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EFFECT OF TEMPERATURE ON SEED GERMINATION OF SEVEN *CALLIGONUM* SPECIES

JUN REN[1, 2, 4], JIN ZIXUE [3], LING TAO[1]

[1]*College of Environmental and Municipal Engineering, Institute of Environmental Ecology, Lanzhou Jiaotong University, Lanzhou 730070, P. R. China*
[2]*College of Life Science, Lanzhou University, Lanzhou730070, Gansu, P. R. China*
[3]*Department of Biology, Shaoxing University, Shaoxing 312000, Zhejiang, P. R. China*
[4]Author for correspondence (Tel: 86 0931 4956563; Fax: 86 0931 4956017;
e-mail: renjun30@hotmail.com)

Abstract

Greenhouse experiments were conducted to study the effects of temperature on the seed germination of 7 *Calligonum* species, dominant shrubs in shifting sand dunes and stabilized sand fields in the northern desert of China. In August 1998, seeds of *Calligonum* were collected and exposed to different temperatures. The results suggested that there is no relationship between the temperature treatment and germination rate. Optimum germination occurred at 20°C. Minimum germination time of 3-4 days was recorded for *C. junceum*, *C. leucocladum*, *C. gobicum* and *C. mongolicum* at 22°C; approximately 3 days for *C. arborescens* and *C. caput-medusae* and 6 days for *C. rubicundum*. The 12°C treatment significantly increased the minimum germination time from those recorded at 20°C and 22°C by about 4-9 days for all seven species. *Calligonum* species germinated readily between 18-22°C. Maximum germination occurred at 20°C in *C. gobicum* and *C. arborescens* and at 22°C in *C. junceum, C. leucocladum, C. rubicundum, C. mongolicum* and *C. caput-medusae*. Seeds germinated faster at higher constant temperatures.

Introduction

In deserts temperature has a major influence on determining the onset of germination. It is a major factor limiting the establishment and regeneration of desert species (Choinski & Tuohy, 1991; Adams, 1999; Baskin & Baskin, 1988; Baskin *et al*., 1999; Budelsky & Galatowitsch, 1999). The pattern of seed germination in relation to temperature plays a key role in the persistence and dynamics of desert plants (Went, 1949; Thompson *et al*., 1977; Meyer *et al*., 1989; Van Assche & Vanlerberghe, 1989; de Villiers *et al*., 1994; Cony & Trione, 1996; Demel & Mulualem, 1996; Demel, 1998; Huang, 1998; Adams, 1999; Budelsky & Galatowitsch, 1999; Tobe *et al*., 2001). However, it is not well understood.

Calligonum species are dominant perennial shrub in active sand dunes and stabilized sand field in the northern desert of China (Mao & Pan, 1986; Tao, 2000). They can grow in mobile sand dunes in extreme drought (Liu, 1985-1990; Mao & Pan, 1986; Ren, 2001). They appear to be suitable for revegetating of deserts due to high tolerance to water deficit. Although little is known about their uses, they have great potential as forage, traditional medicine, halting desert encroachment and stabilizing sand dunes (Liu, 1985-1990; Tao, 2000) and has therefore attracted some attention. Information is available about their botanical characteristics, cultivation 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.*, 2001; Ren, 2001; Ren *et al*., 2002).

However, there is little information on the germination responses of *Calligonum* species in relation to the variation in ambient temperature (Yu and Wang, 1998).

Calligonum species occupy a typical arid environment of summer drought and winter precipitation (Mao & Pan, 1986). They are non-serotinous species, few seeds are stored on the adult shrubs, and most seeds are exposed to unfavorable temperatures over summer. They have appreciable soil seed bank (Tao, 2000), and it is difficult to raise seedlings from seeds due to the presence of water soluble inhibitors in the seed coat (Yu & Wang, 1998; Tao, 2000; Ren, 2001) like in many other arid species (Koller *et al*., 1964; Mott, 1974; Wang *et al*., 1997).

