

## Research Note

### Seed mass and germination in Asteraceae species of Argentina

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(Accepted July 2009)

#### Summary

The effects of seed mass variation on germination percentage, mean time to germinate and light requirements for germination in seven Asteraceae species of Argentina were studied. Germination, mean time to germinate and light requirements were significantly different among species at both 20°C and 25/10°C temperature regimes. In general, larger (> 1.0 mg) and intermediate-mass seeds ( $0.1 \leq 1.0$  mg) species showed higher germination, lower mean time to germinate and less light requirement than smaller-mass seeds (< 0.1 mg). However, these responses can vary with the species and temperature regime under which germination is tested. These results could help to explain the field observations for variation in seedling establishment between species and populations studied.

#### Experimental and discussion

Seed mass variation has important consequences in critical processes such as seed dispersal, germination, emergence, survival and/or competitive ability (Hendrix, 1984; Milberg *et al.*, 2000; Pearson *et al.*, 2002; Daws *et al.*, 2007). Large seeds may give rise to better competitors due to greater germination percentage, less light requirement for germination and greater emergence from deeper depths; but small seeds may gain a competitive advantage due to a greater dispersal capacity that increases the probability of reaching favourable microsites (Wulff, 1986; Milberg *et al.*, 2000; Pearson *et al.*, 2002; Jankowska-Blaszczuk and Daws, 2007). The northern and central regions of Argentina are characterized by high dicotyledon diversity (Juárez *et al.*, 2007). Some species, such as in the family Asteraceae, studied here, have a high aromatic and medicinal value (Barboza *et al.*, 2006) and are being collected by the nutritional, pharmaceutical and cosmetic

industries (Nuñez and Cantero, 2000). Consequently, many of them are threatened by over collecting, and also by current land use changes. Knowledge of the factors that trigger seed germination of endemic, rare and threatened species may contribute to an improved understanding of the phenomenon of rarity, and at the same time assist conservation management decisions for these species (Navarro and Guitián, 2003; Ramírez-Padilla and Valverde, 2005). Consequently, we investigated the effect of light and temperature regime on germination of seven Asteraceae species for which knowledge on their seed biology is limited. Intact achenes (hereafter seeds) of *Baccharis medullosa* D.C., *B. dracunculifolia* D.C., *Eupatorium buniifolium* Kook. & Arn., *Flourensia campestris* Griseb., *F. oolepis* S.F. Blake, *Parthenium hysterophorus* L. and *Zinnia peruviana* L. were collected from different provinces of Argentina (table 1). Seeds were collected between March and May 2005, from a minimum of 30 individual plants per species and stored at 15°C and 15% relative humidity prior to the experiment in July 2005.

Seed mass was determined by weighting 25 individual seeds for each species. According to the differences registered in the comparison of mean seed mass among species (Two-way ANOVA test,  $F_{6, 168} = 764.18$ ,  $p < 0.0001$ , table 1), species were grouped in three classes: smaller-seeded species ( $< 0.1$  mg), intermediate-seeded species ( $\geq 0.1 \leq 1.0$  mg) and larger-seeded species ( $> 1.0$  mg). Viability of five replicates of 20 seeds per species was assessed using the tetrazolium chloride (TZ) staining technique (ISTA, 2003) and for all species it ranged from 70 to 100%.

Four replicates of 25 seeds per species and population were sown on the surface of 1% agar in water at 20°C and 25/10°C under white light (8 h light/16 h dark) and complete darkness. In the alternating temperature, the light period coincided with the elevated temperature period. Germination in the light treatment was recorded daily for 30 days. Darkness was achieved by wrapping dishes in aluminium foil, and germination was recorded once at the end of the experiment. For all species, germination was defined as radicle emergence by  $> 1$  mm. Germination percentages recorded in light and in darkness were used to calculate an index of light requirement, the so called 'relative light germination' (RLG; see Milberg *et al.*, 2000):

$$RLG = G_l / (G_d + G_l)$$

where  $G_l$  is the germination percentage in light and  $G_d$  the germination percentage in darkness. RLG represents a range of values from 0 (germination only in darkness) to 1 (germination only in light). Mean time to germinate (MTG), in days was also calculated, thus:

$$\Sigma (D n) / \Sigma n$$

where  $n$  was the number of seeds that germinated on day  $D$  and  $D$  was the number of days from the beginning of germination test.

