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Research Note

Seed germination of *Echinopsis schickendantzii* (Cactaceae): the effects of constant and alternating temperatures

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Summary

The effects of constant and alternating temperatures on seed germination in the Cactaceae have been reported to vary, probably as a result of the different temperature regimes used and the species considered. We determined the cardinal temperatures for, and evaluated the effects of a wide range of constant and alternating temperatures on, seed germination of the South American cactus, *Echinopsis schickendantzii* Web. The base, optimum and maximum temperatures were 7°C, 26.8°C and 49°C, respectively. The proportion of seeds that germinated and the germination rates were not only significantly different at constant and alternating temperatures but also among all temperature regimes considered. The highest proportion of seeds to germinate occurred at 15°, 20°, 30° and 30/15°C whereas the highest germination rates occurred at 25°, 30°, 30/20°, 35/20° and 40/25°C, with no significant differences between the highest values at constant and alternating temperatures. In the sub-optimal temperature range for germination rate, the thermal time to 50% germination was 98°C-days. The results indicate that the seeds have no obligate requirement for alternating temperature for germination.

Experimental and discussion

Seed germination and seedling establishment of Cactaceae are both processes that occur sporadically in the natural environments, being mainly dependent on soil water availability (Godínez-Alvarez *et al.,* 2003). However, temperature is also an important factor in the germination of cacti seeds (Rojas-Aréchiga et al., 1998; Rojas-Aréchiga and Vázquez-Yanes, 2000; Yang *et al.,* 2003; Ortega-Baes and Rojas-Aréchiga, 2007).

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Cacti seeds generally have an optimal temperature for germination of ca. 25°C, being negatively affected by extreme (> 40°C) temperatures (Rojas-Aréchiga *et al.,* 1998; Rojas Aréchiga and Vázquez-Yanes, 2000; Yang *et al.,* 2003; Ortega-Baes and Rojas Aréchiga, 2007). Nevertheless, the effects of constant and alternating temperatures on cacti seed germination have been contradictory. Some studies have reported that alternating temperatures induce seed germination (Fearn, 1974, 1981; Godínez-Álvarez and Valiente-Banuet, 1998), while others showed that they can inhibit germination of cacti seeds (Rojas-Aréchiga *et al.,* 1998; Rojas-Aréchiga *et al.,* 2001; Ortega-Baes and Rojas Aréchiga, 2007). In addition, a third group of studies have reported no difference in germination with constant or alternating temperature regimes (Ruedas *et al.,* 2000; Godínez-Álvarez and Valiente-Banuet, 1998; De la Barrera and Nobel, 2003; Ramírez-Padilla and Valverde, 2005). These contradictions in the temperature responses may be attributed to a limited number of temperature regimes being trialled, particularly for alternating temperatures (Rojas Aréchiga *et al.,* 1998; Rojas-Aréchiga and Vazquez-Yanes, 2000; Ortega-Baes and Rojas-Aréchiga, 2007).

Cardinal temperatures (base, optimum and maximum temperatures) describe the range of temperatures over which germination can occur (Bewley and Black, 1994). Generally, cardinal temperatures for a particular species depend on environmental conditions in which it is adapted and seeds normally germinate when environmental conditions for seedling establishment are assured (Alvarado and Bradford, 2002). Since seeds of desert plants are exposed to daily alternating temperatures after dissemination, higher seed germination at alternating temperatures might be expected (Baskin and Baskin, 1998; Probert, 2000). However, cactus seedling establishment generally occurs under nurse plants, where the amplitude of soil temperature fluctuations (the maximum and minimum) is less extreme (Godínez-Alvarez *et al.,* 2003). In this context, we might expect seeds to germinate better under conditions of relatively stable temperatures. In this paper, we tested this hypothesis by studying the effects of temperature regimes on seed germination and identified the cardinal temperatures of *Echinopsis schickendantzii* Web, an Andean cactus with non dormant and positively photoblastic seeds (Ortega-Baes *et al.,* 2010a, b).

Since the results obtained on constant and alternating temperatures effects may be a consequence of the limited number of temperature ranges used, we evaluated a greater number of constant and alternating temperature regimens.