After dispersal seeds of *Calligonum* are exposed to high temperature and drought stress. Many other arid zone species also lack dormancy mechanisms, and once the water and temperature requirements for germination are available germination begins (Went, 1949; Baskin & Baskin, 1988; Choinski & Tuohy, 1991; Gutterman, 1993; Baskin *et al*., 1999; Huang & Gutterman, 2000). The role of temperature and the relationship between decreasing germination rate and percentage germination with increasing water stress are generally well established for a wide range of grasses, herbs and shrubs (de Villiers *et al*., 1994; Meyer *et al*., 1989; Mott, 1974; Adams, 1999; Budelsky & Galatowitsch, 1999; Wang *et al*., 1998), and relatively few woody desert plants have been investigated (Went, 1949; Choinski & Tuohy, 1991; Cony & Trione, 1996; Huang, 1998). The effects of temperature on the germination time and germination percentage of *Calligonum* seeds are variable and not well understood.

For natural and artificial desert vegetation, it is the relationship between the number of emerged seedlings and the number of subsequently emerging seedlings that is important in terms of stability and successful establishment (Zhang, 1992). This is particularly of prime importance for desert plant seeds that have to cope with unpredictable climatic conditions. Any strategy, which reduces the time lag between the beginning of water imbibition and germination of seeds is likely to be critical for successful seedling recruitment (Ren, 2001). The impacts of temperature on germination of *Calligonum* seeds were therefore investigated since understanding of these factors is crucial for the successful management of these long-lived desert plants.

Materials and Methods

Seed collection and preparation

Seeds of 7 *Calligonum* species (*C. junceum* (Fisch. Et Mey.) Borszcz., *C. leucocladum* (Schrenk) Bge., *C. rubicundum* Bge., *C. gobicum* (Bge.) A. Los., *C. mongolicum* Turcz., *C. arborescens* Litv., and *C. caput-medusae* Schrenk.) were collected from at least 10 plants per species in August and September, 1998 at Shapotou Desert Research and Experimental Station of the Chinese Academy of Sciences (37°32′ N, 105°02′ E, 1339m a.s.l.), Ningxia province, China (Tao, 2000). The aborted and predated seeds were discarded. Intact plump seeds were surface sterilized with Na-hypochlorite. Seed viability was variable, but generally between 15-20% (Tao 2000; Yu & Wang 1998). All treatments consisted of three replicates of 100 seeds of each species and seeds were considered germinated as soon as the radicle emerged. Germination was recorded for 30 days except where specified. Ungerminated seeds were soaked in water at 30°C for 24 hours to test their viability using tetrazolium chloride test. Seeds were cut and the embryo soaked in 1% tetrazolium chloride for 24 hours at 30°C. Pink embryos were scored as viable. Germination was expressed as the percentage of germinated viable seeds.

The experiments were conducted during March-April 1999. The germination data were subjected to statistical analyses. All percentage data were modified according to angular (arcsin(x)) for analysis. One-way ANOVA and Duncan multiple comparison tests were carried out using STATISTICA software (Statsoft, 1993).

Temperature treatments

Seeds were placed in the temperature-controlled chambers for germination in natural light at 12, 18, 20, or 22°C constant temperature and two alternating temperatures of 30°/16°C and 25°/12°C on 12-hourly cycles and represented approximate mean maximum and minimum ambient temperatures for summer and spring/autumn in Shapotou region (Li, 1999).