At a constant 20°C, germination (%), MTG and RLG, were significantly different among species (Two-way ANOVA test,  $F_{2,25} = 10.4$ ,  $p < 0.001$ ,  $F_{2,25} = 9.5$ ,  $p < 0.001$ , and  $F_{2,25} = 39.6$ ,  $p < 0.001$ , table 2). Germination was higher in larger and intermediate-seeded species than *B. dracunculifolia* (smaller-seeded species), whereas RLG was higher in both smaller than in larger and intermediate-seeded species. For all species, germination started around the third day and finished by the seventh day. MTG was also longer in *B. dracunculifolia* than in the other species (table 2).

Table 1. Details of the seven Asteraceae species studied including provenance, location, status, mean ( $\pm$  SE) seed mass and seed mass classification.

Species	Province	Location	Status	Seed mass (mg)	Seed mass class
<i>Bacharis medullosa</i>	Entre Ríos	31° 55' S; 58° 17' W	native	0.061 $\pm$ 0.004	Smaller
<i>Bacharis dracunculifolia</i>	Entre Ríos	31° 55' S; 58° 17' W	native	0.067 $\pm$ 0.005	Smaller
<i>Eupatorium buntifolium</i>	Entre Ríos	31° 55' S; 58° 17' W	native	0.121 $\pm$ 0.008	Intermediate
<i>Parthenium hysterophorus</i>	Salta	25° 05' S; 65° 30' W	native	0.466 $\pm$ 0.026	Intermediate
<i>Zinnia peruviana</i>	Salta	25° 05' S; 65° 30' W	native	4.186 $\pm$ 0.219	Larger
<i>Flourensia campestris</i>	Córdoba	30° 57' S; 64° 35' W	endemic	3.661 $\pm$ 0.209	Larger
<i>Flourensia oolepis</i>	Córdoba	30° 51' S; 64° 32' W	endemic	5.795 $\pm$ 0.345	Larger

Table 2. Seed mass class and mean ( $\pm$  SE) germination in light, mean time to germinate (MTG) and relative light germination (RLG) under two temperature regimes, for each species.

Species	20°C			25/10°C		
	Germination in light (%)	MTG (day)	RLG	Germination in light (%)	MTG (day)	RLG
Smaller						
<i>Bacharis medullosa</i>	78 $\pm$ 1.15	4.81 $\pm$ 0.14	0.73 $\pm$ 0.04	65 $\pm$ 4.72	6.77 $\pm$ 0.17	0.79 $\pm$ 0.05
<i>Bacharis dracunculifolia</i>	52 $\pm$ 7.46	7.20 $\pm$ 0.29	0.86 $\pm$ 0.04	43 $\pm$ 7.65	9.86 $\pm$ 0.46	0.70 $\pm$ 0.10
Intermediate						
<i>Eupatorium buntifolium</i>	77 $\pm$ 5.74	4.20 $\pm$ 0.12	0.52 $\pm$ 0.04	78 $\pm$ 8.52	6.56 $\pm$ 0.33	0.61 $\pm$ 0.05
<i>Parthenium hysterophorus</i>	99 $\pm$ 1.00	3.67 $\pm$ 0.03	0.56 $\pm$ 0.01	98 $\pm$ 1.15	3.92 $\pm$ 0.08	0.64 $\pm$ 0.03
Larger						
<i>Zinnia peruviana</i>	96 $\pm$ 2.06	3.96 $\pm$ 0.10	0.59 $\pm$ 0.01	44 $\pm$ 5.26	6.70 $\pm$ 0.31	0.44 $\pm$ 0.06
<i>Flourensia campestris</i>	91 $\pm$ 3.42	5.76 $\pm$ 0.15	0.53 $\pm$ 0.02	93 $\pm$ 2.61	7.61 $\pm$ 0.36	0.51 $\pm$ 0.01
<i>Flourensia oolepis</i>	89 $\pm$ 2.94	5.84 $\pm$ 0.11	0.50 $\pm$ 0.01	91 $\pm$ 1.97	7.83 $\pm$ 0.38	0.49 $\pm$ 0.004

