NOTES

Effect of pH on Germination of the Endemic Heather, *Erica andevalensis*

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INTRODUCTION

Erica andevalensis Cabezudo and Rivera is an edaphic endemic heather of the Andévalo, a geographically defined section of the Huelva province in southwestern Spain (Cabezudo 1987). The scarcity of available ecological and biological data about this species and its strict endemic nature led the authorities of the Andalusian Regional Government to include *E. andevalensis* on the list of endangered species in southwestern Spain subject to Recovery Plans (Aparicio 1995). This species grows mostly in the outskirts of mines as well as on the banks of the Tinto and Odiel rivers that run through the Andévalo region (López-Archilla et al. 1994). This area has been subject to intensive mining since ancient times and has had an enormous environmental impact due to the dispersal of different substances throughout this region. Thus, the extreme conditions of the Tinto and Odiel rivers, such as high concentration of metals and low pH, are related to the high pyretic content (Rodriguez et al. 2007). Sarmiento et al. (2005) found pH values between 2.6 and 4.7 at the Odiel river in July and November, respectively, and Aparicio (1995) recorded pH ~3 in some areas along the Tinto banks. The effects of extreme low pH values on *E. andevalensis* seed germination are unknown, however, despite the fact that seed germination is the initial and most crucial stage in the life cycle of plants (Ibañez and Passera 1997) and that it determines seedling establishment and subsequent development. Consequently, we tested the effect of pH on seed germination of *E. andevalensis* under controlled laboratory conditions. Knowledge of the ability of *E. andevalensis* seeds to germinate under different pH conditions is an essential pre-requisite to successfully manage and restore existing populations.

MATERIALS AND METHODS

Seeds of *E. andevalensis* were collected during July 2006 from banks of the Odiel River in the Andévalo region, (37°41'N, 06°39'W; southwest Spain). *E. andevalensis* is a perennial scrub species growing to 1.5 m tall. The flowers are produced on an umbel from July to September and the fruit is a small capsule containing hundreds of seeds that are slowly scattered (Cabezudo 1987). Seeds were stored in the dark for 3 months at 4 °C because Aparicio (1995) reported they required a cooling pre-treatment to germinate.

In October 2006, after the chilling period, four 25-seed replicates were placed separately on filter paper in 5-cm Petri dishes and submerged in 3 ml of five different pH treatment solutions (2, 3, 4, 5 and 7) obtained by mixing distilled water with the appropriate concentrations of either HCl or NaOH. Dishes were wrapped in paraffin to avoid the loss of water through evaporation, placed in a germinator (ASL Aparatos Científicos M-92004, Madrid, Spain), and subjected to a regime of 10 h of light (25 °C, 400-700 nm, 35 μmol photon m^{-2} s^{-1}) and 14 h of darkness (12 °C) for 40 days. This regime was chosen to simulate the temperatures in Andévalo during spring, when this species germinates. The dishes were inspected daily and germinated seeds were counted and removed. Radicle appearance was considered evidence of seed germination.

In addition, in October 2006 a recovery experiment was carried out to determine whether different pH values inhibited or damaged the seeds. Four 25-seeds replicates were submerged for 30 d in 3 ml of the pH treatment solutions 2, 3, 4 and 5 under dark conditions and kept at previously noted temperature conditions. After continuous exposure to these pretreatments, seeds were submerged in 3 ml of distilled water in new Petri dishes and kept for 40 d at the temperature and light conditions described above. Germinated seeds were counted and removed daily during this period.

Four characteristics of germination were determined: final germination percentage, number of days to first germination, number of days to final germination, and mean time to germinate (MTG). MTG was calculated using the equation:

$$MTG = \Sigma_i(n_i \times d_i)/N$$

where n is the number of seeds germinated until day i; d is the incubation period in days and N is the total number of seeds germinated in the treatment (Redondo-Gómez et al. 2007). Lower MTG values indicate more rapid germination.