Results

Minimum time to the onset of germination

The minimum time to the onset of germination (T_{min}) was significantly different among temperature treatments in all 7 species (one-way ANOVA: $F_{5,12} = 5.96$, $p<0.01$ for *C. junceum*; $F_{5,12} = 19.87$, $p<0.0001$ for *C. leucocladum*; $F_{5,12} = 17.03$, $p<0.0001$ for *C. rubicundum*; $F_{5,12} = 10.94$, $p<0.001$ for *C. gobicum*; $F_{5,12} = 7.02$, $p<0.01$ for *C. mongolicum*; $F_{5,12} = 25.13$, $p<0.01$ for *C. arborescens*; $F_{5,12} = 22.87$, $p<0.01$ for *C. caput-medusae*). The shortest T_{min} (approximately 3~4 days) occurred at 20°C constant temperature for *C. junceum, C. leucocladum, C. gobicum* and *C. mongolicum*. The shorter T_{min} was approximately 3 days for *C. arborescens* and *C. caput-medusae* and 5 days for *C. rubicundum* at 30°/16°C alternating temperature treatment (Fig. 1, Table 1). Increases in time occurred at lower temperatures comparing. The 12°C treatment significantly increased the minimum germination time from that recorded at 20°C and 22°C by about 4-9 days for *C. leucocladum, C. rubicundum, C. gobicum, C. arborescens* and *C. caput-medusae* (Fig. 1, Table 1). The summer alternating temperature (30°/16°C) significantly increased T_{min} of *C. leucocladum* from 18°C, 20°C, 22°C and spring/autumn alternating temperature (25°/12°C) by 5~7 days. The spring/autumn alternating temperature significantly increased T_{min} of *C. caput-medusae* from 20°C by 3 days (Fig. 1, Table 1). The T_{min} of all species increased at the 12°C treatment. Compared to 20°C and 22°C, T_{min} of all species (except *C. leucocladum* at 30°/16°C) was not affected by alternating temperature treatments (Table 1).

The T_{min} was significantly different among species at 12°C, 18°C, 20°C and 30°/16°C temperature treatments (one-way ANOVA: $F_{6,18} = 18.89$, $p<0.0001$ at 12°C temperature treatment; $F_{6,18} = 5.04$, $p<0.01$ at 18°C temperature treatment; $F_{6,18} = 8.38$, $p<0.001$ at 20°C temperature treatment; $F_{6,18} = 20.44$, $p<0.0001$ at 30°/16°C alternating temperature treatment). At the 22°C and the spring/autumn alternating temperature, the minimum germination time was not significantly different among all 7 species ($F_{6,18} = 1.92$, $p>0.05$ at 22°C temperature treatment; $F_{6,18} = 2.71$, $p>0.05$ at 25°/12°C alternating temperature treatment). At 12°C and 20°C, the T_{min} of *C. rubicundum* was significantly longer than other six species (Table 1).

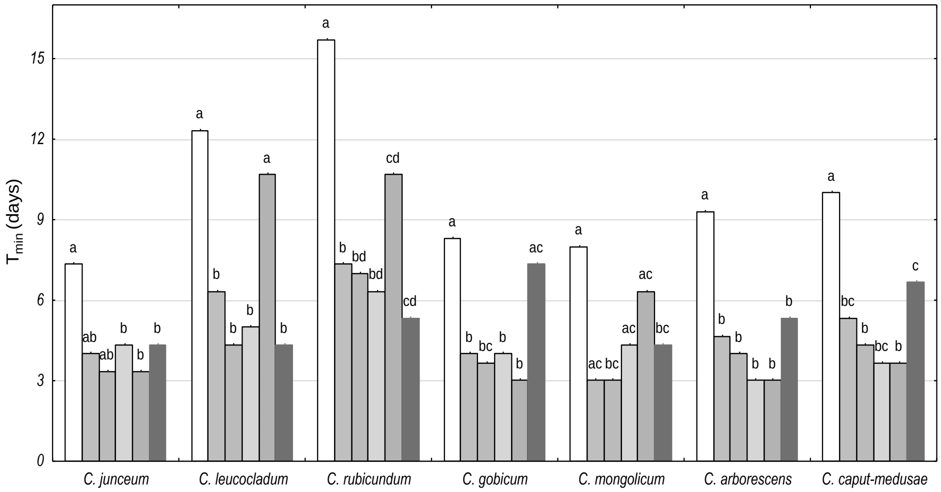

Fig. 1. T_{min} (days) for seed germination of seven *Calligonum* species from different temperature treatments. Values with the same superscript letters are not significantly different among treatments at P<0.05. Temperature treatments: (□) 12°C; (▨) 18°C; (▧) 20°C; (☰) 22°C; (▦) 30°/16°C; (■) 25°/12°C.

