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Seed mass, seed production, germination and seedling traits in two phenological types of *Bidens pilosa* (Asteraceae)

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Abstract. *Bidens pilosa* L. is a summer annual that shows a particular phenological pattern in the Córdoba mountains, Argentina. Some individuals start flowering 1 month after germination (early type), but most of the population starts flowering 4 months after germination (normal type). The aims of this study were to (1) analyse whether differences in flowering phenology affect seed mass and seed production, and (2) assess whether possible differences in seed traits of the two parental phenological types would affect germinability, germination rate, seedling growth and flowering phenology of offspring under laboratory conditions. The study showed that the numbers of seeds per capitulum and per plant were greater in the normal type than in the early type plants. This can be related to plant height, since in the field, normal-type plants are larger than early type plants. However, early type plants produced heavier seeds than normal-type plants. Germination rate was faster in the early type seeds, but total germination was higher in the normal ones. Seedling growth, in terms of height and the date of first flowering, did not differ between the phenological types of parental seed sources. Our results showed that differences in flowering phenology were associated with seed mass and seed production differences. Seed mass appears to be related to germination characteristics but not to seedling growth nor to flowering phenology of offspring.

Introduction

Plant flowering phenology varies not only between species in a community but also between individuals of the same species (Rathcke and Lacey 1985). Flowering phenology can affect seed traits (Cavers and Steel 1984; Wolfe and Burns 2001), which are important components of plant life histories (Ellison 2001). Ågren (1989), Wolfe (1995) and Vaughton and Ramsey (1998) showed that seed mass decreases throughout the flowering period. Seed mass variation may bring about an optimal seed shadow (Janzen 1977) or minimise the risk of failure in heterogeneous environments (Venable and Brown 1988). It also has important effects on seedling traits such as timing of germination, emergence, stress tolerance, survivorship, seedling size, seedling competitive ability and recruitment (Schaal 1980; Winn 1988; Platenkamp and Shaw 1993; Reader 1993; Leishman *et al.* 2000; Simons and Johnston 2000; Susko and Lovett-Doust 2000; Jacquemyn *et al.* 2001; Moles and Westoby 2002). Both seed mass and seedling characteristics have important effects on population dynamics, particularly in annual plants inhabiting unpredictable environments in both space and time (Venable and Brown 1988).

Bidens pilosa L. (Asteraceae) is a common annual herb in the grasslands and shrublands of the Córdoba mountains, central Argentina (Cantero *et al.* 2001). Although native to temperate and tropical America, it is considered a noxious weed in many ecosystems of the world (Marzocca 1976; Holm *et al.* 1977). It exhibits two flowering–fruiting events during the growing season. Some plants, no more than 10% of the population, begin their flowering very early, only 1 or 2 months after germination (hereafter referred to as 'early type'), while most of the population (>90% of the individuals) has a flowering–fruiting event in February or March (Fig. 1), 4 months after germination (hereafter referred to as 'normal type') (D. E. Gurvich, unpubl. data). For both phenological types the peak of germination occurs in early November, right after the first rains. The two phenological types coexist in the same community.

The aims of our study were to (1) analyse differences in seed traits (seed mass, seed number per capitulum and per plant) between both *B. pilosa* phenological types, and (2) assess whether differences in seed traits between phenological types may influence seedling traits (germinability, germination rate, seedling growth

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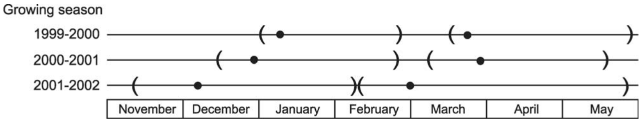

Fig. 1. Flowering phenology of *Bidens pilosa* for three consecutive growing seasons. Parentheses indicate the flowering amplitude, while dots between parentheses show the occurrence of flowering peak. Flowering peak is defined as the week with the highest number of flowering individuals, which coincidently hold the highest number of flowers. The consecutive flowering events in a given growing season correspond to the early and normal phenological types.

and flowering phenology) of offspring under controlled laboratory conditions.

Materials and methods

Study site

The study area is located in Cuesta Blanca (31°30′S, 64°35′W) in the Córdoba mountains of central Argentina. The site is located 880 m asl, and has a mean annual precipitation of 720 mm mainly in the warm season, from September to April (De Fina 1992). The climate is temperate, with a mean annual temperature of 15°C, and several frost events from May to September. The area is an open shrubland dominated by the shrub *Acacia caven* (Molina) Molina and the tussock grass *Stipa eriostachya* H.B.K. (Zak and Cabido 2002), and corresponds to the Chaco Serrano biogeographic district (Cabrera 1976).

