the highest seedling emergence occurred from 2 cm burial depth. There was a significant decline in emergence at 4, 6 and 8 cm burial depths for *C. rubicundum*, *C. mongolicum*, *C. chinese*, *C. caput-medusae*, *C. arborescens* and *C. potaninii* (Fig. 1). There was a significant negative correlation between the mean percent seedling emergence and burial depth for each species (*C. junceum*: $y = 70 \cdot 95 - 5 \cdot 33x$, $r^2 = 0 \cdot 96$, $F_{1,5} = 116 \cdot 05$, $p = 0 \cdot 0001$; *C. leucocladum*: $y = 20 \cdot 95 - 1 \cdot 75x$, $r^2 = 0 \cdot 86$, $F_{1,5} = 31 \cdot 70$, $p = 0 \cdot 0025$; *C. rubicundum*: $y = 47 \cdot 86 - 3 \cdot 94x$, $r^2 = 0 \cdot 90$, $F_{1,5} = 45 \cdot 38$, $p = 0 \cdot 0011$; *C. densum*: $y = 64 \cdot 82 - 5 \cdot 01x$, $r^2 = 0 \cdot 91$, $F_{1,5} = 53 \cdot 01$, $p = 0 \cdot 0008$; *C. mongolicum*: $y = 18 \cdot 39 - 1 \cdot 61x$, $r^2 = 0 \cdot 75$, $F_{1,5} = 15 \cdot 15$, $p = 0 \cdot 0115$; *C. chinese*: $y = 47 \cdot 80 - 3 \cdot 77x$, $r^2 = 0 \cdot 93$, $F_{1,5} = 64 \cdot 55$,
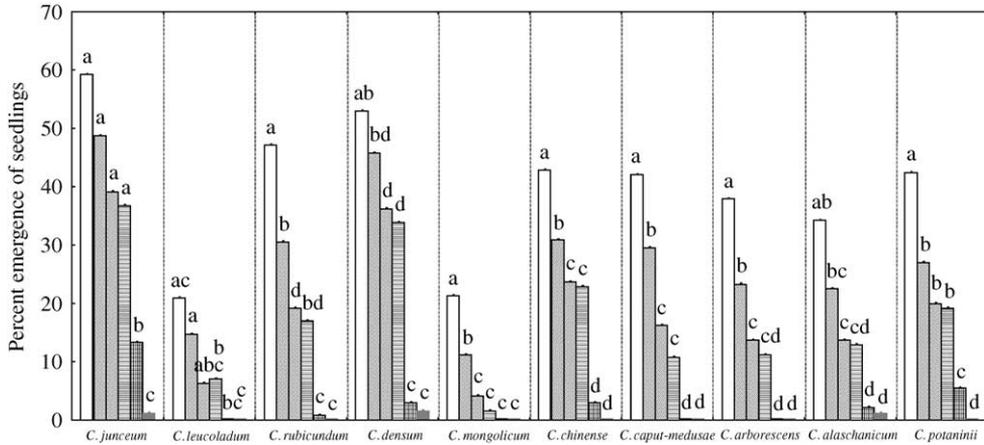
Figure 1. Percent emergence of seedlings of ten *Calligonum* species from different burial depths in sand. Values with the same superscript letters are not significantly different at $p < 0.05$ according to Duncan's multiple comparison test. Burial depth; (☐) 2 cm; (▨) 1 cm; (▨) 6 cm; (▭) 8 cm; (▥) 10 cm; (▬) 12 cm.

$p = 0.0005$; *C. caput-medusae*: $y = 42.92 - 3.60x$, $r^2 = 0.89$ $F_{1,5} = 39.89$, $p = 0.0015$; *C. arborescens*: $y = 37.20 - 3.11x$, $r^2 = 0.88$, $F_{1,5} = 35.72$, $p = 0.0019$; *C. alaschanicum*: $y = 34.76 - 2.80x$, $r^2 = 0.91$, $F_{1,5} = 51.08$, $p = 0.00083$ *C. potaninii*: $y = 44.35 - 3.50x$, $r^2 = 0.93$, $F_{1,5} = 69.50$, $p = 0.0004$). At all burial depths, seedling emergence of *C. junceum* and *C. densum* performed higher than other species, *C. leucocladum* and *C. mongolium* presented lower percent emergence (Fig. 1).