Seeds that did not germinate during the germination experiment were subjected to the tetrazolium test to assess the viability of the embryo. Seeds were kept under water for 16 h at a constant temperature of 25 °C. Seeds were then submerged in a 1% aqueous solution of 2,3,5-triphenil-tetrazolium chloride, pH 7, in darkness for 24 h at a constant temperature of 25 °C. Seeds were then dissected and the embryo was analyzed using a magnifying glass (Bradbeer 1998).

Statistical analyses were carried out using Statistica v. 6.0 (Statsoft Inc.). Data were analyzed using Student's t-test for

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independent samples and one-way analysis of variance (*F*-test). Data were first tested for normality with the Kolmogorov-Smirnov test and for homogeneity of variance with the Brown-Forsythe test and then were arcsine transformed to normalize the error distribution for analysis of variance. Significant test results were subjected to Tukey's test to identify important contrasts.

RESULTS AND DISCUSSION

The pH value had a significant impact on germination of *E. andevalensis* (Table 1; Figure 1). Seeds did not germinate when pH was 2, but final germination percentage varied from 5 to 10% over a pH range of 3 to 7 (Table 1). Generally, germination increased as pH increased, and final germination was highest at pH 7. These results agree with the general trend for heathland species as reported by Roem et al. (2002). Overall, *E. andevalensis* showed very low final germination percentage. These results disagree with those reported by Aparicio (1995), who stated that ~80% of seeds germinated in distilled water after a 3-month chilling pretreatment, conditions that are comparable to the pH 7 treatment in our experiment. Variability in the germination response of seeds from different provenance is well known and have been previously reported for *Erica* species. For example, Cruz et al. (2003) found differences of ~60% in germination of *E. australis* seeds from different populations. Valbuena and Vera (2002) also found variation in the effect of light on seed germination of different species of *Erica*, and even among individuals of the same species, demonstrating the wide range of responses to disturbance. In this way, taxa of *E. andevalensis* could demonstrate differential germination because seeds of different individuals may respond differently to a given stimulus. In contrast, a pH of 7 delayed the beginning and ending of germination, and MTG was highest in distilled water (Table 1). Thus, pH values from 3 to 5 stimulated germination; germination speed was highest under acidic conditions. Keeley and Fotheringham (1998) suggested that scarification with acidic solution could induce germination by increasing oxygen uptake. In this way, acid treatment of seeds could have increased the permeability of their seed coats, allowing the entry of oxygen-saturated water.

Low pH values did not affect seed viability during the experimental period (P > 0.05). Nonetheless, pH 2 and 3 solutions appeared to induce dormancy in *E. andevalensis* (Figure 1) because final germination of seeds removed after 1 month in these low-pH solutions was similar to those in distilled water (*t*-test, P > 0.05; Table 2). The ability to preclude germination at pH 2 could be a strategy used by this plant to avoid germination during adverse conditions and to take advantage of periods when establishment conditions are ideal (i.e., 'windows of germination' [Noe and Zedler 2001], or 'windows of opportunity' [Eriksson and Fröborg 1996]) because pH values increase after the rainfalls. Finally, the number of days to first and final germination and MTG were independent of pH in the recovery experiment (P > 0.05; Table 2), so pH pretreatments did not induce faster germination.