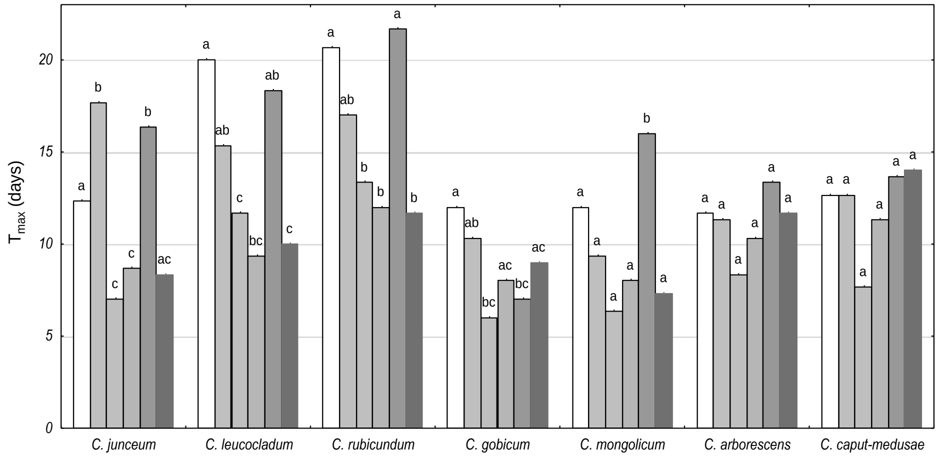

Fig. 2. T_{max} (days) for seed germination of seven *Calligonum* species from different temperature treatments. Values with the same superscript letters are not significantly different among treatments at P<0.05. Temperature treatments: (□) 12°C; (▨) 18°C; (▧) 20°C; (☰) 22°C; (▦) 30°/16°C; (■) 25°/12°C.

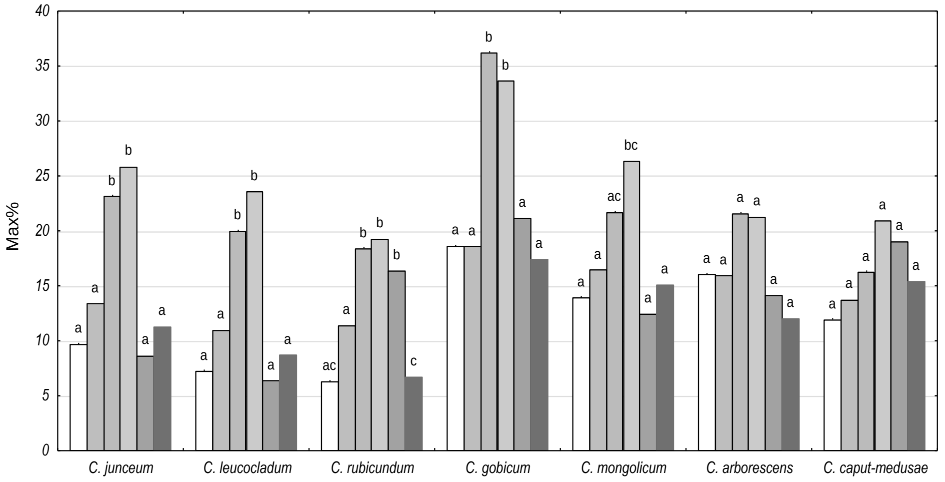

Fig. 3. Max% of seed germination of seven *Calligonum* species from different temperature treatments. Values with the same superscript letters are not significantly different among treatments at P<0.05. Temperature treatments: (▭) 12°C; (▨) 18°C; (▧) 20°C; (☰) 22°C; (⊞) 30°/16°C; (■) 25°/12°C.

Table 1. Month and year of seed collection, as well as seed size and dry mass of ten *Calligonum* species. Seed size and seed dry mass are means (± sd) of ten randomly selected seeds from each species (Tao, 2000).