Plant species

Bidens pilosa is a summer annual plant widely distributed in central and northern Argentina and neighbouring countries (Zuloaga and Morrone 1996). It is considered a weed in tropical and subtropical regions of the world (Cabrera 1963). In the study area, *B. pilosa* is the dominant annual herb, with as much as 40% of the total cover towards the end of the summer when it reaches the peak of its growth (D. E. Gurvich, unpubl. data). It is a self-compatible herb (Sun and Ganders 1990) with ectozoocorous dispersion of seeds. For convenience, the one-seeded fruits or achenes are referred to as ‘seeds’ in the text. Species nomenclature follows Zuloaga and Morrone (1996). The particular phenological pattern herein reported arose from a 3-year study of the reproductive and vegetative phenology of the plant community (44 species) at the study site (Fig. 1; D. E. Gurvich, unpubl. data). Thus, more than 180 individuals of *B. pilosa* were studied annually. The percentage of individuals (8–10%) presenting the early phenological type was similar between years.

Seed mass and seed number measurements

For both phenological types, all measurements were made at the beginning of the fruit-ripening period (29 November for early type, 20 March for normal type) to standardise the ontogenetic stages for both phenological types. For all measurements, individuals were selected randomly within the study site.

We measured plant height in the field of 15 individuals of each phenological type. To obtain seed number per capitulum, we collected 1–3 capitula with developing seeds, thus avoiding an underestimation due to dispersal, from 21 individuals of each phenological type. Also, we counted the total number of capitula in all the stages, from early developed buds to mature capitula, from 15 individuals of each phenological type. For seed mass determination, we collected 1–3 mature capitula from 15 individuals of each phenological type. Seeds were air-dried at 25°C for several days, and stored in the dark at room temperature in seed envelopes until measurements and experimental work began. The total number of seeds collected from the 15 individuals of the early and normal phenological types was 213 and 447, respectively. These seeds were weighed individually to the nearest 0.1 mg. For an estimation of the number of seeds produced per plant, the average number of seeds per capitulum was multiplied by the average number of capitula produced per plant of each phenological type.

Germination experiment

Seeds used for this experiment were taken from those previously used to determine seed mass, and 157 and 334 seeds from the early and normal types, respectively, were distributed on wet filter paper in Petri dishes (90 mm diameter). The number of seeds in each dish varied between 18 and 24. Petri dishes were placed in a room at constant temperature (25°C) and for 12 h of continuous fluorescent light ($\text{PAR} = 24.87 \pm 5.61$ µmol m^2 s^{-1}). The germination experiment started on 27 April 2002. Germinated seeds (radicle emergence) were counted and removed daily. The experiment was finished after 18 days, at which time we observed no further germination. Seeds that did not germinate within this time span were considered dead or not viable. Germination rate was calculated according to Milberg *et al*. (1996): the number of seeds that germinated on Day 1 was multiplied by one, the number on Day 2 by two, and so forth. The average number of days taken before the seeds germinated was calculated by dividing the sum of these terms by the total number of germinated seeds.

Greenhouse experiment

Seeds for this experiment were taken from those previously used to determine seed mass. Twenty-three and 48 seedlings (replicates, previously germinated in Petri dishes) of the early and normal types, respectively, were transplanted to 0.43-L plastic pots filled with commercial garden soil. Height, used here as a measure of growth, was recorded weekly. We also recorded the date of the beginning of flowering of each individual. Measurements on each individual began when the cotyledons fully expanded. All pots were placed in a greenhouse at random positions under a 20–30°C/10–15°C day/night temperature regime and natural photoperiod. Soil in the pots was watered daily. The greenhouse experiment started on 19 June and finished on 13 September 2002.

Data analysis

We tested differences in the measured variables (seed mass, seed number per capitulum and per plant, and plant height) between phenological types by *t*-tests (InfoStat 2002). The χ^2-test was used to test differences in germination percentage between phenological types (InfoStat 2002). ANOVA for repeated measures was used to test differences in growth between both phenological types (InfoStat 2002).