At the alternating temperature, germination, MTG and RLG, were also significantly different among species (Two-way ANOVA test,  $F_{2,25} = 6.6$ ,  $p = 0.005$ ,  $F_{2,25} = 11.24$ ,  $p < 0.001$ , and  $F_{2,25} = 15.8$ ,  $p < 0.001$ , table 2). With the exception of *Z. peruviana*, the other larger and intermediate-seeded species showed higher germination than smaller-seeded species. Smaller-seeded species showed higher light requirements for germination (RLG) than the other species and *P. hysterothorus* (intermediate-seeded) showed shorter MTG than the others species. Overall though, alternating temperature lengthened MTG in all species. For *B. medullosa* and *Z. peruviana*, germination percentages were also lower under alternating compared to constant temperature (table 2).

Several studies have reported that larger seeds have greater reserves which enable faster emergence and greater initial seedling size, with higher tolerance to shade, drought and physical damage (see Leishman *et al.*, 2000). In this case, we found that larger (*Z. peruviana*, *F. campestris* and *F. oolepis*) and intermediate-seeded species (*E. buniifolium* and *P. hysterothorus*) had higher germination percentages and lower mean times to germinate than smaller-seeded species (*B. medullosa* and *B. dracunculifolia*). However, these germination responses and also light requirements may vary with the temperature regime under which germination is tested (see Probert, 2000) and species studied. For all species, mean time to germinate was longer at alternating temperature, and particularly for *B. medullosa* and *Z. peruviana* germination percentage was lower in alternating than in constant temperature. Thus, these species would germinate better at constant than at alternating temperature, although, a wide range of temperatures would be necessary to identify the temperature effects on seed germination characteristics.

In relation to light requirements for germination, our results showed that species with smaller seeds were more likely to require light for germination than intermediate and larger-seeded species, independently of temperature regime. This relationship between light requirement and seed mass has been reported previously for several herbs and tree species from Europe and Central America (Milberg *et al.*, 2000; Pearson *et al.*, 2002; Jankowska-Blaszczuk and Daws, 2007). It has been proposed that light requirements function as a depth-sensing mechanism, ensuring that small seeds germinate only when close to the soil surface, whilst large seeds which can germinate from much greater depths would be indifferent to light. These results support field observations for *B. dracunculifolia*, *B. medullosa* and *E. buniifolium* for which seedling establishment has been associated with post-fire environments (Galíndez *et al.*, 2008). For *P. hysterothorus* and *Z. peruviana* their lower light requirement for germination, high germination percentages and shorter mean times to germinate may partly explain their wide distribution in different habitats (Tamado *et al.*, 2002). However, in the cases of *F. campestris* and *F. oolepis* (both endemic species) factors other than seed germination could explain their distribution patterns.

In conclusion, species with larger and intermediate-sized (mass) seeds showed higher germination percentage and lower mean times to germinate than smaller-seeded species. Percentage and time of germination were influenced by the temperature regime under which germination was tested. Smaller-seeded species were more likely to require light for germination than the other species, independently of the temperature regimes used. Nevertheless, another study including seeds of several populations of each species would

be necessary to evaluate if these results are consistent throughout the distribution of the different studied species and in this way to elucidate if the responses obtained are species-specific.

## Acknowledgements

We are grateful to Mariana Silva, Carlos Chicharro, Walter Martin, Pompeya Schattenhofer and Ezequiel Sotola who helped to collect seed. Financial support for this research was provided by The Millennium Seed Bank Project of the Royal Botanic Gardens, Kew, UK and ANPCyT PICT 01979-2006.

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