Species	Date of seed collection	Length of seed (mm) (mean ± sd)	Diameter of seed (mm) (mean ± sd)	Seed mass (mg) (mean ± sd)
C. junceum	24-Aug-98	11.07 (± 0.76)	9.24 (± 0.99)	0.08 (± 0.01)
C. leucocladum	13-Aug-98	12.53 (±0. 97)	9.95 (± 1.81)	0.07 (± 0. 01)
C. rubicundum	3-Sep-98	16.70 (± 1.62)	14.17 (± 1.43)	0.16 (± 0.02)
C. gobicum	29-Aug-98	11.01 (± 0.90)	8.86 (± 1.12)	0.06 (± 0.01)
C. mongolicum	24-Aug-98	13.32 (± 1.35)	9.32 (± 1.48)	0.10 (± 0.02)
C. arborescens	10-Aug-98	20.32 (± 4.42)	16.34 (± 4.01)	0.15 (± 0.03)
C. caput-medusae	10-Aug-98	21.66 (± 2.31)	18.18 (± 3.05)	0.15 (± 0.01)

Time to maximum germination

The time to maximum germination (T_{max}) was significantly different among temperature treatments in five species (one-way ANOVA: $F_{5,12}$ = 17.44, $p<0.0001$ for *C. junceum*; $F_{5,12}$ = 17.65, $p<0.0001$ for *C. leucocladum*; $F_{5,12}$ = 13.75, $p<0.001$ for *C. rubicundum*; $F_{5,12}$ = 8.47, $p<0.01$ for *C. gobicum*; $F_{5,12}$ = 12.97, $p<0.001$ for *C. mongolicum*). For the other two species the T_{max} was not significantly different among temperature treatments ($F_{5,12}$ = 1.73, $p>0.2$ for *C. arborescens*; $F_{5,12}$ = 3.92, $p>0.01$ for *C. caput-medusae*). The shortest T_{max} (approximately 6~8 days) occurred at 20°C for *C. junceum, C. gobicum, C. mongolicum, C. arborescens* and *C. caput-medusae*. The shortest T_{max} was approximately 9 days for *C. leucocladum* and 12 days for *C. rubicundum* at 22°C (Fig. 2, Table 1). Significant lag in T_{max} occurred at the lower temperature (12°C) from that recorded at 20°C and 22°C by about 5-11 days for *C. junceum, C. leucocladum* and *C. rubicundum* (Fig. 2, Table 1). The summer alternating

temperature significantly increased T_{max} of *C. junceum, C. leucocladum, C. rubicundum* and *C. mongolicum* from 20°C and 22°C and spring/autumn alternating temperature by 5~10 days (Fig. 2, Table 1). The T_{max} of all species was shortened at 20°C and 22°C treatments. The summer alternating temperature significantly delayed T_{max} of *C. junceum, C. leucocladum, C. rubicundum* and *C. mongolicum* from 20°C by about 7~10 days (Fig. 2, Table 1).

The T_{max} was significantly different among species at 12°C, 18°C, 20°C and 30°/16°C (one-way ANOVA: $F_{6,18} = 17.53$, $p<0.0001$ at 12°C; $F_{6,18} = 6.96$, $p<0.01$ at 18°C; $F_{6,18} = 9.35$, $p<0.001$ at 20°C; $F_{6,18} = 12.09$, $p<0.0001$ at 30°/16°C). At the 22°C and the spring/autumn alternating temperature (25°/12°C) the T_{max} was not significantly different among species ($F_{6,18} = 2.78$, $p>0.05$ at 22°C; $F_{6,18} = 1.36$, $p>0.2$ at 25°/12°C; Table 1). But according to results of multiple comparisons test, the T_{max} was not significantly different between any two species at 12°C, 18°C and 22°C temperature treatments (Table 1). At 20°C the T_{max} of *C. rubicundum* was significantly longer than other six species. Significant decreases in T_{max} occurred at 30°/16°C for *C. gobicum* compared with the other 6 species (Table 1).