Results

Seed mass and seed number measurements

All measured variables differed significantly between phenological types (Fig. 2). Plant height in the field was 2.3 times greater in the normal type than in the early type (Fig. 2*a*). The number of seeds per capitulum was 2.4 times higher in the normal than in the early type (Fig. 2*b*). Total number of capitula per plant was almost seven times greater in the normal type than in the early type (Fig. 2*c*). The estimated number of seeds produced per plant was 90 and 1508 for the early and normal types, respectively. Seed mass showed an inverse pattern: seeds from early type plants were heavier

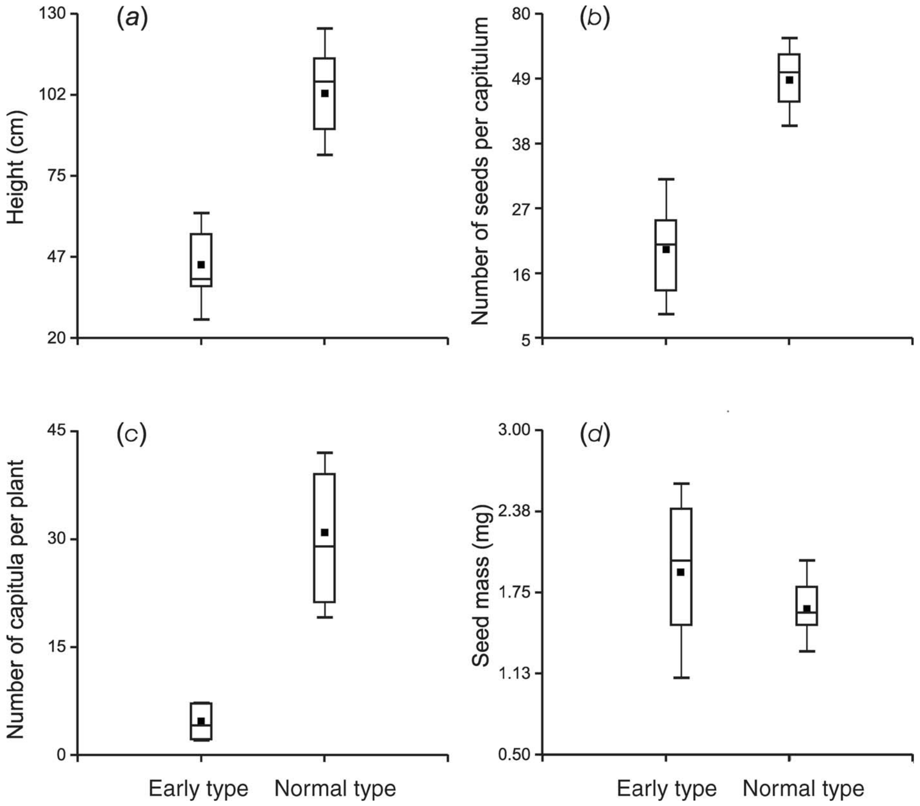

Fig. 2. Comparison of plant height and reproductive traits for the two phenological types of *Bidens pilosa* found in the field. (*a*) Plant height in the field ($n = 15$ for each phenological type), (*b*) number of seeds per capitulum ($n = 21$ for each phenological type), (*c*) number of capitula produced per plant ($n = 15$ for each phenological type), and (*d*) seed mass ($n = 213$ and 447 for the early and normal types, respectively) for the phenological types. In all cases, differences are significant at the 0.0001 probability level (*t*-test). The box plot shows the distribution of the values by the medians (central line), the mean (dots), the 25% and 75% quartiles (box) and the ranges (whiskers).

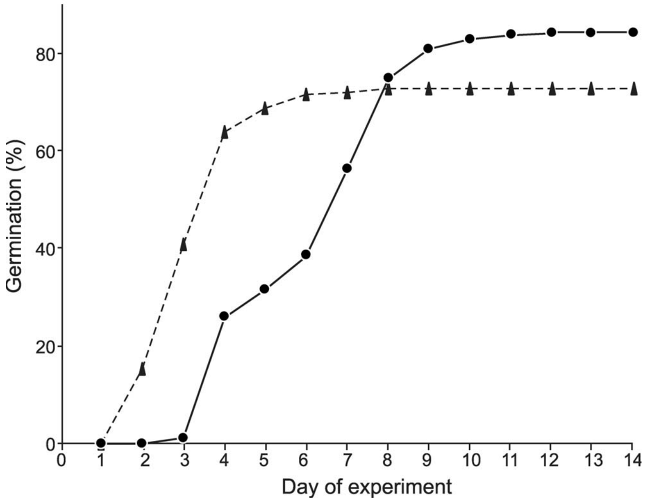

Fig. 3. Cumulative percentage of germination for each phenological type (early type = triangles, normal type = circles). $n = 157$ (early type seeds) and 334 (normal-type seeds). The germination rate was 3.44 and 6.41 for the early and normal types, respectively. Differences in total percentage of germination between types were statistically significant ($\chi^2 = 10.13$, $P = 0.0015$).