Seed germination

The germination of seeds occurred at all burial depths from 2 to 14 cm for each species (Fig. 2). A two-way ANOVA showed that seed germination was significantly affected by different species ($F_9 = 30.1610$, $p < 0.0001$) and burial depth ($F_6 = 45.6794$, $p < 0.0001$) but not by the interaction between species and burial depth (Table 1). For all ten species, a large percentage of seeds germinated from 2 and 4 cm burial depths (Fig. 2). For all species, the highest seed germination occurred from 2 cm burial depth. There was a significant decline in emergence at 6, 8, 10, 12 and 14 cm burial depths for *C. rubicundum*, *C. densum*, *C. mongolicum*, *C. caput-medusae*, *C. arborescens* and *C. potaninii*. There was a significant decline in germination at 14 cm burial depth (Fig. 2). At all burial depths, *C. leucocladum* and *C. mongolicum* both performed lower percent germination and *C. juceum* and *C. densum* both performed higher percent germination. There were no significant differences in seed germination between all burial depths for *C. leucocladum* (Fig. 2). There was a significant negative correlation between the mean percent germination and burial depth for each species (*C. junceum*: $y = 68.87 - 3.18x$, $r^2 = 0.85$, $F_{1,6} = 29.45$, $p = 0.0029$; *C. leucocladum*: $y = 23.81 - 1.14x$, $r^2 = 0.80$, $F_{1,6} = 19.96$, $p = 0.0066$; *C. rubicundum*: $y = 48.51 - 2.78x$, $r^2 = 0.92$, $F_{1,6} = 59.16$, $p = 0.0006$; *C. densum*: $y = 64.29 - 3.21x$, $r^2 = 0.92$, $F_{1,6} = 58.08$, $p = 0.0006$; *C. mongolicum*: $y = 23.57 - 1.82x$, $r^2 = 0.88$, $F_{1,6} = 36.46$, $p = 0.0018$; *C. chinese*: $y = 44.17 - 1.53x$, $r^2 = 0.89$, $F_{1,6} = 41.60$, $p = 0.0013$; *C. caput-medusae*: $y = 45.65 - 3.07x$, $r^2 = 0.92$ $F_{1,6} = 58.61$, $p = 0.0006$; *C. arborescens*: $y = 41.07 - 2.57x$, $r^2 = 0.93$, $F_{1,6} = 69.34$,
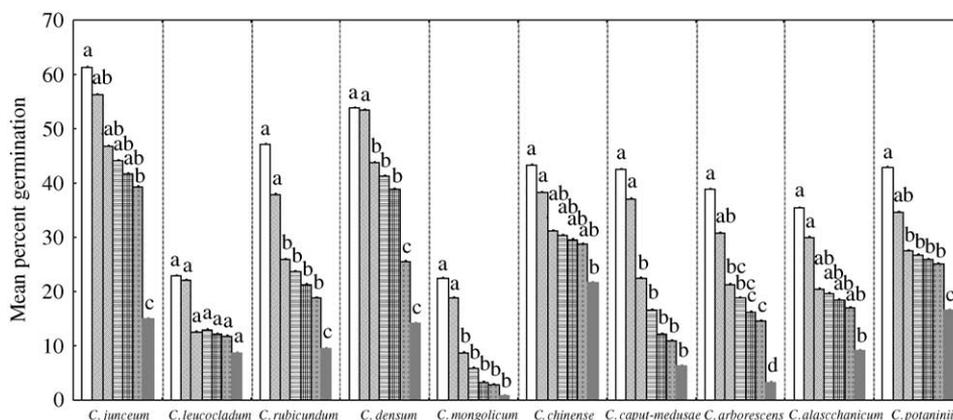
Figure 2. The percent of seed germination of ten *Calligonum* species from different burial depths in sand. Values with the same superscript letters are not significantly different at $p < 0.05$ according to Duncan's multiple comparison test. Burial depth: (☐) 2 cm; (▨) 4 cm; (▨) 6 cm; (▤) 8 cm; (▦) 10 cm; (■) 12 cm; (▨) 14 cm.

