BIOLOGIA PLANTARUM 47 (2): 269-272, 2003/4

BRIEF COMMUNICATION

Effect of temperature regimes on germination of dimorphic seeds of *Atriplex prostrata*

C.T. CARTER*, L.S. BROWN and I.A. UNGAR

Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701-2979, USA

Abstract

Dimorphic seeds of *Atriplex prostrata* were removed from cold dry storage monthly over a one year period to test for fluctuations in seed dormancy and germination rate. For each seed type, four replicates of 25 seeds were exposed to four alternating night/day temperature regimes mimicking seasonal fluctuations in Ohio: 5/15 °C; 5/25 °C; 15/25 °C and 20/35 °C with a corresponding 12-h photoperiod (20 µmol m^{-2} s^{-1}; 400 - 700 nm). We found a significant three-way interaction of seed size, temperature and month for both percent germination and the rate of germination. Large seeds showed the greatest germination at the 20/35 °C and 5/25 °C temperature regimes and small seeds at the 5/25 °C regime. Large seeds had greater germination at all temperatures as compared to small seeds. Large seeds had the fastest germination rates at 20/35 °C followed by 5/25 °C whereas small seeds had the fastest rates at 5/25 °C followed by 20/35 °C.

Additional key words: endogenous rhythms, seed dormancy, seed storage.

Atriplex prostrata Boucher ex DC (syn: *A. triangularis* Willd.) (*Chenopodiaceae*) is a halophytic annual that produces dimorphic seeds. Large brown seeds range from 1.5 - 3.0 mm in diameter while small black seeds range from 1.0 - 2.0 mm (Gleason and Cronquist 1991). Large seeds also have a thinner seed coat and are less dormant than small black seeds (Khan and Ungar 1984). Seeds within the *Chenopodiaceae* have physiological dormancy and typically need a wet cold stratification (over-wintering) treatment to promote germination (Baskin and Baskin 1998).

Many investigations have focused on the germinability of stored seeds at a given point in time or on growth regulators that may be used to break dormancy thereby promoting germination (Priestley 1986, Ungar 1991, Bewley and Black 1994). Gutterman (1980/1981) found that seeds of the desert species *Mesembryanthemum nodiflorum* exhibited an annual endogenous germination rhythm during dry storage at room temperature. Seeds had high germination in the winter and low germination in the summer months, which mimicked their annual germination and dormancy cycle in nature. Short term exposure to cold indicated that there was a 14-d endogenous germination rhythm in seeds of *Distichlis spicata* (Amen 1970). No studies, however, have assessed the endogenous rhythms, or dormancy cycles, of seeds that have been kept in cold storage under constant temperatures over long periods of time. Theoretically, under constant temperatures, the number of seeds that germinate at any one time would be the same as any other point in time, unless seeds lose viability during storage. We wish to investigate whether there are endogenous rhythms in dimorphic seeds of *A. prostrata* that have been stored under a constant low temperature that influence their germination responses to fluctuating temperature treatments at different times of the year.

Physiologically mature fruiting structures of *Atriplex prostrata* Boucher ex DC were collected from an inland salt marsh in Rittman, Ohio, USA, during October, 1998. Fruits were dried at room temperature for 10 d, stored dry at 4 °C in the dark in a 500 cm^3 polyethylene container and removed from storage monthly over a 12 month period beginning in April, 2000. No data were recorded for September, 2000.

Received 18 March 2002, *accepted* 3 December 2002.
Acknowledgements: This project was supported by a grant from the National Science Foundation (USA) to I.A. Ungar.

* Corresponding author, fax: (+740) 593 1130, e-mail: ct346390@ohio.edu

Seeds were removed from their bracts after a 2 mm sieve was used to separate large and small fruits (utricles). Twenty-five large or small seeds were placed in tight-fitting 50 × 9 mm plastic Petri dishes (*Gelman No. 7232*) with 2 layers of *Whatman No. 5* filter paper and 2 cm^3 distilled water. Four replicates of each seed type were exposed to four alternating temperature regimes (5/15 °C; 5/25 °C; 15/25 °C and 20/35 °C) to mimic seasonal temperatures with a 12-h photoperiod (20 μmol m^{-2} s^{-1}; 400-700 nm) in a *Percival*™ incubator (Boone, Iowa, USA). Germination was recorded at 2 d intervals over a 20 d period. Emergence of the radicle was the criterion used to assess germination.

A three-way fixed-effects general linear model (*GLM*) *ANOVA* was performed on arcsine square root transformed data to assess the effects of seed size, month and temperature on germination. When significant differences were found, a Bonferroni post-hoc test was used to assess differences among means.