Final percentage of viable seeds germination

The final percentage of germination of viable seeds (Max%) was significantly different among temperature treatments in five species (one-way ANOVA: $F_{5,12} = 12.44$, $p<0.001$ for *C. junceum*; $F_{5,12} = 14.88$, $p<0.0001$ for *C. leucocladum*; $F_{5,12} = 26.29$, $p<0.0001$ for *C. rubicundum*; $F_{5,12} = 21.64$, $p<0.0001$ for *C. gobicum*; $F_{5,12} = 8.57$, $p<0.01$ for *C. mongolicum*). The Max% for other two species was not significantly different among temperature treatments ($F_{5,12} = 2.15$, $p>0.1$ for *C. arborescens*; $F_{5,12} = 4.53$, $p>0.01$ for *C. caput-medusae*). For *C. gobicum* and *C. arborescens*, Max% occurred at 20°C, while Max% for *C. junceum, C. leucocladum, C. rubicundum, C. mongolicum* and *C. caput-medusae* occurred at 22°C (Fig. 3, Table 1). The spring/autumn alternating temperature significantly decreased Max% of *C. rubicundum* from 20°C and 22°C by about 12% (Fig. 3). Significant increases in Max% occurred at 20°C and 22°C compared with the other 4 treatments for *C. junceum, C. leucocladum* and *C. gobicum* (Fig. 3).

The Max% was significantly different among species at 12°C, 20°C, 30°/16°C and 25°/12°C (one-way ANOVA: $F_{6,18} = 11.23$, $p<0.001$ at 12°C; $F_{6,18} = 5.02$, $p<0.01$ at 20°C; $F_{6,18} = 16.65$, $p<0.0001$ at 30°/16°C; $F_{6,18} = 11.23$, $p<0.001$ at 25°/12°C). At 18°C and 22°C the Max% was not significantly different among species ($F_{6,18}= 3.22$, $p>0.01$ at 18°C; $F_{6,18} = 4.42$, $p>0.01$ at 22°C). At any given temperature treatment, the Max% of *C. gobocum* was greater than the other six species, and significant increases in Max% occurred at 20°C compared with the other 5 treatments (Table 1).

Discussion

No complex dormancy mechanisms have been found for *Calligonum* species (Yu & Wang, 1998). Seed germination of *Calligonum* was affected by change in temperature. They germinated readily at temperatures between 18-22°C. The highest percentage of final germination of all viable seeds occurred at 20°C for *C. gobicum* and *C. arborescens*, at 22°C for *C. junceum, C. leucocladum, C. rubicundum, C. mongolicum* and *C. caput-medusae*. In the field, this range of temperatures occurs during spring and summer (Li, 1999), and is likely to restrict the germination of *Calligonum* species to these seasons. The seven species are found on distinctly different soil types and in different climatic conditions (Mao & Pan, 1986). *Calligonum* species did not show similar responses of germination triggering time to simulated field seasonal temperatures.

The lower temperature treatment significantly delayed the onset of germination. Seeds germinated faster at higher constant temperatures where maximum germination percentage occurred. Compared to the higher constant temperature treatments, lower alternating temperature treatments both reduced germination time and percentage of seeds. Similar responses in germination under higher temperatures were reported for other desert species (Khan & Weber, 1986; Khan & Ungar, 1999).

The difference in germination abilities of the 7 *Calligonum* species at different temperature treatments may partially explain the variation of their habitats. The low capacity of *Calligonum* to germinate at lower temperature is consistent with their more frequent occurrences in a sandy region with higher summer soil temperatures (Mao, 1984; Liu, 1985-1990; Mao & Pan, 1986). Under all temperature treatments, *C. gobicum* exhibited higher germination percentage and faster germination rate than the other species. This behavior has practical implications in countries like China where different temperature conditions prevail as a result of variable topography in the country. Therefore, the species can be artificially propagated by seed in different regions of the country.

Under the conditions of ideal temperature, the length of germination period (G_{period}) is a prime important factor for regeneration and seedling recruitment of desert plant species (Koller *et al*., 1964; Fenner, 1991; Gutterman, 1993; Chen & Maun, 1998), although its contribution to final germination percentage is still in dispute.

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