$p = 0.0004$; *C. alaschanicum*: $y = 36.67 - 1.90x$, $r^2 = 0.90$, $F_{1,6} = 44.14$, $p = 0.0012$; *C. potaninii*: $y = 42.68 - 1.78x$, $r^2 = 0.87$, $F_{1,6} = 32.55$, $p = 0.0023$).

Days of first emergence

A two-way ANOVA showed a significant effect of burial depth ($F_5 = 511.7878$, $p < 0.0001$), different species ($F_9 = 14.1113$, $p < 0.0001$) and the interaction between depth and species ($F_{54} = 2.8237$, $p < 0.0001$) on the number of days to first emergence of seedling which reflects the rate of emergence (Table 1). For all species, the fastest seedling emergence occurred from 2 cm burial depth, there was a significant increase in days of first emergence at 4, 6, 8, 10, 12 and 14 cm burial depths (Fig. 3). There was a significant positive correlation between the number of days to first emergence and burial depth for each species (*C. junceum*: $y = -1.88 + 2.08x$, $r^2 = 0.95$, $F_{1,5} = 71.32$, $p = 0.0011$; *C. leucocladum*: $y = 3.72 + 2.10x$, $r^2 = 0.88$, $F_{1,5} = 28.98$, $p = 0.0058$; *C. rubicundum*: $y = -0.60 + 2.38x$, $r^2 = 0.95$, $F_{1,5} = 71.86$, $p = 0.0011$; *C. densum*: $y = -0.82 + 2.01x$, $r^2 = 0.94$, $F_{1,5} = 63.49$, $p = 0.0013$; *C. mongolicum*: $y = 2.08 + 2.33x$, $r^2 = 0.96$, $F_{1,5} = 93.10$, $p = 0.0006$; *C. chinese*: $y = 0.57 + 2.08x$, $r^2 = 0.95$, $F_{1,5} = 78.27$, $p = 0.0009$; *C. caput-medusae*: $y = -1.51 + 2.55x$, $r^2 = 0.97$, $F_{1,5} = 128.51$, $p = 0.0003$; *C. arborescens*: $y = -0.99 + 2.42x$, $r^2 = 0.92$, $F_{1,5} = 45.84$, $p = 0.0025$; *C. alaschanicum*: $y = 1.15 + 2.01x$, $r^2 = 0.94$, $F_{1,5} = 65.91$, $p = 0.0013$, *C. potaninii*: $y = 1.37 + 2.00x$, $r^2 = 0.89$, $F_{1,5} = 33.30$, $p = 0.0045$).