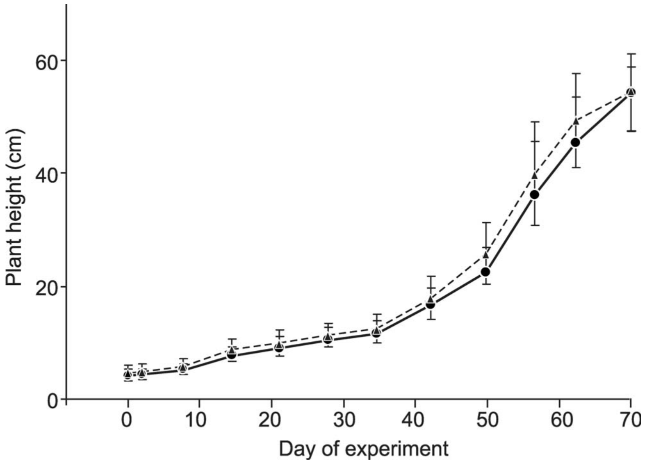

Fig. 4. Mean growth in height for both phenological types under laboratory conditions. $n = 23$ and 48 plants for the early and normal types, respectively (early type = triangles, normal type = circles). Differences were not significant ($F = 1.17$, $P = 0.29$; ANOVA for repeated measures).

(1.92 mg) than those from normal-type individuals (1.63 mg) (Fig. 2*d*). Within the phenological types, seed mass varied 6.2-fold in early type (0.5–3.1 mg) and 2.5-fold in normal type (0.9–2.3 mg). Mean seed mass per plant (seed mass × number of seeds per capitulum × number of capitula per plant) was 14 times greater in the normal (2458 mg) than in the early type (174 mg).

Germination experiment

Sprouting began on Day 2 for the early type and on Day 3 for the normal-type seeds. Germination rate was 3.44 and 6.41 days for the early and normal-type seeds, respectively (Fig. 3). However, total seed germination was higher in normal-type seeds than in early type seeds (84.7% compared with 72.6%; Fig. 3). Seeds germinated until Days 8 (early type) and 13 (normal type).

Greenhouse experiment

There was no significant difference in plant growth (height) between seedlings of both types (Fig. 4). Flowering began during Week 6 in seedlings of both phenological types. On Day 44, 71% of the early type and 39% of normal-type individuals had at least one capitulum. A week later, 86% of the early type and 91% of the normal-type offspring had at least one capitulum, while the following week, on Day 58, all offspring from both types had capitula.

The beginning of flowering of offspring observed during the experiment was intermediate between that observed in the field for both the early and normal type (4 and 18 weeks, respectively).

Discussion

Our data show that seed production and mass differ significantly between *B. pilosa* phenological types. Early type plants produce heavier, but much fewer, seeds, both per capitulum and per plant, than normal-type plants. Although we do not rule out other factors (see Obeso 1993; Wolfe 1995; Vaughton and Ramsey 1998; Susko and Lovett-Doust 2000) affecting seed mass in an annual plant such as *B. pilosa*, our study shows that a differential flowering phenology could be linked to it.

The differences in number of seeds per capitulum and per plant and in mean seed mass per plant can be accounted for by differences in plant height in the field between phenological types. Thus, larger normal-type plants produce more seeds per capitulum and more capitula per plant than early type plants. In terms of total seed mass produced by plant, the normal type produce 14 times more seed mass than the early type. Ollerton and Lack (1998) found in *Lotus corniculatus* that larger plants produce more fruit than smaller ones. Susko and Lovett-Doust (2000) reported for *Alliaria petiolata* that larger plants from warmer southern locations had greater fruit and seed production than smaller plants from colder northern sites. Regarding the difference in seed mass between both phenological types, it could be the result of a trade-off between seed number and seed mass (Smith and Fretwell 1974; Venable 1992; Wolfe 1995; Vaughton and Ramsey 1998).