Rate of germination ($\Sigma G/t$) was calculated using a modified Timson's index (Timson 1965, Khan and Ungar 1984) where G = percentage of seed germination at 2 d intervals and t = number of observations (10). The maximum possible value for our data was 100 (*e.g.* 1000/10), where 100 indicates that all seeds germinated on the first day and a value of zero indicates that no seeds germinated over the 20 d period. A three-way fixed effects *GLM ANOVA* was performed to assess the effects of month, seed size and temperature on the rate of germination. A Bonferroni post-hoc test was implemented when differences among means were found. All statistical analyses were performed in *NCSS 2000* (Hintze 1998).

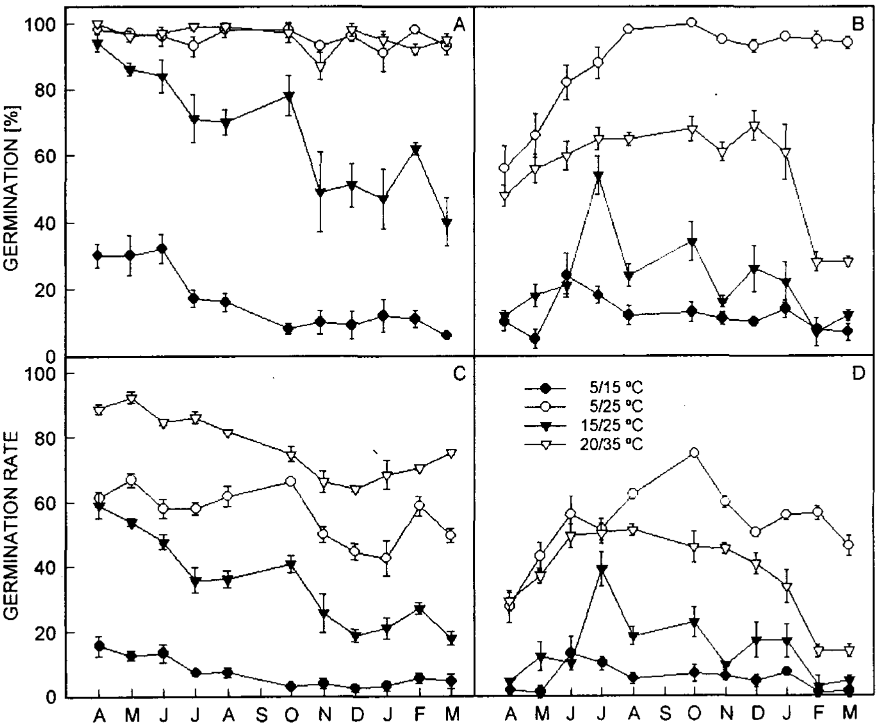

Fig. 1. Percent germination (mean ± SE) and rate of germination (modified Timson's Index) (mean ± SE) for large (*A,C*) and small (*B,D*) seeds of *Atriplex prostrata* for four temperature regimes.

There was a significant three-way interaction of seed size, month and temperature ($F = 3.27$; $P < 0.01$; power = 0.99) for overall germination. Large seeds had the highest germination at 5/25 °C and 20/35 °C whereas small seeds had the highest germination at 5/25 °C. Both seed types had the lowest germination at 5/15 °C (Fig. 1*A,B*). Large seeds had an overall greater germination for all temperatures as compared to small seeds (Fig. 1*A,B*). Large seeds showed greater germination during spring months, whereas small seeds showed greater germination during late summer and early fall months.

A significant three-way interaction of seed size, month and temperature ($F = 5.22$; $P < 0.01$; power = 1.0) was also found for the rate of germination. Large seeds had the fastest rate of germination at 20/35 °C followed by 5/25 °C whereas small seeds had the fastest rate of germination at 5/25 °C followed by 20/35 °C. Both seed types showed the slowest rate of germination at 5/15 °C (Fig. 1*C, D*). The pattern of total germination and rate of germination varied by month (Fig. 1).

Even though the temperature regimes used in our study reflect seasonal temperatures in Ohio, the differences found in germination for both large and small seeds may be attributed to the differing amplitudes between the night and day temperatures. The greatest germination occurred in temperatures where the difference between night and day temperatures was at least 15 °C. Thompson and Grime (1983) found that stimulation of germination for *Rumex obtusifolius*, *Urtica dioica* and *Fimbristylis littoralis* occurred when the amplitude of diurnal temperatures was increased. It has also been hypothesized that sensitivity to amplitude of diurnal temperatures can serve as a depth sensing mechanism for seeds buried in soil or under water (Thompson and Grime 1983, Pons and Schröder 1986). Seedlings produced by small seeds would not reach the surface if they were buried too deeply in the soil. Our results differ from Khan *et al.* (2001) who determined that germination of seeds of the halophyte *Sarcobatus vermiculatus* showed little change at different temperature regimes.

