Seed germination ecology of the perennial *Euphorbia boetica*, an endemic spurge of the southern Iberian Peninsula

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Patterns of seed germination of the endemic perennial spurge *Euphorbia boetica* were studied in two populations in southern Spain. We investigated whether its seeds have any type of dormancy, and whether diverse factors such as temperature, darkness, or acid scarification affect germination of the seeds. Freshly matured seeds of *E. boetica* do not germinate; they need a dry after-ripening period of up to 3 months to reach maximum germination, which indicates that the seeds have a non-deep physiological dormancy. Seeds of this species seem to present a certain tolerance to fire, since many of them survived after exposure to the mildest treatment simulating fire conditions. Finally, darkness and acid scarification had no effect on seed germination. The high germination capacity of *E. boetica* seeds both in natural conditions and under diverse treatments suggests that germination *per se* does not represent a crucial phase in the life cycle of this rare species.

Introduction

Germination is a crucial phase in the life cycle of a plant, greatly affecting its fitness. In order for the seeds of a species to germinate, they must be in suitable environmental conditions, which usually favour the growth and establishment of the seedlings (Harper 1977). However, viable seeds of some species do not germinate even though conditions are favourable. Six types of dormancy are known to occur in seeds: physiological, morphological, morphophysiological, physical, chemical, and mechanical (for more details *see* Nikolaeva 1977).

The genus *Euphorbia* contains approximately 1000–1600 species and is distributed mainly in subtropical and warm areas (Steinmann & Porter 2002). Most of the studied species of this genus are annuals, and their seeds have physiological dormancy (Brenchley & Warington 1930, Heit 1942, Capon & van der Asdall 1967, Kivilaan & Bandurski 1981, Washitani & Masuda 1990, Kigel *et al*. 1992) or are non-dormant (van der Rooden *et al*. 1970, Brecke 1995). Only a few perennial species have been studied. Seeds of *E. esula* have physiological dormancy that was broken by dry storage at room temperature (after-ripening) for three months (Best *et al*.

1980), In contrast, seeds of *E. caducifolia*, *E. characias*, and *E. nicaeensis* were not dormant (Sen & Chatterji 1966, Gómez & Espadaler 1997, Narbona 2002).

Euphorbia boetica, a perennial spurge endemic to the southern half of the Iberian Peninsula (Benedí *et al*. 1997), is considered an endangered species in some areas (Henández-Bermejo & Clemente 1994). This species grows in pine woods or on acid soils at very low altitudes (0–100 m; Benedí *et al*. 1997). Flowering occurs in early spring, and seed dispersion takes place in early-mid summer. This species produces an average of 76 capsules per individual (Narbona 2002), and each capsule has three seeds, each weighing between 3 and 7 mg (Narbona *et al*. 2005a). *Euphorbia boetica* is a diplochorous species: it has a primary explosive dispersal system and a secondary dispersal system by ants, since its seeds have a caruncle (Narbona *et al*. 2005a). Although seeds have a caruncle while in the fruit, all of them lose it during explosive dispersal (Narbona *et al*. 2005a). In various populations in southern Spain, carunculate seeds are dispersed by ants, and both carunculate and ecarunculate seeds are collected by granivorous ants (Narbona 2002).

The main aim of this study was to investigate the germination variability of *E. boetica* seeds and their ability to germinate under diverse environmental conditions. The specific objectives were (1) to determine if freshly matured seeds are dormant, and (2) to analyze whether different factors such as temperature, darkness, or acid scarification affect germination.