Mature fruits were randomly collected from five plants at El Sunchal (25°10'S; 65°49'W; Salta, Argentina) in March 2005 (summer). The climate is semi-arid with a mean annual rainfall of 667 mm (Bianchi and Yáñez, 1992). Annual mean temperature is 10.2°C, with 14.6°C being the average summer temperature and 4.4°C the average winter temperature (Bianchi, 1996). Immediately after fruit collection, seeds were manually extracted and set to germinate in order to know their viability. Four replicates of 25 seeds were sown on agar (1%) in Petri dishes and were incubated in controlled incubators (25°C with a 8 h-light/16 h-dark photoperiod) for 30 days. Germination was 86 ± 5%. Subsequently, seeds were stored in paper bags in darkness and under laboratory conditions until the experiments began (August 2005). Eight constant temperatures: 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40°C; and seven alternating temperatures: 15/5°, 20/10°, 25/15°, 30/15°, 30/20°, 35/20° and 40/25°C were selected. These temperatures were selected taking to account: 1) temperature regimens in which cacti germinate (Rojas Aréchiga and Vázquez-Yanes, 2000), 2) the need for a wide range of temperatures to estimate cardinal temperatures and 3) temperatures present at the study site during potential seed germination period (summer). For each temperature regime, four replicates of 25 seeds per treatment were sown on the surface of 1% agar in water in 50 mm Petri dishes and incubated with an 8 h-light/16 h-dark photoperiod (cold white fluorescent light, 20 µmol/m^2/s; Baskin and Baskin 1998). In the alternating temperature regimes, the light period coincided with the highest temperature. Germination was defined as radicle protrusion through the seed coats by > 1 mm and the proportion of germinated seeds was recorded daily for 30 days.

To estimate the cardinal temperatures, germination curves were plotted for each constant temperature regime. Subsequently, the time periods (t) taken to reach 50% of the final germination percentage at each temperature were estimated, and the reciprocal ($1/t_{50}$) or germination rate was plotted against each temperature. The thermal time for germination was estimated from the reciprocal of the slope of the change in germination rate between the base and optimum temperatures (Pritchard and Manger, 1990).

Germination proportions were compared between constant temperatures, alternating temperatures and between all temperatures considered in the experiment, using ANOVA on arcsine squared root transformed data. Means were compared by *post hoc* DGC's test (Di Rienzo *et al.,* 2002). The same analysis was performed for germination rates ($1/T_{50}$). Treatments with no germination recorded in all replicates were not considered for the ANOVA.

The $1/t_{50}$ increased linearly with temperature above 7°C (base temperature) to a sharply defined optimum at 26.8°C, then decreased linearly to 49°C (maximum temperature, figure 1). The thermal time for germination in the sub-optimal temperature range was 98°C-days.

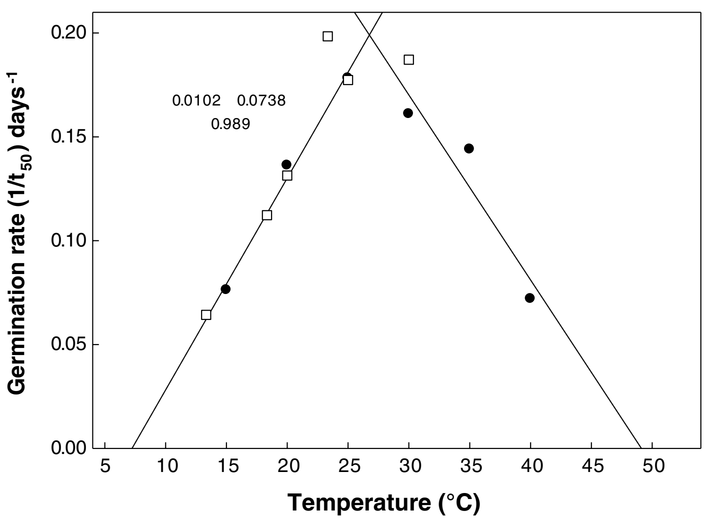

Figure 1. Germination rate ($1/t_{50}$) of *Echinopsis schickendantzii* seeds for each constant temperature (●) and alternating temperature (□: average temperature).

Significant differences were found among constant temperatures ($F = 25.32$, $p < 0.0001$, figure 1), with the highest $1/t_{50}$ registered at 25 and 30°C. The mean germination rate was significantly different among alternating temperatures ($F = 84.68$, $p < 0.0001$). The highest $1/t_{50}$ were obtained at 30/20, 40/25 and 35/20°C, which have average temperatures corresponding to 24°, 31° and 26°C, respectively (figure 1). Comparing across all the experimental temperatures we found significant differences among them ($F = 41.12$, $P < 0.0001$; figure 1). Germination rates were higher at 25°, 30°, 25/15°, 35/20° and 30/20°C than at the other temperatures.