From the variables measured in the germination and greenhouse experiments, only germination rate and percentage of germination differed significantly between the parental phenological types. Seedling growth and flowering date did not differ between phenological types. Seeds from early type plants germinate faster but show a lower germinability than seeds from the normal-type individuals. Milberg *et al*. (1996) found that although different seed mass fractions differed in germinability and germination rate, the magnitude and direction of such differences are difficult to foresee. According to Baskin and Baskin (1998), the rate and percentage of germination could increase, decrease or remain unaffected by differences in seed size.

Although it has been reported that differences in seed mass can produce seedlings with differences that persist into adulthood (Simons and Johnston 2000), in many cases this does not happen. Hence, the variation evident at the earliest point of germination, and immediately after, may be of great significance for the initial establishment in a population, but not for plant survivorship until reproductive maturity (Seiwa 1998; Vaughton and Ramsey 1998; Susko and Lovett-Doust 2000). Therefore, a faster germination rate of seeds could improve the establishment of early type seedlings during the following growing season, which could be crucial when normal-type individuals die as a result of an uncommon drought (see below).

Since variance in seed mass of the early type is very high (see Fig. 2*d*), we cannot discard the possibility that the results of the germination and greenhouse experiments could also have been affected by this rather than simply by the difference in the mean of seed mass between phenological types. Further research is needed to separate the effects of mean seed mass from those that could be related to their high variance, mainly in seeds from the early type plants.

The fact that offspring from both phenological types, growing under the same environmental conditions, did not show differences in growth and in the timing of their flowering, discounts possible genetic or maternal effects as responsible for the phenological pattern observed in the field (Roach and Wulff 1987). It is well known that stress events, such as drought or heat, can trigger an early flowering (Larcher 1995). Probably, micro-environmental differences in soil moisture and temperature would trigger an early flowering in some of many plants growing close together.

This phenotypic plasticity in flowering phenology not only allows some individuals growing in poorer patches to produce seeds, but also ensures that at least some of the plants would produce seeds in atypically unfavourable years. Although the number of seeds produced by these early flowering plants is much smaller than that produced by the normal plants, it could be enough for the recovery of the population after an uncommon drought event.

It is remarkable that the only annual plant showing this phenological pattern in the study area is *B. pilosa,* it being also the most abundant annual. This could be due to the fact that *B. pilosa* is not drought tolerant, showing a loss of leaf turgor at –1.3 Mpa (D. E. Gurvich, unpubl. data), which means that even with a relatively high soil water content, plants stop growing, and if drought persists they could eventually die (Salisbury and Ross 1992). Other annual plants common in the area, such as *Tagetes minuta* L., *Zinnia peruviana* (L.) L. and *Conyza bonariensis* (L.) Cronquist, seem to be more drought tolerant than *B. pilosa*. The phenological pattern observed in *B. pilosa* could therefore be crucial for maintaining its dominance over the other annual plants. Knowing that there is a trade-off between stress tolerance, such as drought resistance, and growth rate (Chapin *et al.* 1993; Aerts and Chapin 2000), it is likely that *B. pilosa* has a higher growth rate than the other annuals, at an expense of reduced drought tolerance. Thus, in a dry year, the other annuals—with a greater drought tolerance—would become dominant, but within a year or two the *B. pilosa* population would recover and become dominant again.

Phenotypic plasticity (e.g. growth rate and size) has been considered an important characteristic of invasive plants (Dean 1998). It would also be possible that plasticity in the flowering phenology (here reported) could play an important role in invasive species, as it promotes the dominance over other annual herbs in their native habitat. Although the bimodal phenological pattern here reported seems to be common in the Córdoba mountains (D. E. Gurvich, pers. obs.) we have no information regarding the phenological behaviour of this species in other regions. Therefore, further research is needed to determine whether the success of *B. pilosa* as an invasive species could be related to its flowering phenology. Also, the study of the phenology of *B. pilosa* and the climate in different regions could give a clue to the environmental factors responsible for this phenological pattern.

To conclude, our study shows that a differential flowering phenology influences seed mass and seed production, as well as germination characteristics in *B. pilosa* in the Córdoba mountains. However, seedling growth and the subsequent flowering of offspring were not affected by parental phenology. Early flowering individuals produce much fewer but heavier seeds than the late-flowering individuals. These early type seeds germinate faster than normal-type seeds. This would confer a better seedling establishment during the following growing season. This particular phenological pattern would be somehow advantageous in the face of climatic variability. Therefore, early flowering individuals, which constitute a minor component of the total population in a normal year, would be crucial in a dry year, thus ensuring that at least some individuals would produce seeds for the following growing season.

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