Material and methods

Seed collection sites

Euphorbia boetica was studied in southwestern Spain in two populations called Hinojos and El Gandul. The El Gandul population is situated on a peneplane (Seville province; 5°47´17´´W, 37°20´16´´N), at an altitude of 45 m on Miocenic detritic limestones, in an area of abandoned farmland without tree cover. The vegetation consists of scattered scrubs (*Chamaerops humilis*, *Heli-*

chrysum stoechas, *Asparagus aphyllus*, *Micromeria graeca*, etc.) and grasses (mainly Poaceae). The Hinojos population is located on a peneplane (Huelva province; 6°25´43´´W, 37°16´14´´N) at an altitude of 80–90 m on Pliocenic sands, and ca. 30 km from the sea. The vegetation consists of a mixed woodland of *Pinus pinea* and *Quercus suber*, with a scrub layer consisting mainly of Cistaceae, Lamiaceae, and Leguminosae. The two sites have a typical dry-summer Mediterranean climate, with an average annual precipitation of 543 mm and 614 mm, and an average annual temperature of 18.1 °C and 16.9 °C (El Gandul and Hinojos, respectively).

General

In July 1998, 2600 seeds were collected from 38 plants in El Gandul, and in July 1999, 900 seeds were collected from 30 plants in Hinojos. Given that this species has vegetative reproduction, we selected plants separated by more than 2 metres from each other, along two 100 metres transects. Seeds were collected from the third and fourth branching levels of the inflorescence (for more details *see* Narbona *et al*. 2005b), and preliminary analysis demonstrates that there are no significant differences in germination between these two levels (E. Narbona unpubl. data). In each population, seeds were mixed, and kept in paper envelopes, in the dark and at laboratory temperatures (ca. 20 °C, relative humidity 50%–70%), until October 1999. In all treatments, a batch of 100 seeds was sown; these seeds were distributed in four replicates, each of 25 seeds. These replicates were placed on permanently moist Whatman #1 filter paper in Petri dishes, and placed in a chamber room at 17/21 °C (night/day, 13/11 hrs). Preliminary tests showed that this temperature range is optimum for germination. Dishes were checked every 1–3 days and germinated seeds were counted and removed. Seeds with a radicle that had emerged more than 0.2 mm were considered to be germinated. Dishes were randomly arranged in the chamber room, and the layout was changed each inspection day to avoid pseudoreplication problems (Hurlbert 1984). The trials lasted 40 days, after which

time dishes were checked for an additional three weeks to confirm that no more germination had occurred (Baskin & Baskin 1998). A tetrazolium test was performed on non-germinated seeds to check their viability (Grabe 1970).

Germination of freshly-matured seeds

To know whether *E. boetica* seeds are dormant, a batch of seeds from Hinojos and another from El Gandul was sown immediately after harvesting. Other batches of seeds, kept in paper envelopes under laboratory conditions, were sown after 3 and 15 months of dry storage.

Seed germination variability with different pre-treatments and conditions

To assess germination capacity of *E. boetica* seeds under diverse natural conditions, we designed some experiments simulating them. These experiments were carried out with seeds from El Gandul, which were sown at 17/21 °C in October 1998. One batch of seeds was left untreated as the "control" group. Other batches were subjected to one of the treatments described below. Germination was assessed as described above.

Germination following thermal shock

Before they were sown, five batches of seeds were placed in an oven, and were subjected to temperatures of 100 °C for 1, 5, and 15 minutes, and 120 °C for 1 and 5 minutes, respectively. These treatments were aimed at simulating the conditions that occur in the soil (0–5 cm depth) following a fire in the Mediterranean area (Trabaud 1979, Mallik & Gimingham 1985). Three further batches of seeds were subjected to temperatures of 50 °C for 4 hours, 50 °C for 4 hours repeated on 4 successive days, and 65 °C for 30 minutes, to simulate the temperatures that are reached in summer at ground level in the Mediterranean area (Kelly *et al.* 1985). The seeds were sown immediately after the treatments were finished.

Sulphuric acid scarification

To simulate passage of seeds through the digestive tract of animals, a batch of seeds was immersed in concentrated sulphuric acid (96%) for five minutes, then washed in abundant water, and immediately sown.

Darkness

In order to determine whether the seeds are capable of germinating in darkness, a batch was placed on Petri dishes, wrapped in two layers of aluminium foil, and left to germinate. The dishes were checked every five days, using a low-intensity green safe light to avoid germination after a small amount of white light (Baskin & Baskin 1998).