Overall differences in the timing of germination may be a mechanism by which dimorphic seeds avoid salinity fluctuations, which tend to be the most pronounced during the spring for inland salt marshes (Ungar 1987). Large seeds all germinate during the spring and summer when the net difference between night and day temperatures is about 20 °C. Small seeds of *A. prostrata*, like those of many other annuals, are known to have a physiological dormancy, preventing germination until environmental or temporal conditions are more favorable (Louda 1989, Fenner 1995). Wertis and Ungar (1986) have shown that small seeds of *Atriplex prostrata* retain about 10 % of their seeds in the soil seed bank. This dormancy allows small seeds to form persistent seed banks that can play an important role in the recovery of an area following disturbance (Thompson 1992). Egan and Ungar (1999) reported that the persistent seed bank in an inland salt marsh played a significant role in the recovery of an area after flooding. The differential in the timing of germination between the large and small seeds permits the germination period to be extended throughout the spring and summer. Wertis and Ungar (1986) noted, however, that in some years later cohorts tend not to reach reproductive maturity, or have higher mortality due to unfavorable environmental conditions. However, small seeds play an essential part in the reestablishment of plants after catastrophic events when environmental conditions are favorable (Egan and Ungar 1999).

The rate of germination for large and small seeds corresponds to the monthly changes in total germination. Large seeds germinated faster when removed from storage in the spring whereas small seeds germinated faster in the summer and fall. These results mimic field conditions in which all large seeds germinate in the spring, whereas small seeds in the seed bank may germinate later in the growing season when conditions become favorable. Our results suggest that the germination response of the dimorphic seeds of *A. prostrata* is not only a response to environmental factors, but also to an internal clocking mechanism which controls the timing of germination for each seed type. Further studies will have to be performed in order to discern whether this endogenous rhythm is affected by environmental conditions to which parent plants are exposed during seed maturation.

References

Amen, R.D., Carter, G.E., Kelly, R.J.: The nature of seed dormancy and germination in the salt marsh grass *Distichlis spicata*. - New Phytol. **69**: 1005-1013, 1970.

Baskin, C.C., Baskin, J.M.: Seeds – Ecology, Biogeography and Evolution of Dormancy and Germination. - Academic Press, San Diego 1998.

Bewley, J.D., Black, M.: Seeds: Physiology of Development and Germination, 2nd Edition. - Plenum Press, New York 1994.

Egan, T.P., Ungar, I.A.: The effects of temperature and seasonal change on the germination of two salt marsh species, *Atriplex prostrata* and *Salicornia europaea*, along a salinity gradient. - Int. J. Plant Sci. **160**: 861-867, 1999.

Fenner, M.: Ecology of seed banks. - In: Kigel, J., Galili, G., (ed.): Seed Development and Germination. Pp. 507-528. Dekker, New York 1995.

Gleason, H.A., Cronquist, A.: Manual of Vascular Plants of Northeastern United States and Adjacent Canada, 2nd Edition. - New York Botanical Garden, Bronx, New York 1991.

Gutterman, Y.: Annual rhythm and position effect in the germinability of *Mesembryanthemum nodiflorum*. - Israel J. Bot. **29**: 93-97, 1980/1981.

Hintze, J.L.: User's Guide 2000. - Number Cruncher Statistical Systems, Kaysville 1998.

Khan, M.A., Ungar, I.A.: The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. – Amer. J. Bot. **71**: 481-489, 1984.

Khan, M.A., Gul, B., Weber, D.J.: Seed germination in relation to salinity and temperature in *Sarcobatus vermiculatus*. - Biol. Plant. **45**: 133-135, 2001.

Louda, S.M.: Predation in the dynamics of seed regeneration. - In: Leck, M.A., Parker, V.T., Simpson, R.L. (ed.): Ecology of Soil Seed Banks. Pp. 25-51. Academic Press, New York 1989.

Pons, T.L., Schröder, H.F.J.M.: Significance of temperature fluctuation and oxygen concentration for germination of the rice field weeds *Fimbristylis littoralis* and *Scirpus juncoides*. - Oecologia **68**: 315-319, 1986.

Priestley, D.A.: Seed Aging: Implications for Seed Storage and Persistence in the Soil. - Comstock Publishing Associates, Ithaca 1986.

Thompson, K.: The functional ecology of seed banks. - In: Fenner, M. (ed.): Seeds – The Ecology of Regeneration in Plant Communities. Pp. 231-258. CAB International, Wallingford 1992.

Thompson, K., Grime, J.P.: A comparative study of germination responses to diurnally-fluctuating temperatures. - J. appl. Ecol. **20**: 141-156, 1983.

Timson, J.: New methods of recording germination data. - Nature **207**: 216-217, 1965.

Ungar, I.A.: Population ecology of halophyte seeds. - Bot. Rev. **53**: 301-334, 1987.

Ungar, I.A.: Ecophysiology of Vascular Halophytes. – CRC Press, Boca Raton 1991.

Wertis, B.A., Ungar, I.A.: Seed demography and seedling survival in a population of *Atriplex triangularis* Willd. – Amer. Midl. Nat. **116**: 152-162, 1986.