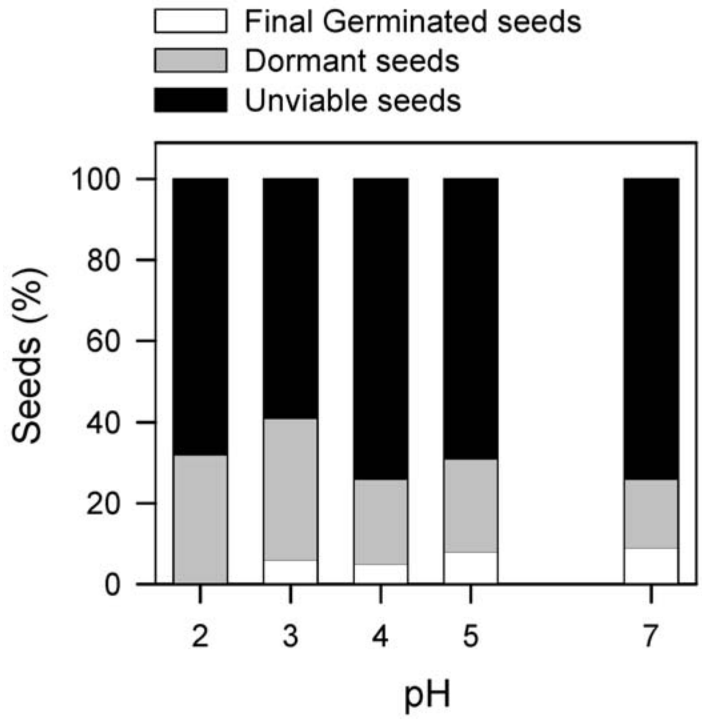

Figure 1. Final germinated, dormant and unviable seeds (%) for *Erica andevalensis* under five pH treatments for 40 d.

TABLE 1. FINAL GERMINATION (%), DAYS TO FIRST GERMINATION, DAYS TO FINAL GERMINATION AND MEAN TIME-TO-GERMINATE (MTG) OF *ERICA ANDEVALENSIS* SEEDS IN FIVE DIFFERENT PH SOLUTIONS FOR 40 D. VALUES REPRESENT MEAN ± SE OF FOUR REPLICATES. MEANS WITH DIFFERENT SUPERSCRIPT LETTERS ARE SIGNIFICANTLY DIFFERENT FROM EACH OTHER (TUKEY TEST; P < 0.05)

	Germination characteristics			
pH treatment	Final percentage	First germination (days)	Final germination (days)	MTG
2	0 ± 0.0^{a}	—	—	—
3	7 ± 2.9^{b}	13 ± 1.2^{a}	22 ± 4.0^{a}	28 ± 8.6^{a}
4	5 ± 2.1^{b}	22 ± 3.2^{ab}	30 ± 4.5^{ab}	29 ± 3.3^{a}
5	8 ± 2.9^{b}	19 ± 0.0^{ab}	25 ± 4.0^{ab}	19 ± 0.0^{a}
7	10 ± 3.8^{b}	26 ± 5.9^{b}	38 ± 2.2^{b}	58 ± 9.0^{b}

TABLE 2. FINAL GERMINATION (%), DAYS TO FIRST GERMINATION, DAYS TO FINAL GERMINATION AND MEAN TIME-TO-GERMINATE (MTG) OF *ERICA ANDEVALENSIS* IN DISTILLED WATER AFTER FOUR PH TREATMENTS FOR 40 D (RECOVERY EXPERIMENT). VALUES REPRESENT MEAN ± SE OF FOUR REPLICATES. MEANS WITH DIFFERENT SUPERSCRIPT LETTERS ARE SIGNIFICANTLY DIFFERENT FROM EACH OTHER (TUKEY TEST; P < 0.05).

	Germination characteristics			
pH treatment	Final percentage	First germination (days)	Final germination (days)	MTG
2	13 ± 1.0^{ab}	12 ± 0.7	28 ± 5.5	30 ± 5.3
3	15 ± 0.9^{a}	14 ± 2.0	26 ± 5.0	28 ± 9.8
4	8 ± 2.2^{ab}	14 ± 1.6	27 ± 6.4	29 ± 5.3
5	6 ± 1.4^{b}	18 ± 1.1	19 ± 1.1	17 ± 1.1

E. andevalensis, typical of heathland species, germinated under a wide range of pH values, even at a pH as low as 3. Furthermore, acidic pH pretreatments showed a stimulative effect on germination of this species, indicating that pH should not be considered a limiting factor for the germination of *E. andevalensis* along the Odiel and Tinto rivers banks, where of the pH soils are strongly acid, especially in summer.

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