Statistical analysis

Mean germination percentage, standard error and mean germination time (t_{50}) were calculated. Mean germination time refers to the number of days taken for 50% of total germination to be achieved (Bewley & Black 1985). Prior to data analysis, the variables "germination percentage" and "t_{50}" were transformed, using square root, arcsine, or arcsine of the square root when they did not fulfil the requirements of normality and homogeneity of variances (Zar 1999). The homogeneity of variances was verified using the Levene test (StatSoft 1999). To check whether the distribution of the data fitted the normal function, the Kolmogorov-Smirnov goodness-of-fit test was used with the Lilliefors correction (StatSoft 1999).

To compare germination percentages of the seeds sown immediately after harvesting with those of the seeds sown after 3 and 15 months, a single-factor ANOVA was used for each population. When the ANOVA showed significant differences, the means of groups were compared using the *t*-test with estimation of the separate variance, as the variance of the groups was not equal (Day & Quinn 1989). Differences in both the germination percentages and t_{50} between treatments were analyzed using a single-factor ANOVA, comparing each treatment with the

control. To compare the percentage of viable seeds with the germination percentage of all batches, a single-factor ANOVA was used.

Results

Germination of freshly matured seeds

Germination of the seeds sown immediately after harvesting was practically null in both populations (Fig. 1). However, seeds in both populations sown after three months germinated (Fig. 1), and germination percentages were not statistically different from those of viability ($P > 0.9$ for both populations). The same result was found with seeds sown after 15 months of dry storage (Fig. 1). For both populations, germination percentages after 3 and 15 moths were not significantly different from each other, but both were significantly different from fresh seeds ($F_{2,9} = 68.9$, $P < 0.00001$ for El Gandul; $F_{2,9} = 257.3$, $P < 0.00001$ for Hinojos).

Seed germination variability with different pre-treatments and conditions

Intact *E. boetica* seeds developed a mucilaginous layer when they were placed into a humid environment. The average germination percentage for the control batch was 73% (Table 1). Both in the control batch and in the pre-treated batches, all the viable seeds germinated ($P > 0.9$).

Treatments that simulated summer soil temperatures (50 °C 4 h, 50 °C 4 h × 4 d, and 60 °C 30 minutes) did not affect seed germination or t_{50} (Table 1 and Fig. 2a). Treatments that simulated the brief passage of fire (1 and 5 minutes at 100 °C and 1 minute at 120 °C) did not affect the germination percentage of the seeds, but intense heat treatments (15 minutes at 100 °C and 5 minutes at 120 °C) caused a drastic reduction in germination percentage (Table 1 and Fig. 2b and c). The seeds heated for 5 and 15 minutes at 100 °C and for 5 minutes at 120 °C showed a significant increase in t_{50} with respect to that of the control, but the seeds heated for 1 minute at 120 °C showed a significant decrease in t_{50} (Table 1 and Fig. 2b and c). Seeds subjected to acid scarifica-
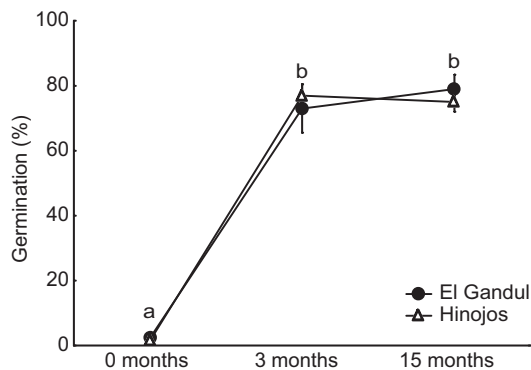

Fig. 1. Seed germination (mean ± SE) in relation to age of *Euphorbia boetica* seeds from El Gandul and Hinojos populations. In each population, the means followed by the same letter are not significantly different at the 5% level probability as determined by the *t*-test with estimation of the separate variance.

tion displayed similar germination percentages and t_{50} as those of the control seeds (Table 1 and Fig. 2c). Seeds subjected to sulphuric acid scarification, unlike those in all other treatments, did not produce a mucilaginous layer. Finally, seeds germinated to a similar percentage and t_{50} in both light and darkness (Table 1).