Significant differences in the proportion of germinated seeds were found among constant temperatures ($F = 181.83$, $p < 0.0001$, figure 2a), with the higher proportions of germinated seeds registered at 15, 20 and 30°C. No germination was recorded at 5 and 10°C over the four-week time course of the experiment, but should have occurred at 10°C after about 5 weeks. The same pattern was recorded among alternating temperatures ($F = 162.41$, $p < 0.0001$; figure 2b). The highest germination proportion was obtained at 30/15°C and no germination was recorded at 15/5°C. When we compared all the experimental temperatures we found significant differences ($F = 31.69$, $P < 0.0001$; figure 2). Germination proportions were higher at 15°, 20°, 30°, 20/10°, 25/15°, 30/15°, 30/20° and 40/25°C than at the other temperatures.

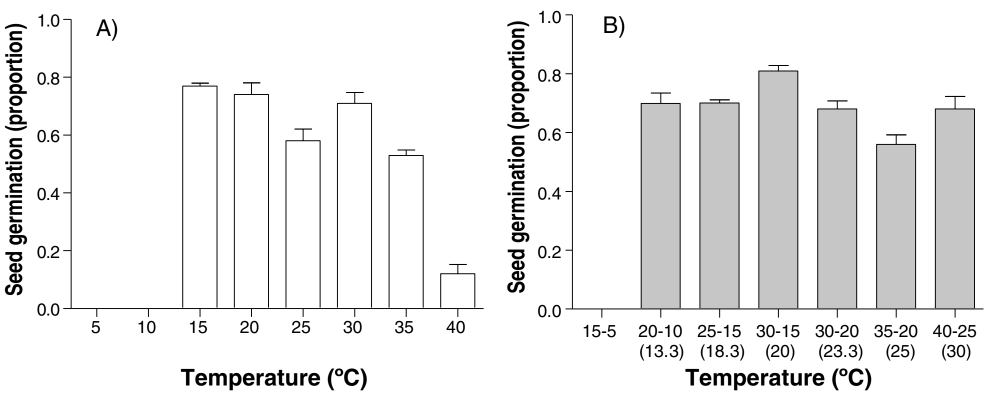

Figure 2. Germination proportion (media ± standard error) of *Echinopsis schickendantzii* seeds at (A) constant and (B) alternating temperatures (average temperature for each alternating temperature).

According to these results, *Echinopsis schickendantzii* seeds can germinate at different rates across a wide range of constant temperatures starting from 7°C up to 49°C, with the optimum temperature at 26.8°C. This optimum temperature is similar to that reported by Nobel (1988) and Rojas-Aréchiga *et al.* (1998), i.e frequently around 25°C for cacti. As for other cactus species, germination was affected by extreme temperatures, particularly in the lower temperature region (Rojas-Aréchiga and Vazquez-Yanes, 2000; Yang *et al.,* 2003; Ortega-Baes and Rojas-Aréchiga, 2007).

In arid environments, temperature fluctuates all day, so we might expect a higher germination under alternating temperatures (Baskin and Baskin, 1998). Alternating temperatures often increase germination (speed and final percentage). However, there is no agreement on whether alternating temperatures can release seeds from the last remnants of dormancy or whether they act as a germination stimulant (Gosling, 2003). In our study, no difference was found among the highest germination proportions at the most efficacious constant or alternating temperatures. This indicates a very efficient germination response. Although the effect of nurse plants is to reduce temperature fluctuations (Godinez-Álvarez *et al.,* 2003), any daily variations in microclimate probably would be beneficial, except when much higher temperatures associated with bare soils without vegetation coverage are reached. At the study site, a high proportion of emerging individuals of *E. schickendantzii* grows under shrubby species (93%, Ortega-Baes, unpublished. data).

In summary, using a wide range of conditions we found little difference between the promotory effects of constant and alternating temperatures on germination in *E. schickendantzii*. To determine whether the behaviour detected in this work actually occurs in natural conditions it would be necessary to carry out specifically designed field experiments. Our results indicate that the thermal time characteristics and cardinal temperatures in this cactus species can be used as the basis for predicting possible changes in seed germination in nature in relation to climate change, assuming moisture is not limiting. In addition, they indicate that the effects reported by other studies may be due to the limited number of temperature regimens used; therefore, cactus species would have efficient germination response to temperature. Whether the temperature response is species-specific or if it is a common response of cactus species, needs to be tested.

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