Seed dormancy

A two-way ANOVA showed that the percent of dormant seeds was significantly affected by species ($F_9 = 5.6029$, $p < 0.0001$) as well as burial depth ($F_6 = 13.5256$, $p < 0.0001$) but not by the interaction between species and burial depth (Table 1). For *C. rubicundum*, *C. leucocladum*, *C. caput-medusae* and *C. arborescens*, the highest number of dormant seeds presented from 14 cm burial depth; there was a significant decline in the number of dormant seeds at 2 and 4 cm burial depths (Fig. 2). There were no significant differences in the percent of dormant seeds between all burial
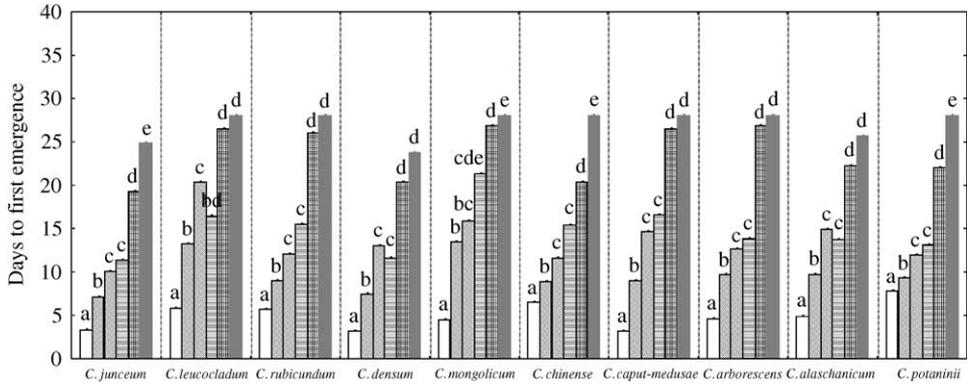
Figure 3. The number of days to first emergence of seedlings of ten *Calligonum* species from different burial depths in sand. Values with the same superscript letters are not significantly different at $p < 0.05$ according to Duncan's multiple comparison test. Burial depth: (☐) 2 cm; (▨) 4 cm; (▨) 6 cm; (▨) 8 cm; (▨) 10 cm; (▨) 12 cm.

Table 1. *The analysis of variance for the effects of different species, depths of burial, and their interaction on seedling emergence (%), seed germination (%), seed dormancy (%) and the number of days to the first emergence of seedling for Calligonum species in a greenhouse. Since no seedling emerged from sand burial treatment of 14 cm depth, this treatment was not included in data analysis of seedling emergence*

Source of variation	SS	df.	MS	F	p
Seedling emergence					
Depth of burial	26·0641	5	4·3440	320·4671	<0·0001
Species	3·9547	9	0·4394	32·4163	<0·0001
Species × depth	1·9275	54	0·0357	2·6332	<0·0001
Seed germination					
Depth of burial	7·5007	6	1·2501	45·6794	<0·0001
Species	7·4288	9	0·8254	30·1610	<0·0001
Species × depth	1·0117	54	0·0187	0·6846	0·9550
Seed dormancy					
Depth of burial	1·6754	6	0·2792	13·5256	<0·0001
Species	1·0411	9	0·1157	5·6029	<0·0001
Species × depth	0·4102	54	0·0076	0·3680	0·99998
Days to the first emergence					
Depth of burial	23·3465	5	4·6693	511·7878	<0·0001
Species	1·1587	9	0·1287	14·1113	<0·0001
Species × depth	1·1593	45	0·0258	2·8237	<0·0001

depths for *C. junceum*, *C. densum*, *C. mongolicum*, *C. chinese*, *C. alaschanicum*, *C. potaninii*; Fig. 4).

Discussion

During a plant life cycle, the seed has the highest resistance to extreme environmental stresses, whereas the seedlings of many plant species are most susceptible (Freas &
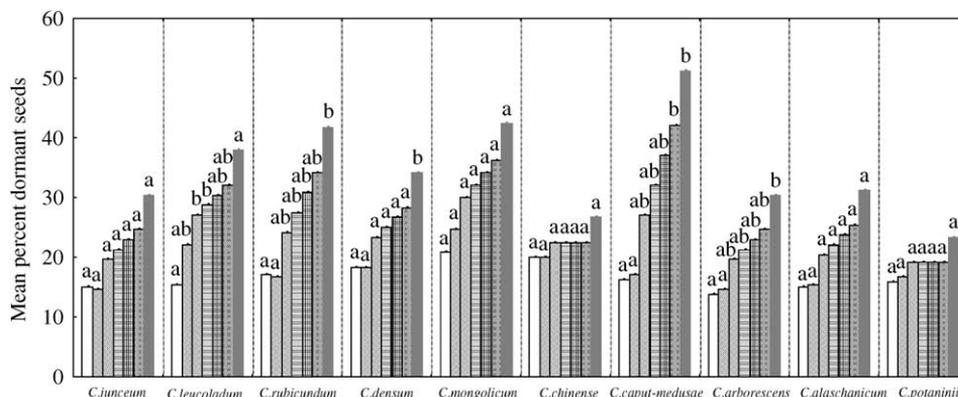
Figure 4. The percent of dormant seeds of ten *Calligonum* species from different burial depths in sand. Values with the same superscript letters are not significantly different at $p < 0.05$ according to Duncan's multiple comparison test. Burial depth: (☐) 2 cm; (▨) 4 cm; (▨) 6 cm; (▤) 8 cm; (▦) 10 cm; (▨) 12 cm; (■) 14 cm.