Discussion

Freshly-matured seeds of *Euphorbia boetica* did not germinate. However, the fact that the seeds

Table 1. Germination percentages and mean times of germination (t_{50}) of *Euphorbia boetica* seeds from El Gandul after several treatments and the significance level of ANOVAs tests comparing each treatment with the control. Mean ± SE are given. n.s.= not significant, *= $P < 0.01$, **= $P < 0.001$.

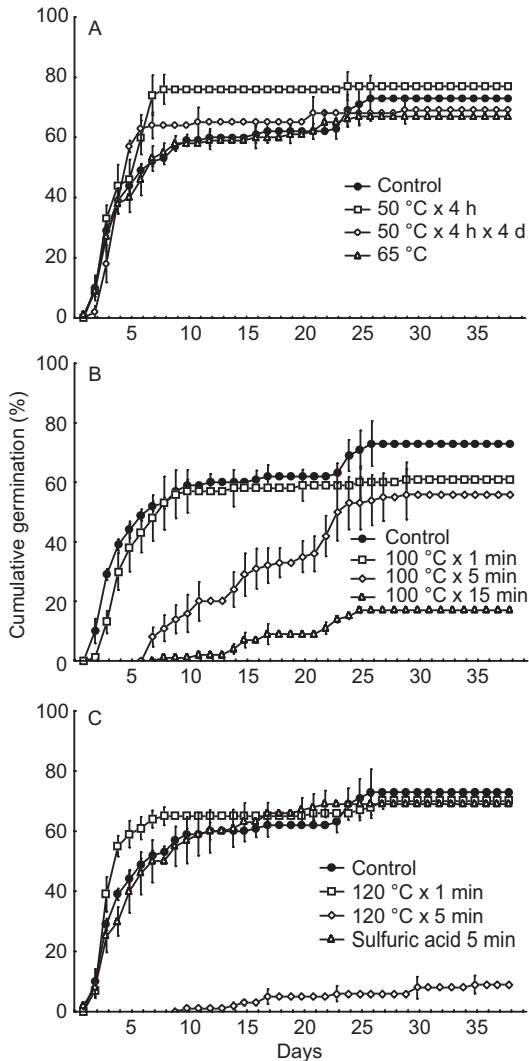|	Germination (%)	t_{50}
Control	73 ± 7.5	7.4 ± 1.3
50 °C 4 h	78 ± 5.3 n.s.	5.6 ± 0.7 n.s.
50 °C 4 h × 4 d	69 ± 4.4 n.s.	5.4 ± 0.7 n.s.
65 °C 30 min	67 ± 3.0 n.s.	6.5 ± 0.9 n.s.
100 °C 1 min	61 ± 11.5 n.s.	6.2 ± 1.0 n.s.
100 °C 5 min	56 ± 17.0 n.s.	16.3 ± 1.0 *
100 °C 15 min	17 ± 1.0 **	18.1 ± 2.0 *
120 °C 1 min	70 ± 2.6 n.s.	5.2 ± 0.5 *
120 °C 5 min	8 ± 3.6 **	22.1 ± 4.5 *
Sulfuric acid	69 ± 1.2 n.s.	6.7 ± 1.5 n.s.
Darkness	66 ± 4.6 n.s.	5.4 ± 0.7 n.s.

Fig. 2. Cumulative temporal pattern of germination of *Euphorbia boetica* seeds from El Gandul subjected to different treatments. Bars represent standard errors of means.

sown under the same temperature conditions after three months of dry storage did germinate indicates that they had non-deep physiological dormancy (Nikolaeva 1977, Baskin & Baskin 1998). Physiological dormancy is common in temperate-climate species, and it has been reported in species of many different families (Nikolaeva 1977, Probert 1992, Baskin & Baskin 1998). Physiological dormancy also has been found in species of the genus *Euphorbia*, in which six months of dry storage were sufficient for the seeds to lose dormancy (Ramakrishan

1965, Baskin & Baskin 1988, Kigel *et al.* 1992). In natural habitats, with lower temperatures during the night and higher ones during the day than those in the laboratory, breaking of physiological dormancy is probably shorter than in the laboratory (Baskin & Baskin 1998). Therefore, because *E. boetica* seeds disperse in June–July, they can germinate in the field with the first rains of autumn (Narbona 2002). As suggested for other species of temperate climate (Baskin *et al.* 1993), the non-deep physiological dormancy of *E. boetica* could prevent germination after summer occasional rains, ensuring germination at the beginning of autumn — the favourable period for the seedling's growth.

Darkness did not affect the germination capacity of *E. boetica* seeds. Similar results have been found in some other *Euphorbia* species (Best *et al.* 1980, Brecke 1995, Narbona *et al.* 2006), while in others germination is favoured by irradiance conditions (Kivilaan & Bandurski 1981, Baskin & Baskin 1988). Seeds collected by granivorous ants may remain underground, in the nest chambers, after the colony dies or moves to a new nest site (Beattie 1985, Vorster *et al.* 1994). Hence, the capacity to germinate in darkness can be an advantage. This would be important in *E. boetica*, as most of its seeds are actively collected by granivorous ants (Narbona 2002).

Fire is a common disturbance in Mediterranean ecosystems (Naveh 1994), and two types of species have been distinguished, depending on their response to fire: seeders and resprouters (Keeley 1991). Seeder species die after the passage of a fire, and their seeds of the bank germinate in the first rainy season following the fire. In addition to loss of physiological dormancy that occurs while seeds are in the soil, thermal shock and/or substances leached from burnt plant material may play a role in promoting germination (Keeley 1991). In contrast, resprouter species do not die after a fire, but elongate their dormant buds, on the lower knots of the stem, generally underground (Keeley 1991). Resprouter species do not usually form seed banks (Parker & Kelly 1989). Post-fire survival by means of resprouting has been documented in some perennial species of *Euphorbia* (Fellows & Newton 1999, Pfab & Witkowski 1999); however, nothing is known

about the response of the seeds of this genus after a fire. *Euphorbia boetica* has the ability to resprout after a fire (E. Narbona pers. obs.). Our results show that seeds of this species seem to have a certain tolerance to fire, since many of them survived after exposure to the mildest fire-simulated treatment. In the field, at a depth of 2.5 cm, the temperature during a fire does not exceed 100 or 120 °C for more than a few minutes (Trabaud 1979, Mallik & Gimingham 1985). Hence, many buried seeds of *E. boetica* could maintain their germination capacity. Thus, *E. boetica* shows an intermediate situation between the two categories (seeders versus resprouters) proposed by Keeley (1991).

Euphorbia boetica seeds resisted sulphuric acid scarification. In nature, acid scarification occurs when seeds are eaten by herbivores or frugivores, and pass through their digestive tract (Janzen 1983). Endozoochory by large mammalian herbivores could be an important mechanism for long-distance dispersal (Pakeman 2001). Recently, it has been documented that the seeds of some Mediterranean dry-fruited shrubs remain viable after passage through the sheep gut (Manzano *et al.* 2005). Olson *et al.* (1997) observed that sheep are important consumers of inflorescences and seeds of *E. esula*, and estimated that between 5% and 24% of the eaten seeds were still viable. Goats have been observed (Narbona 2002) to consume fruits of *E. nicaeensis*, a species closely related to *E. boetica*. Although our treatment did not include any grinding effect to simulate the natural passage through an animal digestive system, the fact that most *E. boetica* seeds germinated after acid scarification suggests that herbivores could act as occasional dispersal agents.

To sum up, *Euphorbia boetica* seeds have non-deep physiological dormancy, which is broken in summer, thus allowing seeds to germinate at the beginning of the wet season when conditions are optimal for seedling growth. Seeds of this species can germinate under diverse situations such as light or darkness, and after high temperature conditions. Further, the high germination capacity of *E. boetica* seeds found in El Gandul and Hinojos populations in the years of the present study, was observed in later years and in other the populations (Narbona 2002); therefore, seed germination *per se* does not appear to represent a crucial phase in the species life cycle.

Acknowledgements

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