Kemp, 1983; Baskin & Baskin, 1993, 1998; Gutterman, 1993). Of the total number of seeds produced by any plant, only a small fraction becomes seedlings. Large losses occur between seed dispersal and seedling emergence because of various reasons. Most plant germination studies have been concerned with the effects of specific environment factors on seed germination and seedling establishment (Meyer & Monsen, 1991; Maun, 1994; Wang *et al.*, 1997; Silvertown, 1999; Benvenuti *et al.*, 2001; Zhang, 2001). Burial depth is an important factor for the behaviors of seed germination and seedling emergence (Harper & Obeid, 1967; Maun & Riach, 1981; Freas & Kemp, 1983; Maun & Lapierre, 1986; Zhang & Maun, 1990; Maun, 1996; Yangful & Maun, 1996; 1998; Huang & Gutterman, 1998; Chen & Kuo, 1999; Chen & Maun, 1999). In this study, two questions were posed: (1) what is the ultimate fate of ten *Calligonum* seeds planted at different depths in sand and (2) is there a difference in the performance of seed germination and seedling emergence among *Calligonum* species. In answer to these questions, all buried seeds may show one of four responses (Maun, 1994): (1) they may germinate and emerge as seedlings, (2) they may germinate but the seedlings are unable to emerge above the sand surface, (3) the seeds may acquire dormancy and become part of the seed bank, or (4) the seeds nay succumb to various mortality factors. In this study, we found evidence for all four possibilities.

For *Calligonum* species in this study, seed germination occurred at all burial depths (2, 4, 6, 8, 10, 12 and 14 cm). But as burial depth increased, both seed germination and seedling emergence decreased, and they both were negatively correlated with burial depth significantly, at the same time their performance was different among species. The experimental data also showed that a proportion of seeds had germinated but the seedling failed to emerge above the surface.

In this experiment, the number of dormant seeds of *Calligonum* species increased with addition of burial depth. A small proportion of seeds acquired dormancy especially at deeper depths in the soil. The suggested causes of dormancy acquisition by seeds are higher soil moisture, lower temperatures, poor gas exchange and higher CO_2 levels (Harper & Benton, 1966; Woolley & Stoller, 1978; Meidan, 1990; Gutterman, 1993; Keeley & Fotheringham, 1997; Baskin & Baskin, 1998; Huang & Gutterman, 1998; Yu & Wang, 1998). The limiting light intensity can affect seed dormancy, but under 2 cm sand burial depth, the light intensity can almost be detected (Wooley & Stoller, 1978; Huang *et al.*, 2001). The acquisition of seed

dormancy at greater burial depths would be ecologically advantageous because seeds would survive in the dormant state in the seed bank (Fenner, 1985; Gutterman, 1993; Baskin & Baskin, 1993, 1998; Wang *et al.*, 1998; Huang & Gutterman, 2000), they are 'ready' to germination as soon as the upper sand layer is removed by wind or runoff, thus exposing them to germinate at the proper sand depth (Maun, 1996; Huang & Gutterman, 1998). However, further examination of ungerminated seed of *Calligonum* species revealed that a large proportion had died during the experimental period. The causes of higher mortality of seeds at greater burial depths are unknown. The mortality of seeds may be caused by fungal infection, water-soluble germination inhibitors in the seed coat, unfavorable microenvironment and low oxygen concentration (Zhang & Maun, 1994; Wang *et al.*, 1997; Huang & Gutterman, 1998; Yu & Wang, 1998).

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